EPA/635/R-09/011F
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TOXICOLOGICAL REVIEW

OF

TRICHLOROETHYLENE

(CAS No. 79-01-6)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2011

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

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GUIDE TO READERS OF THIS DOCUMENT

Due to the length of the TCE toxicological review, it is recommended that Chapters 1 and 6 be read prior to Chapters 2–5.

Chapter 1 is the standard introduction to an IRIS Toxicological Review, describing the purpose of the assessment and the guidelines used in its development.

Chapter 2 is an exposure characterization that summarizes information about TCE sources, releases, media levels, and exposure pathways for the general population (occupational exposure is also discussed to a lesser extent).

Chapter 3 describes the toxicokinetics and physiologically based pharmacokinetic (PBPK) modeling of TCE and metabolites (PBPK modeling details are in Appendix A).

Chapter 4 is the hazard characterization of TCE. Section 4.1 summarizes the evaluation of epidemiologic studies of cancer and TCE (qualitative details in Appendix B; meta-analyses in Appendix C). Each of the Sections 4.2–4.9 provides a self-contained summary and syntheses of the epidemiologic and laboratory studies on TCE and metabolites, organized by tissue/type of effects, in the following order: genetic toxicity, central nervous system (CNS), kidney, liver, immune system, respiratory tract, reproduction and development, and other cancers. Additional details are provided in Appendix D for CNS effects and in Appendix E for liver effects. Section 4.10 summarizes the available data on susceptible lifestages and populations. Section 4.11 describes the overall hazard characterization, including the weight of evidence for noncancer effects and for carcinogenicity.

Chapter 5 is the dose-response assessment of TCE. Section 5.1 describes the dose-response analyses for noncancer effects, and Section 5.2 describes the dose-response analyses for cancer. Additional computational details are described in Appendix F for noncancer dose-response analyses, Appendix G for cancer dose-response analyses based on rodent bioassays, and Appendix H for cancer dose-response analyses based on human epidemiologic data.

Chapter 6 is the summary of the major conclusions in the characterization of TCE hazard and dose response.

Appendix I contains the summary of EPA‘s response to major external peer review and public comments.

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LIST OF ABBREVIATIONS AND ACRONYMS

[^{14}C]TCE	[^{14}C]-radiolabeled TCE
1,2-DCVC	S-(1,2-dichlorovinyl)-L-cysteine
17-β-HSD	17-β-hydroxy steroid dehydrogenase
8-OHdG	8-hydroxy-2‘ deoxyguanosine
ACO	acyl CoA oxidase
ADAF	age-dependent adjustment factor
ADME	absorption, distribution, metabolism, and excretion
AIC	Akaike‘s Information Criteria
ALL	acute lymphoblastic leukemia
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANA	antinuclear antibodies
ANCA	antineutrophil-cytoplasmic antibody
ANOVA	analysis of variance
AOAA	a beta-lyase inhibitor
ASD	autism spectrum disorder
ASPEN	Assessment System for Population Exposure Nationwide
AST	aspartate aminotransferase
ATF-2	activating transcription factor 2
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area-under-the-curve
AV	atrioventricular
AVC	atrioventricular canal
AZ DHS	Arizona Department of Health Services
BAER	brainstem auditory-evoked response
BAL	bronchoalveolar lavage
BMD	benchmark dose
BMDL	benchmark dose lower bound
BMDS	BenchMark Dose Software
BMI	body mass index
BMR	benchmark response
BUN	blood urea nitrogen
CA DHS	California Department of Health Services
CH	chloral hydrate
CI	confidence interval
CLL	chronic lymphocytic leukemia
CNS	central nervous system
CO_2	carbon dioxide
CoA	coenzyme A
cRfC	candidate RfC
cRfD	candidate RfD
CRT	choice reaction time
CYP	cytochrome P450

DAL	dichloroacetyl lysine
$DASO_2$	diallyl sulfone
DBP	dibutyl phthalate
DCA	dichloroacetic acid
DCAA	dichloroacetic anhydride
DCAC	dichloroacetyl chloride
DCE	dichloroethylene
DCVC	S-dichlorovinyl-L-cysteine (collectively, the 1,2- and 2,2- isomers)
DCVG	S-dichlorovinyl-L-glutathione (collectively, the 1,2- and 2,2- isomers)
DEHP	di(2-ethylhexyl) phthalate
DHEAS	dehydroepiandrosterone sulphate
DNA	deoxyribonucleic acid
DNP	dinitrophenol
DPM	disintegrations per minute
dsDNA	double-stranded DNA
EC_x	concentration of the chemical at which x% of the maximal effect is produced
EEG	electroencephalograph
EPA	U.S. Environmental Protection Agency
ERG	electroretinogram
ESRD	end stage renal disease
FAA	fumarylacetoacetate
FDVE	fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether
FMO	flavin mono-oxygenase
FOB	functional observational battery
FSH	follicle-stimulating hormone
G6PDH	glucose 6-p dehydrogenase
GABA	gamma-amino butyric acid
G-CSF	granulocyte colony stimulating factor
GD	gestation day
GGT	γ-glutamyl transpeptidase or γ-transpeptidase
GI	gastrointestinal
GIS	geographic information system
GSD	geometric standard deviation
GSH	glutathione
GSSG	oxidized GSH
GST	glutathione-S-transferase
GT	glutamyl transferase
H&E	hematoxylin and eosin
H_2O	water
HCC	hepatocellular carcinoma
hCG	human chorionic gonadotropin
HCl	hydrochloric acid
HDL-C	high density lipoprotein-cholesterol
HEC	human equivalent concentration

HED	human equivalent dose
$HgCl_2$	mercuric chloride
HH	Hamberger and Hamilton
HPLC	high-performance liquid chromatography
HPT	hypothalamic-pituitary-testis
i.a.	intra-arterial
i.p.	intraperitoneal
i.v.	intravenous
IARC	International Agency for Research on Cancer
ICC	intrahepatic cholangiocarcinoma
ICD	International Classification of Disease
ICRP	The International Commission on Radiological Protection
idPOD	internal dose points of departure
IDR	incidence density ratio
IFN	interferon
IgE	immunoglobulin E
IGF-II	insulin-like growth factor-II (gene)
IL	interleukin
IPCS	International Programme on Chemical Safety
IUGR	intrauterine growth restriction
JEM	job-exposure matrix
JTEM	job-task-exposure matrix
LC	lethal concentration
LCL	lower confidence limit
LDH	lactate dehydrogenase
LEC_x	lowest effective concentration corresponding to an extra risk of x%
LH	luteinizing hormone
lnPBC	blood-air partition coefficient
lnQCC	cardiac output
lnVMAXC	VMAX for oxidation
lnVPRC	ventilation-perfusion ratio
LOAEL	lowest-observed adverse effect level
LOH	loss of heterozygosity
LORR	loss of righting reflex
MA	maleylacetone
MA DPH	Massachusetts Department of Public Health
MAA	maleylacetoacetate
MCA	monochloroacetic acid
MCMC	Markov chain Monte Carlo
MCP	methylclofenapate
MDA	malondialdehyde
MLE	maximum likelihood estimate
MNU	methyl nitrosourea

MS	mass spectrometry
MSW	multistage Weibull
NAcDCVC	N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate-oxidase
NAG	N-acetyl-β-D-glucosaminidase
NAS	National Academy of Sciences
NAT	N-acetyl transferase
NCI	National Cancer Institute
NF-κB	nuclear factor kappa-light-chain enhancer of activated B cells
NHL	non-Hodgkin lymphoma
NK	natural killer
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NPMC	nonpurified rat peritoneal mast cells
NRC	National Research Council
NSATA	National-Scale Air Toxics Assessment
NTP	National Toxicology Program
NYS DOH	New York State Department of Health
ODE	ordinary differential equation
OECD	Organization for Economic Co-operation and Development
OFT	outflow tract
OP	oscillatory potential
OR	odds ratio
OR_{adj}	adjusted odds ratio
PAS	periodic acid-Schiff
PBPK	physiologically based pharmacokinetics
PCEs	polychromatic erythrocytes
PCNA	proliferating cell nuclear antigen
PCO	palmitoyl-CoA oxidase
PCR	polymerase chain reaction
p-cRfC	PBPK model-based candidate RfCs
p-cRfD	PBPK model-based candidate RfDs
PEG 400	polyethylene glycol 400
PFC	plaque-forming cell
PFU	plaque-forming units
PMR	proportionate mortality ratio
PND	postnatal day
PO_2	partial pressure oxygen
POD	point of departure
PPAR	peroxisome proliferator activated receptor
RBL-2H3	rat basophilic leukemia

RCC	renal cell carcinoma
RfC	inhalation reference concentration
RfD	oral reference dose
RNA	ribonucleic acid
RR	relative risk
RRm	summary RR
RT	reaction time
S9	metabolic activation system
SBA	serum bile acids
SC	sensitivity coefficient
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SEER	Surveillance, Epidemiology, and End Results
SES	socioeconomic status
SGA	small for gestational age
SHBG	sex-hormone binding globulin
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SNP	single nucleotide polymorphism
SRBC	sheep red blood cells
SRT	simple reaction time
SSB	single-strand breaks
SSCP	single stand conformation polymorphism
ssDNA	single-stranded DNA
TaClo	tetrahydro-beta-carbolines
TBARS	thiobarbiturate acid-reactive substances
TCA	trichloroacetic acid
TCAA	trichloroacetaldehyde
TCAH	trichloroacetaldehyde hydrate
TCE	trichloroethylene
TCOG	trichloroethanol-glucuronide conjugate
TCOH	trichloroethanol
ThX	T-helper Type X
TNF	tumor necrosis factor
TRI	Toxics Release Inventory
TSEP	trigeminal somatosensory evoked potential
TTC	total trichloro compounds
TWA	time-weighted average

U.S. EPA	U.S. Environmental Protection Agency
UCL	upper confidence limit
UDS	unscheduled DNA synthesis
UF	uncertainty factor
USGS	United States Geological Survey
U-TCA	urinary-TCA
U-TTC	urinary total trichloro-compounds
VEGF	vascular endothelial growth factor
VEP	visual evoked potential
VHL	von Hippel-Lindau
VLivC	liver volume
VOC	volatile organic compound
VSCC	voltage sensitive calcium channel
W	wakefulness
WHO	World Health Organization
YFF	fluorescent Y-bodies

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to trichloroethylene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of trichloroethylene.

The intent of Chapter 6, *Major Conclusions in the Characterization of Hazard and Dose Response,* is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of the data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA‘s IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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This document has been reviewed by U.S. EPA scientists, reviewers from other Federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to U.S. EPA. A summary and U.S. EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix I.

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ACKNOWLEDGMENTS

Drafts of Section 3.3 (TCE metabolism) were prepared for the U.S. EPA by Syracuse Research Corporation under contract. Additional support, including literature searches and retrievals and drafts of Appendix D were prepared for the U.S. EPA by the Oak Ridge Institute for Science and Education (ORISE) through interagency agreement number DW-89939822-01-0 with the U.S. Department of Energy (DOE). ORISE is managed by Oak Ridge Associated Universities under a contract with DOE. The PBPK modeling sections of this report are dedicated to the memory of Fred Power (1938–2007). His keen analytical mind will be greatly missed, but his gentle heart and big smile will be missed even more.

Additionally, we gratefully acknowledge Terri Konoza and Ellen Lorang of NCEA for their management of the document production and reference/citation management processes. Technical editing support was provided by ICF International; IntelliTech Systems, Inc.; ECFlex, Inc.; and Syracuse Research Corporation.

EXECUTIVE SUMMARY

There is substantial potential for human exposure to trichloroethylene (TCE), as it has a widespread presence in ambient air, indoor air, soil, and groundwater. At the same time, humans are likely to be exposed to a variety of compounds that are either metabolites of TCE or which have common metabolites or targets of toxicity. Once exposed, humans, as well as laboratory animal species, rapidly absorb TCE, which is then distributed to tissues via systemic circulation, extensively metabolized, and then excreted primarily in breath as unchanged TCE or carbon dioxide, or in urine as metabolites.

Based on the available human epidemiologic data and experimental and mechanistic studies, it is concluded that TCE poses a potential human health hazard for noncancer toxicity to the central nervous system, kidney, liver, immune system, male reproductive system, and the developing fetus. The evidence is more limited for TCE toxicity to the respiratory tract and female reproductive system. Following U.S. Environmental Protection Agency (U.S. EPA, 2005b) *Guidelines for Carcinogen Risk Assessment*, TCE is characterized as "~~c~~*arcinogenic in humans by all routes of exposure*." This conclusion is based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer. The human evidence of carcinogenicity from epidemiologic studies of TCE exposure is strong for non-Hodgkin Lymphoma but less convincing than for kidney cancer, and more limited for liver and biliary tract cancer. Less human evidence is found for an association between TCE exposure and other types of cancer, including bladder, esophageal, prostate, cervical, breast, and childhood leukemia, breast. Further support for the characterization of TCE as "~~c~~*arcinogenic in humans by all routes of exposure*" is derived from positive results in multiple rodent cancer bioassays in rats and mice of both sexes, similar toxicokinetics between rodents and humans, mechanistic data supporting a mutagenic mode of action for kidney tumors, and the lack of mechanistic data supporting the conclusion that any of the mode(s) of action for TCE-induced rodent tumors are irrelevant to humans.

As TCE toxicity and carcinogenicity are generally associated with TCE metabolism, susceptibility to TCE health effects may be modulated by factors affecting toxicokinetics, including lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status, lifestyle, and nutrition status. In addition, while these some of these factors are known risk factors for effects associated with TCE exposure, it is not known how TCE interacts with known risk factors for human diseases.

For noncancer effects, the most sensitive types of effects, based either on human equivalent concentrations/doses or on candidate inhalation reference concentrations (RfCs)/oral reference doses (RfDs), appear to be developmental, kidney, and immunological (adult and developmental) effects. The neurological and reproductive effects appear to be about an order of

magnitude less sensitive, with liver effects another two orders of magnitude less sensitive. The RfC of **0.0004 ppm** (0.4 ppb or 2 μg/m^3) is based on route-to-route extrapolated results from oral studies for the critical effects of heart malformations (rats) and immunotoxicity (mice). This RfC value is further supported by route-to-route extrapolated results from an oral study of toxic nephropathy (rats). Similarly, the RfD for noncancer effects of **0.0005 mg/kg/day** is based on the critical effects of heart malformations (rats), adult immunological effects (mice), and developmental immunotoxicity (mice), all from oral studies. This RfD value is further supported by results from an oral study for the effect of toxic nephropathy (rats) and route-to-route extrapolated results from an inhalation study for the effect of increased kidney weight (rats). There is high confidence in these noncancer reference values, as they are supported by moderate-to-high confidence estimates for multiple effects from multiple studies.

For cancer, the inhalation unit risk is **2×10^{-2} per ppm [4×10^{-6} per μg/m^3]**, based on human kidney cancer risks reported by Charbotel et al. (2006) and adjusted, using human epidemiologic data, for potential risk for NHL and liver cancer. The oral unit risk for cancer is **5×10^{-2} per mg/kg/day**, resulting from physiologically based pharmacokinetic model-based route-to-route extrapolation of the inhalation unit risk based on the human kidney cancer risks reported in Charbotel et al. (2006) and adjusted, using human epidemiologic data, for potential risk for NHL and liver cancer. There is high confidence in these unit risks for cancer, as they are based on good quality human data, as well as being similar to unit risk estimates based on multiple rodent bioassays. There is both sufficient weight of evidence to conclude that TCE operates through a mutagenic mode of action for kidney tumors and a lack of TCE-specific quantitative data on early-life susceptibility. Generally, the application of age-dependent adjustment factors (ADAFs) is recommended when assessing cancer risks for a carcinogen with a mutagenic mode of action. However, because the ADAF adjustment applies only to the kidney cancer component of the total risk, it is likely to have a minimal impact on the total cancer risk except when exposures are primarily during early life.

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of trichloroethylene (TCE). IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of ppm or $\mu g/m^3$) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per ppm or $\mu g/m^3$ in air breathed.

Development of these hazard identification and dose-response assessments for TCE has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: EPA Guidelines and Risk Assessment Forum technical panel reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986a), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental*

Toxicity Risk Assessment (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994b), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994a), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005e), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006b), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006a).

The literature search strategy employed for this compound was based on the chemical name, Chemical Abstracts Service Registry Number (CASRN), and multiple common synonyms. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. Primary, peer-reviewed literature identified through December 2010 was included where that literature was determined to be critical to the assessment. The relevant literature included publications on trichloroethylene which were identified through Toxicology Literature Online (TOXLINE), the U.S. National Library of Medicine's MEDLINE, the Toxic Substance Control Act Test Submission Database (TSCATS), the Registry of Toxic Effects of Chemical Substances (RTECS), the Chemical Carcinogenesis Research Information System (CCRIS), the Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART/ETIC), the Environmental Mutagens Information Center (EMIC) and Environmental Mutagen Information Center Backfile (EMICBACK) databases, the Hazardous Substances Data Bank (HSDB), the Genetic Toxicology Data Bank (GENE-TOX), Chemical abstracts, and Current Contents. Other information, including health assessments developed by other organizations, review articles, and independent analyses of the health effects data were retrieved and may be included in the assessment where appropriate. It should be noted that references have been added to the Toxicological Review after the external peer review in response to peer reviewer‘s comments and for the sake of completeness. These references have not changed the overall qualitative and quantitative conclusions.

In addition to using peer-reviewed, published scientific literature, the preparation of this toxicological review considered the advice to EPA from a 2002 SAB peer review report (SAB, 2002), a 2006 NRC consultation report (NRC, 2006), and a 2011 SAB peer review report (SAB, 2011), as well as comments from the public and other federal Agencies (weblinks).

2. EXPOSURE CHARACTERIZATION

The purpose of this exposure characterization is to summarize information about TCE sources, releases, media levels, and exposure pathways for the general population (occupational exposure is also discussed to a lesser extent). It is not meant as a substitute for a detailed exposure assessment for a particular risk assessment application. While this section primarily addresses TCE, it also includes some information on a number of related compounds. These related compounds include metabolites of TCE and other parent compounds that produce similar metabolites as shown in Table 2-1. The first column in this table lists the principal TCE metabolites in humans (trichloroethanol, trichloroethanol-glucuronide, and trichloroacetic acid) as well as a number of minor metabolites (ATSDR, 1997c). The subsequent columns list parent compounds that can produce some of the same metabolites. The metabolic reaction pathways are much more complicated than implied here and it should be understood that this table is intended only to provide a general understanding of which parent compounds lead to which TCE metabolites. Exposure to the TCE-related compounds can alter or enhance TCE's metabolism and toxicity by generating higher internal metabolite concentrations than would result from TCE exposure by itself. This characterization is based largely on earlier work by Wu and Schaum (2001, 2000), but also provides updates in a number of areas.

Table 2-1. TCE metabolites and related parent compounds[a]

TCE metabolites	Parent compounds				
	Tetrachloro-ethylene	1,1-Dichloro-ethane	1,1,1-Tri-chloroethane	1,1,1,2-Tetra-chloroethane	1,2-Dichloro-ethylene
Oxalic acid				X	X
Chloral	X				
Chloral hydrate	X				
Monochloroacetic acid	X	X	X	X	X
Dichloroacetic acid	X	X		X	
Trichloroacetic acid	X		X	X	
Trichloroethanol	X		X	X	
Trichloroethanol-glucuronide	X		X	X	

[a]X indicates that the parent compound can produce the corresponding metabolite (Hazardous Substances Data Bank, http://toxnet.nlm.nih.gov./cgi-bin/sis/htmlgen?HSDB).

2.1. ENVIRONMENTAL SOURCES

TCE is a stable, colorless liquid with a chloroform-like odor and chemical formula C_2Cl_3H as diagrammed in Figure 2-1 (Hawley and Lewis, 2001). Its chemical properties are listed in Table 2-2.

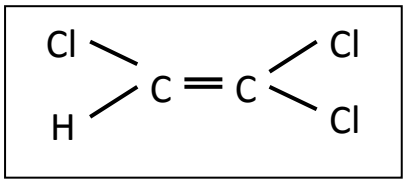


Figure 2-1. Molecular structure of TCE.

Table 2-2. Chemical properties of TCE

Property	Value	Reference
Molecular weight	131.39	Lide et al. (1998)
Boiling point	87.2°C	Lide et al. (1998)
Melting point	−84.7°C	Lide et al. (1998)
Density	1.4642 at 20°C	Budavari (1996)
Solubility	1,280 mg/L water at 25°C	Horvath et al. (1999)
Vapor pressure	69.8 mmHG @ 25°C	Boublik et al. (1984)
Vapor density	4.53 (air = 1)	Budavari (1996)
Henry's law constant	9.85×10^{-3} atm-cu m/mol @ 25°C	Leighton and Calo (1981)
Octanol/water partition coefficient	log K_{ow} = 2.61	Hansch et al. (1995)
Air concentration conversion	1 ppb = 5.38 µg/m^3	HSDB (2002)

TCE has been produced commercially since the 1920s in many countries by chlorination of ethylene or acetylene. Its use in vapor degreasing began in the 1920s. In the 1930s, it was introduced for use in dry cleaning. This use was largely discontinued in the 1950s and was replaced with tetrachloroethylene (ATSDR, 1997c). More recently, 80−90% of TCE production worldwide is used for degreasing metals (IARC, 1995a). It is also used in adhesives, paint-stripping formulations, paints, lacquers, and varnishes (SRI, 1992). A number of past uses in cosmetics, drugs, foods, and pesticides have now been discontinued including use as an extractant for spice oleoresins, natural fats and oils, hops, and decaffeination of coffee (IARC, 1995a), and as a carrier solvent for the active ingredients of insecticides and fungicides, and for spotting fluids (ATSDR, 1997c; WHO, 1985). The production of TCE in the United States peaked at 280 million kg (616 million pounds) in 1970 and declined to 60 million kg (132 million pounds) in 1998 (USGS, 2006). In 1996, the United States imported 4.5 million kg (10 million pounds) and exported 29.5 million kg (65 million pounds) (Chemical Marketing Reporter, 1997). Table 2-3 summarizes the basic properties and principal uses of the TCE related compounds.

Table 2-3. Properties and uses of TCE related compounds

	Water solubility (mg/L)	Vapor pressure (mmHG)	Uses	References
Tetrachloroethylene	150	18.5 @25°C	Dry cleaning, degreasing, solvent	Wu and Schaum (2001)
1,1,1-Trichloroethane	4,400	124 @25°C	Solvents, degreasing	Wu and Schaum (2001)
1,2-Dichloroethylene	3,000−6,000	273−395 @30°C	Solvents, chemical intermediates	Wu and Schaum (2001)
1,1,1,2-Tetrachloroethane	1,100	14 @25°C	Solvents, but currently not produced in United States	HSDB, 2002; Wu and Schaum (2001)
1,1-Dichloroethane	5,500	234 @25°C	Solvents, chemical intermediates	Wu and Schaum (2001)
Chloral	High	35 @20°C	Herbicide production	Wu and Schaum (2001)
Chloral hydrate	High	NA	Pharmaceutical production	Wu and Schaum (2001)
Monochloroacetic acid	High	1 @43°C	Pharmaceutical production	Wu and Schaum (2001)
Dichloroacetic acid	High	<1 @20°C	Pharmaceuticals, not widely used	Wu and Schaum (2001)
Trichloroacetic acid	High	1 @50°C	Herbicide production	Wu and Schaum (2001)
Oxalic acid	220,000	0.54 @105°C	Scouring/cleaning agent, degreasing	HSDB (2002)
Dichlorovinyl cysteine	Not available	Not available	Not available	
Trichloroethanol	Low	NA	Anesthetics and chemical intermediate	Hawley and Lewis (2001)

Releases of TCE from nonanthropogenic activities are negligible (HSDB, 2002). Most of the TCE used in the United States is released to the atmosphere, primarily from vapor degreasing operations (ATSDR, 1997c). Releases to air also occur at treatment and disposal facilities, water treatment facilities, and landfills (ATSDR, 1997c). TCE has also been detected in stack emissions from municipal and hazardous waste incineration (ATSDR, 1997c). TCE is on the list for reporting to U.S. EPA's Toxics Release Inventory (TRI). Reported releases into air predominate over other types and have declined over the period 1994−2004 (see Table 2-4).

Table 2-4. TRI releases of TCE (pounds/year)

Yr	On-site fugitive air	On-site stack air	Total on-site air emissions	On-site surface water discharges	Total on-site underground injection	Total on-site releases to land	Total off-site disposal or other releases	Total on- and off-site disposal or other releases
1994	15,018,818	15,929,943	30,948,761	1,671	288	4,070	96,312	31,051,102
1995	12,498,086	13,784,853	26,282,939	1,477	550	3,577	74,145	26,362,688
1996	10,891,223	10,995,228	21,886,451	541	1,291	9,740	89,527	21,987,550
1997	9,276,150	8,947,909	18,224,059	568	986	3,975	182,423	18,412,011
1998	6,769,810	6,504,289	13,274,099	882	593	800	136,766	13,413,140
1999	5,861,635	4,784,057	10,645,692	1,034	0	148,867	192,385	10,987,978
2000	5,485,493	4,375,516	9,861,009	593	47,877	9,607	171,952	10,091,038
2001	4,968,282	3,453,451	8,421,733	406	98,220	12,609	133,531	8,666,499
2002	4,761,104	3,436,289	8,197,393	579	140,190	230	139,398	8,477,790
2003	3,963,054	3,121,718	7,084,772	595	90,971	150,642	66,894	7,393,873
2004	3,040,460	3,144,980	6,185,440	216	123,637	2	71,780	6,381,075
2005	2,733,983	2,893,168	5,627,152	533	86,817	4,711	60,074	5,779,287
2006	2,816,241	2,795,184	5,611,425	482	0	77,339	90,758	5,780,004

Source: EPA TRI Explorer, http://www.epa.gov/triexplorer/trends.htm.

Under the National-Scale Air Toxics Assessment (NSATA) program, EPA has developed an emissions inventory for TCE (U.S. EPA, 2007a). The inventory includes sources in the United States plus the Commonwealth of Puerto Rico and the U.S. Virgin Islands. The types of emission sources in the inventory include large facilities, such as waste incinerators and factories and smaller sources, such as dry cleaners and small manufacturers. Figures 2-2 and 2-3 show the results of the 1999 emissions inventory for TCE. Figure 2-2 shows the percent contribution to total emissions by source category. A variety of sources have TCE emissions with the largest ones identified as halogenated solvent cleaners and metal parts and products. Figure 2-3 shows a national map of the emission density (tons/square miles/year) for TCE. This map shows the highest densities in the far west and northeastern regions of the United States. Emissions range from 0 to 4.12 tons/square miles/year.

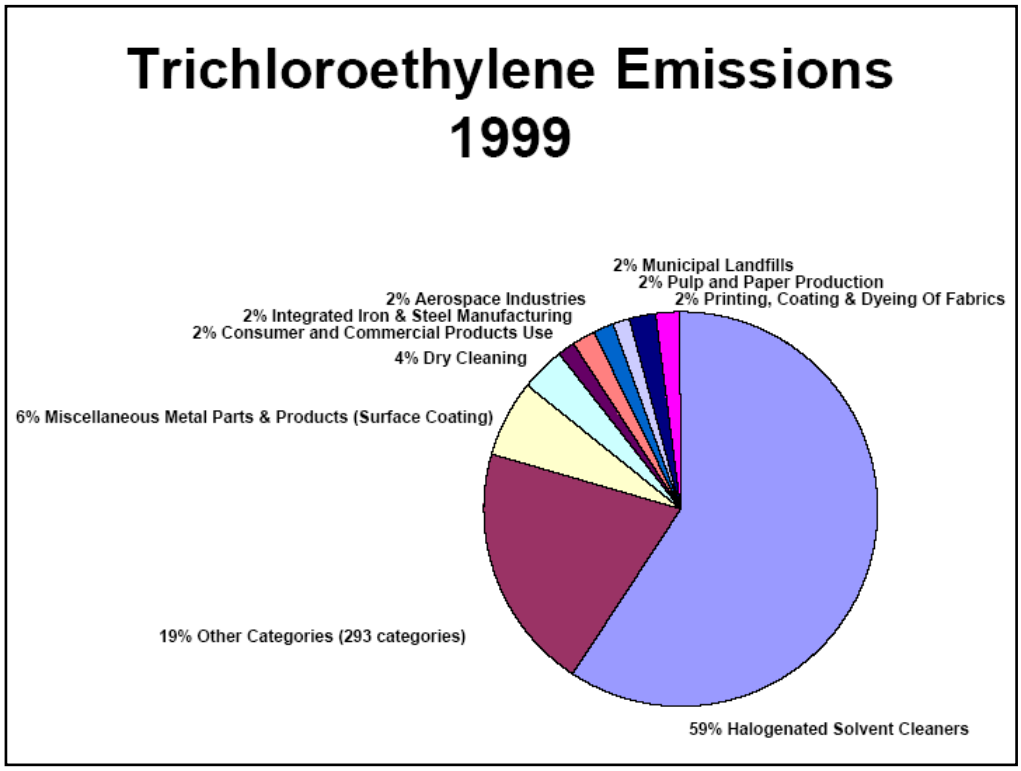


Figure 2-2. Source contribution to TCE emissions.

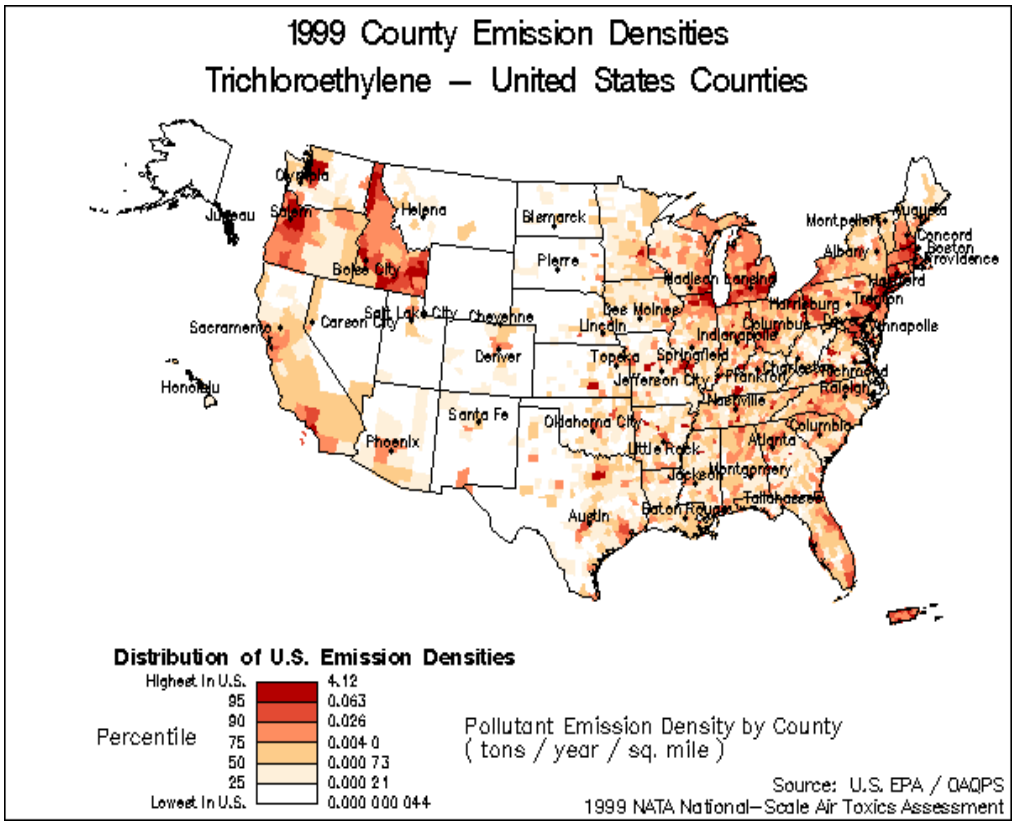


Figure 2-3. Annual emissions of TCE.

2.2. ENVIRONMENTAL FATE

2.2.1. Fate in Terrestrial Environments

The dominant fate of TCE released to surface soils is volatilization. Because of its moderate water solubility, TCE introduced into soil (e.g., landfills) also has the potential to migrate through the soil into groundwater; this is confirmed by the relatively frequent detection of TCE in groundwater. Biodegradation in soil and groundwater may occur at a relatively slow rate (half-lives on the order of months to years) (Howard et al., 1991).

2.2.2. Fate in the Atmosphere

In the atmosphere, TCE is expected to be present primarily in the vapor phase, rather than sorbed to particulate, because of its high vapor pressure. Some removal by scavenging during wet precipitation is expected because of its moderate water solubility. The major degradation process affecting vapor-phase TCE is photo-oxidation by hydroxyl radicals. Photolysis in the atmosphere proceeds very slowly, if at all. TCE does not absorb ultraviolet light at wavelengths of <290 nm and thus, will not directly photolyze. Based on measured rate data for the vapor phase photo-oxidation reaction with hydroxyl radicals, the estimated half-life of TCE in the atmosphere is on the order of 1–11 days with production of phosgene, dichloroacetyl chloride (DCAC), and formyl chloride. Under smog conditions, degradation is more rapid (half-life on the order of hours) (HSDB, 2002; Howard et al., 1991).

2.2.3. Fate in Aquatic Environments

The dominant fate of TCE released to surface waters is volatilization (predicted half-life of minutes to hours). Bioconcentration, biodegradation, and sorption to sediments and suspended solids are not thought to be significant (HSDB, 2002). TCE is not hydrolyzed under normal environmental conditions. However, slow photo-oxidation in water (half-life of 10.7 months) has been reported (HSDB, 2002; Howard et al., 1991).

2.3. EXPOSURE CONCENTRATIONS

TCE levels in the various environmental media result from the releases and fate processes discussed in Sections 2.1 and 2.2. No statistically based national sampling programs have been conducted that would allow estimates of true national means for any environmental medium. A substantial amount of air and groundwater data, however, has been collected as well as some data in other media, as described below.

2.3.1. Outdoor Air—Measured Levels

TCE has been detected in the air throughout the United States. According to ATSDR (1997c), atmospheric levels are highest in areas concentrated with industry and population, and

lower in remote and rural regions. Table 2-5 shows levels of TCE measured in the ambient air at a variety of locations in the United States.

Table 2-5. Concentrations of TCE in ambient air

Area	Yr	Concentration (µg/m^3)	
		Mean	Range
Rural			
Whiteface Mountain, New York[a]	1974	0.5	<0.3−1.9
Badger Pass, California[a]	1977	0.06	0.005−0.09
Reese River, Nevada[a]	1977	0.06	0.005−0.09
Jetmar, Kansas[a]	1978	0.07	0.04−0.11
All rural sites	1974−1978		0.005−1.9
Urban and suburban			
New Jersey[a]	1973−1979	9.1	ND−97
New York City, New York[a]	1974	3.8	0.6−5.9
Los Angeles, California[a]	1976	1.7	0.14−9.5
Lake Charles, Louisiana[a]	1976−1978	8.6	0.4−11.3
Phoenix, Arizone[a]	1979	2.6	0.06−16.7
Denver, Colorado[a]	1980	1.07	0.15−2.2
St. Louis, Missouri[a]	1980	0.6	0.1−1.3
Portland, Oregon[a]	1984	1.5	0.6−3.9
Philadelphia, Pennsylvania[a]	1983−1984	1.9	1.6−2.1
Southeast Chicago, Illinois[b]	1986−1990	1.0	
East St. Louis, Illinois[b]	1986−1990	2.1	
District of Columbia[c]	1990−1991	1.94	1−16.65
Urban Chicago, Illinois[d]	pre−1993	0.82−1.16	
Suburban Chicago, Illinois[d]	pre−1993	0.52	
300 cities in 42 states[e]	pre−1986	2.65	
Several Canadian Cities[f]	1990	0.28	
Several United States Cities[f]	1990	6.0	
Phoenix, Arizona[g]	1994−1996	0.29	0−1.53
Tucson, Arizona[g]	1994−1996	0.23	0−1.47
All urban/suburban sites	1973−1996		0−97

[a]IARC (1995a).
[b]Sweet (1992).
[c]Hendler (1992).
[d]Scheff (1993).
[e]Shah (1988).
[f]Bunce (1994).
[g]Zielinska-Psuja (1998).

ND = nondetect

More recent ambient air measurement data for TCE were obtained from EPA's Air Quality System database at the AirData Web site: http://www.epa.gov/air/data/index.html (2007b). These data were collected from a variety of sources including state and local environmental agencies. The data are not from a statistically based survey and cannot be assumed to provide nationally representative values. The most recent data (2006) come from 258 monitors located in 37 states. The means for these monitors range from 0.03 to 7.73 µg/m^3

and have an overall average of 0.23 µg/m^3. Table 2-6 summarizes the data for the years 1999–2006. The data suggest that levels have remained fairly constant since 1999 at about 0.3 µg/m^3. Table 2-7 shows the monitoring data organized by land setting (rural, suburban, or urban) and land use (agricultural, commercial, forest, industrial, mobile, and residential). Urban air levels are almost 4 times higher than rural areas. Among the land use categories, TCE levels are highest in commercial/industrial areas and lowest in forest areas.

Table 2-6. TCE ambient air monitoring data (µg/m^3)

Yr	Number of monitors	Number of states	Mean	Standard deviation	Median	Range
1999	162	20	0.30	0.53	0.16	0.01−4.38
2000	187	28	0.34	0.75	0.16	0.01−7.39
2001	204	31	0.25	0.92	0.13	0.01−12.90
2002	259	41	0.37	1.26	0.13	0.01−18.44
2003	248	41	0.35	0.64	0.16	0.02−6.92
2004	256	37	0.32	0.75	0.13	0.00−5.78
2005	313	38	0.43	1.05	0.14	0.00−6.64
2006	258	37	0.23	0.55	0.13	0.03−7.73

Source: EPA's Air Quality System database at the AirData Web site: http://www.epa.gov/air/data/index.html.

Table 2-7. Mean TCE air levels across monitors by land setting and use (1985–1998)

	Rural	Suburban	Urban	Agricul-tural	Com-mercial	Forest	Indus-trial	Mobile	Resi-dential
Mean concentration (µg/m^3)	0.42	1.26	1.61	1.08	1.84	0.1	1.54	1.5	0.89
n	93	500	558	31	430	17	186	39	450

Source: EPA's Air Quality System database at the AirData Web site: http://www.epa.gov/air/data/index.html.

2.3.2. Outdoor Air—Modeled Levels

Under the National-Scale Air Toxics Assessment program, EPA has compiled emissions data and modeled air concentrations/exposures for the Criteria Pollutants and Hazardous Air Pollutants (U.S. EPA, 2007a). The results of the 1999 emissions inventory for TCE were discussed earlier and results presented in Figures 2-2 and 2-3. A computer simulation model known as the Assessment System for Population Exposure Nationwide (ASPEN) is used to estimate toxic air pollutant concentrations (http://www.epa.gov/ttnatw01/nata/aspen.html). This model is based on the EPA's Industrial Source Complex Long Term model which simulates the behavior of the pollutants after they are emitted into the atmosphere. ASPEN uses estimates of toxic air pollutant emissions and meteorological data from National Weather Service Stations to

estimate air toxics concentrations nationwide. The ASPEN model takes into account important determinants of pollutant concentrations, such as:

- rate of release;
- location of release;
- the height from which the pollutants are released;
- wind speeds and directions from the meteorological stations nearest to the release;
- breakdown of the pollutants in the atmosphere after being released (i.e., reactive decay);
- settling of pollutants out of the atmosphere (i.e., deposition); and
- transformation of one pollutant into another (i.e., secondary formation).

The model estimates toxic air pollutant concentrations for every census tract in the continental United States, the Commonwealth of Puerto Rico and the U.S. Virgin Islands. Census tracts are land areas defined by the U.S. Bureau of the Census and typically contain about 4,000 residents each. Census tracts are usually smaller than 2 square miles in size in cities but much larger in rural areas.

Figure 2-4 shows the results of the 1999 ambient air concentration modeling for TCE. The county median air levels range from 0 to 3.79 $\mu g/m^3$ and an overall median of 0.054 $\mu g/m^3$. They have a pattern similar to the emission densities shown in Figure 2-3. These NSATA modeled levels appear lower than the monitoring results presented above. For example, the 1999 air monitoring data (see Table 2-6) indicates a median outdoor air level of 0.16 $\mu g/m^3$ which is about 3 times as high as the modeled 1999 county median (0.054 $\mu g/m^3$). However, it should be understood that the results from these two efforts are not perfectly comparable. The modeled value is a median of county levels for the entire United States which includes many rural areas. The monitors cover many fewer areas (n = 162 for 1999) and most are in nonrural locations. A better analysis is provided by EPA (2007a) which presents a comparison of modeling results from NSATA to measured values at the same locations. For 1999, it was found that formaldehyde levels were underestimated at 79% of the sites (n = 92). Thus, while the NSATA modeling results are useful for understanding geographic distributions, they may frequently underestimate ambient levels.

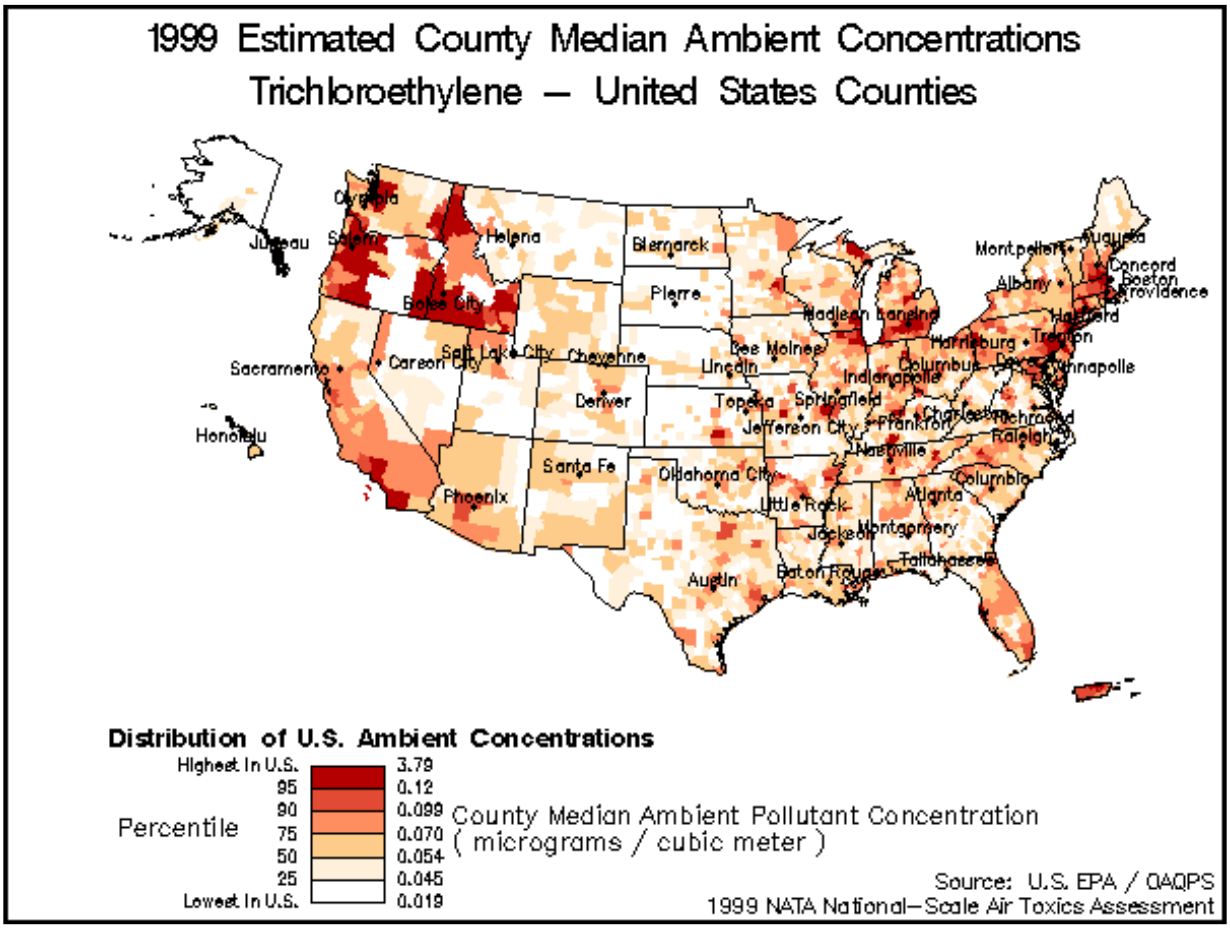


Figure 2-4. Modeled ambient air concentrations of TCE.

2.3.3. Indoor Air

TCE can be released to indoor air from use of consumer products that contain it (i.e., adhesives and tapes), vapor intrusion (migration of volatile chemicals from the subsurface into overlying buildings) and volatilization from the water supply. Where such sources are present, it is likely that indoor levels will be higher than outdoor levels. A number of studies have measured indoor levels of TCE:

- The 1987 EPA Total Exposure Assessment Methodology study (Wallace, 1987) showed that the ratio of indoor to outdoor TCE concentrations for residences in Greensboro, NC, was about 5:1.
- In two homes using well water with TCE levels averaging 22–128 µg/L, the TCE levels in bathroom air ranged from <500–40,000 µg/m^3 when the shower ran <30 minutes (Andelman, 1985).
- Shah and Singh (1988) report an average indoor level of 7.2 µg/m^3 based on over 2,000 measurements made in residences and workplaces during 1981−1984 from various locations across the United States.
- Hers et al. (2001) provides a summary of indoor air TCE measurements at locations in United States, Canada, and Europe with a range of <1–165 µg/m^3.

- Sapkota et al. (2005) measured TCE levels inside and outside of the Baltimore Harbor Tunnel toll booths during the summer of 2001. Mean TCE levels were 3.11 μg/m^3 indoors and 0.08 μg/m^3 outdoors based on measurements on 7 days. The authors speculated that indoor sources, possibly dry cleaning residues on uniforms, were the primary source of the indoor TCE.
- Sexton et al. (2005) measured TCE levels inside and outside residences in Minneapolis/St. Paul metropolitan area. Two day samples were collected over three seasons in 1999. Mean TCE levels were 0.5 μg/m^3 indoors (n = 292), 0.2 μg/m^3 outdoors (n = 132) and 1.0 μg/m^3 based on personal sampling (n = 288).
- Zhu et al. (2005) measured TCE levels inside and outside of residences in Ottawa, Canada. Seventy-five homes were randomly selected and measurements were made during the winter of 2002/2003. TCE was above detection limits in the indoor air of 33% of the residences and in the outdoor air of 19% of the residences. The mean levels were 0.06 μg/m^3 indoors and 0.08 μg/m^3 outdoors. Given the high frequency of nondetects, a more meaningful comparison can be made on basis of the 75th percentiles: 0.08 μg/m^3 indoors and 0.01 μg/m^3 outdoors.

TCE levels measured indoors have been directly linked to vapor intrusion at two sites in New York:

- TCE vapor intrusion has occurred in buildings/residences near a former Smith Corona manufacturing facility located in Cortlandville, New York. An extensive sampling program conducted in 2006-2007 has detected TCE in groundwater (up to 22 μg/L), subslab gas (up to 1,000 μg/m^3), and indoor air (up to 34 μg/m^3) (NYSDEC, 2007).
- Evidence of vapor intrusion of TCE has also been reported in buildings and residences in Endicott, New York. Sampling in 2003 showed total volatile organic compounds (VOCs) in soil gas exceeding 10,000 μg/m^3 in some areas. Indoor air sampling detected TCE levels ranging from 1 to 140 μg/m^3 (Meyers, 2003).

Little et al. (1992) developed attenuation coefficients relating contaminants in soil gas (assumed to be in chemical equilibrium with the groundwater) to possible indoor levels as a result of vapor intrusion. On this basis they estimated that TCE groundwater levels of 540 μg/L, (a high contamination level) could produce indoor air levels of 5–500 μg/m^3. Vapor intrusion can be an important contributor to indoor levels in situations where residences are located near soils or groundwater with high contamination levels. EPA (2002c) recommends considering vapor intrusion when volatiles are suspected to be present in groundwater or soil at a depth of <100 feet. Hers et al. (2001) concluded that the contribution of VOCs from subsurface sources relative to indoor sources is small for most chemicals and sites.

2.3.4. Water

A number of early (pre-1990) studies measured TCE levels in natural water bodies (levels in drinking water are discussed later in this section) as summarized in Table 2-8.

Table 2-8. Concentrations of TCE in water based on pre-1990 studies

Water type	Location	Yr	Mean (µg/L)	Median (µg/L)	Range (µg/L)	Number of samples	Reference
Industrial effluent	United States	1983		0.5		NR	IARC (1995a)
Surface waters	United States	1983		0.1		NR	IARC (1995a)
Rainwater	Portland, Oregon	1984	0.006		0.002−0.02	NR	Ligocki et al. (1985)
Groundwater	Minnesota	1983			0.2−144	NR	Sabel and Clark (1984)
	New Jersey	1976			≤1,530	NR	Burmaster et al. (1982)
	New York	1980			≤3,800	NR	Burmaster et al. (1982)
	Pennsylvania	1980			≤27,300	NR	Burmaster et al. (1982)
	Massachusetts	1976			≤900	NR	Burmaster et al. (1982)
	Arizona				8.9−29	NR	IARC (1995a)
Drinking water	United States	1976			0.2−49		IARC (1995a)
	United States	1977			0−53		IARC (1995a)
	United States	1978			0.5−210		IARC (1995a)
	Massachusetts	1984			max. 267		IARC (1995a)
	New Jersey	1984	23.4		max. 67	1130	Cohn et al. (1994b)
	California	1985			8–12	486	EPA, (1987)
	California	1984	66			486	EPA, (1987)
	North Carolina	1984	5			48	EPA, (1987)
	North Dakota	1984	5			48	EPA, (1987)

NR = not reported

According to IARC (1995a), the reported median concentrations of TCE in 1983−1984 were 0.5 µg/L in industrial effluents and 0.1 µg/L in ambient water. Results from an analysis of the EPA STORET Data Base (1980−1982) showed that TCE was detected in 28% of 9,295 surface water reporting stations nationwide (ATSDR, 1997c). A more recent search of the STORET database for TCE measurements nationwide during 2008 in streams, rivers and lakes indicated three detects (0.03–0.04 µg/L) out of 150 samples (STORET Database, http://www.epa.gov/storet/dbtop.html).

ATSDR (1997c) has reported that TCE is the most frequently reported organic contaminant in groundwater and the one present in the highest concentration in a summary of ground water analyses reported in 1982. It has been estimated that between 9 and 34% of the drinking water supply sources tested in the United States may have some TCE contamination. This estimate is based on available Federal and State surveys (ATSDR, 1997c).

Squillace et al. (2004) reported TCE levels in shallow groundwater based on data from the National Water Quality Assessment Program managed by United States Geological Survey (USGS). Samples from 518 wells were collected from 1996 to 2002. All wells were located in

residential or commercial areas and had a median depth of 10 m. The authors reported that approximately 8.3% of the well levels were above the detection limit (level not specified), 2.3% were above 0.1 µg/L and 1.7% were above 0.2 µg/L.

As part of the Agency's first Six-Year Review, EPA obtained analytical results for over 200,000 monitoring samples reported at 23,035 public water systems (PWS) in 16 states (U.S. EPA, 2003c). Approximately 2.6% of the systems had at least one sample exceed a minimum reporting level of 0.5 µg/L; almost 0.65% had at least one sample that exceeds the maximum contaminant level of 5 µg/L. Based on average system concentrations estimated by EPA, 54 systems (0.23%) had an average concentration that exceeded the maximum contaminant level. EPA's statistical analysis to extrapolate the sample result to all systems regulated for TCE resulted in an estimate of 154 systems with average TCE concentrations that exceed the maximum contaminant level.

TCE concentrations in ground water have been measured extensively in California. The data were derived from a survey of water utilities with more than 200 service connections. The survey was conducted by the California Department of Health Services (CDHS, 1986). From January 1984 through December 1985, untreated water from wells in 819 water systems were sampled for organic chemical contamination. The water systems use a total of 5,550 wells, 2,947 of which were sampled. TCE was found in 187 wells at concentrations up to 440 µg/L, with a median concentration among the detects of 3.0 µg/L. Generally, the wells with the highest concentrations were found in the heavily urbanized areas of the state. Los Angeles County registered the greatest number of contaminated wells (149).

A second California study collected data on TCE levels in public drinking water (Williams et al., 2002). The data were obtained from the CA DHS. The data spanned the years 1995–2001 and the number of samples for each year ranged from 3,447 to 4,226. The percent of sources that were above the detection limit ranged from 9.6 to 11.7 per year (detection limits not specified). The annual average detected concentrations ranged from 14.2 to 21.6 µg/L. Although not reported, the overall average concentration of the samples (assuming an average of 20 µg/L among the samples above the detection limit, 10% detection rate and 0 for the nondetects) would be about 2 µg/L.

The USGS (2006) conducted a national assessment of 55 VOCs, including TCE, in ground water. A total of 3,500 water samples were collected during 1985−2001. Samples were collected at the well head prior to any form of treatment. The types of wells sampled included 2,400 domestic wells and 1,100 public wells. Almost 20% of the samples contained one or more of the VOCs above the assessment level of 0.2 µg/L. The detection frequency increased to over 50% when a subset of samples was analyzed with a low level method that had an assessment level of 0.02 µg/L. The largest detection frequencies were observed in California, Nevada, Florida, the New England States, and Mid-Atlantic states. The most frequently detected VOCs

(>1% of samples) include TCE, tetrachloroethylene, 1,1,1-trichloroethane (methyl chloroform), 1,2 dichloroethylene, and 1,1-dichloroethane. Findings specific to TCE include the following:

- Detection frequency was 2.6% at 0.2 µg/L and was 3.8% at 0.02 µg/L.
- The median concentration was 0.15 µg/L with a range of 0.02–100 µg/L.
- The number of samples exceeding the maximum contaminant level (5 µg/L) was six at domestic wells and nine at public wells.

USGS (2006) also reported that four solvents (TCE, tetrachloroethylene, 1,1,1-trichloroethane and methylene chloride) occurred together in 5% of the samples. The most frequently occurring two-solvent mixture was TCE and tetrachloroethylene. The report stated that the most likely reason for this co-occurrence is the reductive dechlorination of tetrachloroethylene to TCE.

2.3.5. Other Media

Levels of TCE were found in the sediment and marine animal tissue collected in 1980−1981 near the discharge zone of a Los Angeles County waste treatment plant. Concentrations were 17 µg/L in the effluent, <0.5 µg/kg in dry weight in sediment, and 0.3−7 µg/kg wet weight in various marine animal tissue (IARC, 1995a). TCE has also been found in a variety of foods. U.S. Food and Drug Administration (FDA) has limits on TCE use as a food additive in decaffeinated coffee and extract spice oleoresins (see Table 2-15). Table 2-9 summarizes data from two sources:

- IARC (1995a) reports average concentrations of TCE in limited food samples collected in the United States.
- Jones and Smith (2003) measured VOC levels in over 70 foods collected from 1996 to 2000 as part of the FDA's Total Diet Program. All foods were collected directly from supermarkets. Analysis was done on foods in a ready-to-eat form. Sample sizes for most foods were in the 2−5 range.

Table 2-9. Levels in food

IARC (1995a)	Fleming-Jones and Smith (2003)
Cheese 3.8 µg/kg Butter and margarine 73.6 µg/kg	Cheese 2−3 µg/kg Butter 7−9 µg/kg Margarine 2−21 µg/kg Cheese pizza 2 µg/kg
Peanut butter 0.5 µg/kg	Nuts 2−5 µg/kg Peanut butter 4−70 µg/kg
	Ground beef 3−6 µg/kg Beef frankfurters 2−105 µg/kg Hamburger 5−9 µg/kg Cheeseburger 7 µg/kg Chicken nuggets 2−5 µg/kg Bologna 2−20 µg/kg Pepperoni pizza 2 µg/kg
	Banana 2 µg/kg Avocado 2−75 µg/kg Orange 2 µg/kg
	Chocolate cake 3−57 µg/kg Blueberry muffin 3−4 µg/kg Sweet roll 3 µg/kg Chocolate chip cookies 2−4 µg/kg Apple pie 2−4 µg/kg Doughnuts 3 µg/kg
	Tuna 9−11 µg/kg
Cereals 3 µg/kg Grain−based foods 0.9 µg/kg	Cereal 3 µg/kg
	Popcorn 4−8 µg/kg French fries 3 µg/kg Potato chips 4−140 µg/kg Coleslaw 3 µg/kg

2.3.6. Biological Monitoring

Biological monitoring studies have detected TCE in human blood and urine in the United States and other countries such as Croatia, China, Switzerland, and Germany (IARC, 1995a). Concentrations of TCE in persons exposed through occupational degreasing operations were most likely to have detectable levels (IARC, 1995a). In 1982, eight of eight human breastmilk samples from four United States urban areas had detectable levels of TCE. The levels of TCE detected, however, are not specified (HSDB, 2002; ATSDR, 1997c).

The Third National Health and Nutrition Examination Survey (NHANES III) examined TCE concentrations in blood in 677 nonoccupationally exposed individuals. The individuals were drawn from the general U.S. population and selected on the basis of age, race, gender and region of residence (IARC, 1995a; Ashley et al., 1994). The samples were collected during 1988−1994. TCE levels in whole blood were below the detection limit of 0.01 µg/L for about 90% of the people sampled (see Table 2-10). Assuming that nondetects equal half of the detection limit, the mean concentration was about 0.017 µg/L.

Table 2-10. TCE levels in whole blood by population percentile

Percentiles	10	20	30	40	50	60	70	80	90
Concentration (µg/L)	ND	ND	ND	ND	ND	ND	ND	ND	0.012

ND = Nondetect, i.e., below detection limit of 0.01 µg/L.

Sources: IARC (1995a); Ashley et al. (1994).

2.4. EXPOSURE PATHWAYS AND LEVELS

2.4.1. General Population

Because of the pervasiveness of TCE in the environment, most people are likely to have some exposure via one or more of the following pathways: ingestion of drinking water, inhalation of outdoor/indoor air, or ingestion of food (ATSDR, 1997c). As noted earlier, the NHANES survey suggests that about 10% of the population has detectable levels of TCE in blood. Each pathway is discussed below.

2.4.1.1. Inhalation

As discussed earlier, EPA has estimated emissions and modeled air concentrations for the Criteria Pollutants and Hazardous Air Pollutants under the National-Scale Air Toxics Assessment program (U.S. EPA, 2007a). This program has also estimated inhalation exposures on a nationwide basis. The exposure estimates are based on the modeled concentrations from outdoor sources and human activity patterns (U.S. EPA, 2005a). Table 2-11 shows the 1999 results for TCE.

Table 2-11. Modeled 1999 annual exposure concentrations (µg/m^3) for TCE

Percentile	Exposure concentration (µg/m^3)		
	Rural areas	Urban areas	Nationwide
5	0.030	0.048	0.038
10	0.034	0.054	0.043
25	0.038	0.065	0.056
50	0.044	0.086	0.076
75	0.053	0.122	0.113
90	0.070	0.189	0.172
95	0.097	0.295	0.262
Mean	0.058	0.130	0.116

Percentiles and mean are based on census tract values.

Source: http://www.epa.gov/ttn/atw/nata/ted/exporisk.html#indb.

These modeled inhalation exposures would have a geographic distribution similar to that of the modeled air concentrations as shown in Figure 2-4. Table 2-11 indicates that TCE inhalation exposures in urban areas are generally about twice as high as rural areas. While these modeling results are useful for understanding the geographic distribution of exposures, they appear to underestimate actual exposures. This is based on the fact that, as discussed earlier, the modeled ambient air levels are generally lower than measured values. Also, the modeled exposures do not consider indoor sources. Indoor sources of TCE make the indoor levels higher than ambient levels. This is particularly important to consider since people spend about 90% of their time indoors (U.S. EPA, 1997). A number of measurement studies were presented earlier that showed higher TCE levels indoors than outdoors. Sexton et al. (2005) measured TCE levels in Minneapolis/St. Paul area and found means of 0.5 μg/m^3 indoors (n = 292) and 1.0 μg/m^3 based on personal sampling (n = 288). Using 1.0 μg/m^3 and an average adult inhalation rate of 13 m^3 air/day (U.S. EPA, 1997) yields an estimated intake of 13 μg/day. This is consistent with ATSDR (1997c), which reported an average daily air intake for the general population of 11−33 μg/day.

2.4.1.2. Ingestion

The median value from the nationwide survey of domestic and public wells by USGS for 1985−2001 is 0.15 μg/L. This value was selected for exposure estimation purposes because it was the most current and most representative of the national population. Using this value and an average adult water consumption rate of 1.4 L/d yields an estimated intake of 0.2 μg/day. [This is from U.S. EPA (1997), but note that U.S. EPA (2004) indicates a mean per capita daily average total water ingestion from all sources of 1.233 L]. This is lower than the ATSDR (1997c) estimate water intake for the general population of 2–20 μg/day. The use of the USGS survey to represent drinking water is uncertain in two ways. First, the USGS survey measured only groundwater and some drinking water supplies use surface water. Second, the USGS measured TCE levels at the well head, not the drinking water tap. Further discussion about the possible extent and magnitude of TCE exposure via drinking water is presented below.

According to ATSDR (1997c), TCE is the most frequently reported organic contaminant in ground water (1997c), and between 9 and 34% of the drinking water supply sources tested in the United States may have some TCE contamination. Approximately 90% of the 155,000 public drinking water systems[1] in the United States are ground water systems. The drinking water standard for TCE only applies to community water systems (CWSs) and approximately 78% of the 51,972 CWSs in the United States are ground water systems (U.S. EPA, 2008a). Although commonly detected in water supplies, the levels are generally low

[1] PWSs are defined as systems which provide water for human consumption through pipes or other constructed conveyances to at least 15 service connections or serves an average of at least 25 people for at least 60 days a year. EPA further specifies three types of PWSs, including CWS)—a PWS that supplies water to the same population year-round.

because, as discussed earlier, maximum contaminant level violations for TCE in public water supplies are relatively rare for any extended period (U.S. EPA, 1998b). The USGS (2006) survey found that the number of samples exceeding the maximum contaminant level (5 µg/L) was six at domestic wells (n = 2,400) and nine at public wells (n = 1,100). Private wells, however, are often not closely monitored and if located near TCE disposal/contamination sites where leaching occurs, may have undetected contamination levels. About 10% of Americans (27 million people) obtain water from sources other than public water systems, primarily private wells (U.S. EPA, 1995b). TCE is a common contaminant at Superfund sites. As of September, 2011, EPA's Superfund program has identified 761 sites with TCE as a contaminant of concern in groundwater, soil or both (CERCLIS Public Access Database). Studies have shown that many people live near these sites: 41 million people live <4 miles from one or more of the nation's NPL sites, and on average 3,325 people live within 1 mile of any given NPL site (ATSDR, 1996b).

Table 2-12 presents preliminary estimates of TCE intake from food. They are based on average adult food ingestion rates and food data from Table 2-9. This approach suggests a total ingestion intake of about 5 µg/d. It is important to consider this estimate as preliminary because it is derived by applying data from very limited food samples to broad classes of food.

Table 2-12. Preliminary estimates of TCE intake from food ingestion

	Consumption rate (g/kg-d)	Consumption rate (g/d)	Concentration in food (µg/kg)	Intake (µg/d)
Fruit	3.4	238	2	0.48
Vegetables	4.3	301	3	0.90
Fish		20	10	0.20
Meat	2.1	147	5	0.73
Dairy products	8	560	3	1.68
Grains	4.1	287	3	0.86
Sweets	0.5	35	3	0.10
Total				4.96

[a]Consumption rates are per capita averages from EPA (1997).
[b]Consumption rates in g/d assume 70 kg body weight.

2.4.1.3. Dermal

TCE in bathing water and consumer products can result in dermal exposure. A modeling study has suggested that a significant fraction of the total dose associated with exposure to volatile organics in drinking water results from dermal absorption (Brown et al., 1984). EPA (2004) used a prediction model based on octanol-water partitioning and molecular weight to derive a dermal permeability coefficient for TCE in water of 0.012 cm/hour. EPA used this value to compute the dermally absorbed dose from a 35 minute shower and compared it to the dose from drinking 2 L of water at the same concentration. This comparison indicated that the

dermal dose would be 17% of the oral dose. Much higher dermal permeabilities were reported by Nakai et al. (1999) based on human skin in vitro testing. For dilute aqueous solutions of TCE, they measured a permeability coefficient of 0.12 cm/hour (26°C). Nakai et al. (1999) also measured a permeability coefficient of 0.018 cm/hour for tetrachloroethylene in water. Poet et al. (2000) measured dermal absorption of TCE in humans from both water and soil matrices. The absorbed dose was estimated by applying a physiologically based pharmacokinetic model to TCE levels in breath. The permeability coefficient was estimated to be 0.015 cm/hour for TCE in water and 0.007 cm/hour for TCE in soil (Poet et al., 2000).

2.4.1.4. Exposure to TCE Related Compounds

Table 2-13 presents adult exposure estimates that have been reported for the TCE related compounds. This table was originally compiled by Wu and Schaum (2001). The exposure/dose estimates are taken directly from the listed sources or derived based on monitoring data presented in the source documents. They are considered "preliminary" because they are generally based on very limited monitoring data. These preliminary estimates suggest that exposures to most of the TCE related compounds are comparable to or greater than TCE itself.

Table 2-13. Preliminary intake estimates of TCE and TCE-related chemicals

Chemical	Population	Media	Range of estimated adult exposures (µg/d)	Range of adult doses (mg/kg-d)	Data sources[a]
Trichloroethylene	General	Air	11–33	1.57×10^{-4}–4.71×10^{-4}	ATSDR (1997c)
	General	Water	2–20[b]	2.86×10^{-5}–2.86×10^{-4}	ATSDR (1997c)
	Occupational	Air	2,232–9,489	3.19×10^{-2}–1.36×10^{-1}	ATSDR (1997c)
Tetrachloroethylene	General	Air	80–200	1.14×10^{-3}–2.86×10^{-3}	ATSDR (1997a)
	General	Water	0.1–0.2	1.43×10^{-6}–2.86×10^{-6}	ATSDR (1997a)
	Occupational	Air	5,897–219,685	8.43×10^{-2}–3.14	ATSDR (1997a)
1,1,1-Trichloroethane	General	Air	10.8–108	1.54×10^{-4}–1.54×10^{-3}	ATSDR (1995)
	General	Water	0.38–4.2	5.5×10^{-6}–6.0×10^{-5}	ATSDR (1995)
1,2-Dichloroethylene	General	Air	1–6	1.43×10^{-5}–8.57×10^{-5}	ATSDR (1996a)
	General	Water	2.2	3.14×10^{-5}	ATSDR (1996a)
Cis-1,2-Dichloroethylene	General	Air	5.4	7.71×10^{-5}	HSDB (1996)
	General	Water	0.5–5.4	7.14×10^{-6}–7.71×10^{-5}	HSDB (1996)
1,1,1,2-Tetrachloroethane	General	Air	142	2.03×10^{-3}	HSDB (2002)
1,1-Dichloroethane	General	Air	4	5.71×10^{-5}	ATSDR (1990)
	General	Water	2.47–469.38	3.53×10^{-5}–6.71×10^{-3}	ATSDR (1990)
Chloral	General	Water	0.02–36.4	2.86×10^{-7}–5.20×10^{-4}	HSDB (1996)
Monochloroacetic acid	General	Water	2–2.4	2.86×10^{-5}–3.43×10^{-5}	EPA (1994c)
Dichloroacetic acid	General	Water	10–266	1.43×10^{-4}–3.80×10^{-3}	IARC (1995a)
Trichloroacetic acid	General	Water	8.56–322	1.22×10^{-3}–4.60×10^{-3}	IARC (1995a)

[a]Originally compiled in Wu and Schaum (2001).
[b]New data from USGS (2006) suggests much lower water intakes, i.e., 0.2 µg/d.

2.4.2. Potentially Highly Exposed Populations

Some members of the general population may have elevated TCE exposures. ATSDR (1997c) has reported that TCE exposures may be elevated for people living near waste facilities where TCE may be released, residents of some urban or industrialized areas, people exposed at work (discussed further below) and individuals using certain products (also discussed further below). Because TCE has been detected in breast milk samples of the general population, infants who ingest breast milk may be exposed, as well. Increased TCE exposure is also a possible concern for bottle-fed infants because they ingest more water on a bodyweight basis than adults (the average water ingestion rate for adults is 21 mL/kg-day and for infants under one year old it is 44 mL/kg-day) (U.S. EPA, 1997). Also, because TCE can be present in soil, children may be exposed through activities such as playing in or ingesting soil.

2.4.2.1. Occupational Exposure

Occupational exposure to TCE in the United States has been identified in various degreasing operations, silk screening, taxidermy, and electronics cleaning (IARC, 1995a). The major use of TCE is for metal cleaning or degreasing (IARC, 1995a). Degreasing is used to remove oils, greases, waxes, tars, and moisture before galvanizing, electroplating, painting, anodizing, and coating. The five primary industries using TCE degreasing are furniture and fixtures; electronic and electric equipment; transport equipment; fabricated metal products; and miscellaneous manufacturing industries (IARC, 1995a). Additionally, TCE is used in the manufacture of plastics, appliances, jewelry, plumbing fixtures, automobile, textiles, paper, and glass (IARC, 1995a).

Table 2-14 lists the primary types of industrial degreasing procedures and the years that the associated solvents were used. Vapor degreasing has the highest potential for exposure because vapors can escape into the work place. Hot dip tanks, where TCE is heated to close to its boiling point of 87°C, are also major sources of vapor that can create exposures as high as vapor degreasers. Cold dip tanks have a lower exposure potential, but they have a large surface area which enhances volatilization. Small bench-top cleaning operations with a rag or brush and open bucket have the lowest exposure potential. In combination with the vapor source, the size and ventilation of the workroom are the main determinants of exposure intensity (NRC, 2006).

Table 2-14. Years of solvent use in industrial degreasing and cleaning operations

Years	Vapor degreasers	Cold dip tanks	Rag or brush and bucket on bench top
~1934−1954	Trichloroethylene (poorly controlled)	Stoddard solvent[a]	Stoddard solvent (general use), alcohols (electronics shop), carbon tetrachloride (instrument shop).
~1955−1968	TCE (poorly controlled, tightened in 1960s)	TCE (replaced some Stoddard solvent)	Stoddard solvent, TCE (replaced some Stoddard solvent), perchloroethylene, 1,1,1-trichloroethane (replaced carbon tetrachloride, alcohols, ketones).
~1969−1978	TCE, (better controlled)	TCE, Stoddard solvent	TCE, perchloroethylene, 1,1,1-trichloro-ethane, alcohols, ketones, Stoddard solvent.
~1979−1990s	1,1,1-Trichloroethane (replaced TCE)	1,1,1-Trichloroethane (replaced TCE), Stoddard solvent	1,1,1-Trichloroethane, perchloroethylene, alcohols, ketones, Stoddard solvent.

[a]A mixture of straight and branched chain paraffins (48%), naphthenes (38%), and aromatic hydrocarbons (14%).

Sources: Stewart and Dosemeci (2005); Bakke et al. (2007).

Occupational exposure to TCE has been assessed in a number of epidemiologic and industrial hygiene studies. Bakke et al. (2007) estimated that the arithmetic mean of TCE occupational exposures across all industries and decades (mostly 1950s, 1970s, and 1980s) was 38.2 ppm (210 mg/m^3). They also reported that the highest personal and area air levels were found in vapor degreasing operations (arithmetic mean of 44.6 ppm or 240 mg/m^3). Hein et al. (2010) developed and evaluated statistical models to estimate the intensity of occupational exposure to TCE (and other solvents) using a database of air measurement data and associated exposure determinants. The measurement database was compiled from the published literature and National Institute for Occupational Safety and Health (NIOSH) reports from 1940 to 1998 (n = 484) and were split between personal (47%)and area (53%) measurements. The predicted arithmetic mean exposure intensity levels for the evaluated exposure scenarios ranged from 0.21 to 3,700 ppm (1.1–20,000 mg/m^3) with a median of 30 ppm (160 mg/m^3). Landrigan et al. (1987) used air and biomonitoring techniques to quantify the exposure of degreasing workers who worked around a heated, open bath of TRI. Exposures were found to be between 22 and 66 ppm (117−357 mg/m^3) on average, with short-term peaks between 76 and 370 ppm (413−2,000 mg/m^3). High peak exposures have also been reported for cardboard workers who were involved with degreasing using a heated and open process (Henschler et al., 1995). Lacking industrial hygiene data and making some assumptions about plant environment and TCE usage, Cherrie et al. (2001) estimated that cardboard workers at a plant in Germany had peak exposures in the range of 200–4,000 ppm (1,100−22,000 mg/m^3) and long-term average exposures of 10–225 ppm (54−1,200 mg/m^3). ATSDR (1997c) reports that the majority of published worker exposure data show time-weighted average concentrations ranging from <50 ppm–100 ppm (<270−540 mg/m^3). NIOSH conducted a survey of various industries from

1981 to 1983 and estimated that approximately 401,000 U.S. employees in 23,225 plants in the United States were potentially exposed to TCE during this timeframe (ATSDR, 1997c; IARC, 1995a). Occupational exposure to TCE has likely declined since the 1950s and 1960s due to decreased usage, better release controls, and improvements in worker protection. Reductions in TCE use are illustrated in Table 2-14, which shows that by about 1980, common degreasing operations had substituted other solvents for TCE.

2.4.2.2. Consumer Exposure

Consumer products reported to contain TCE include wood stains, varnishes, and finishes; lubricants; adhesives; typewriter correction fluids; paint removers; and cleaners (ATSDR, 1997c). Use of TCE has been discontinued in some consumer products (i.e., as an inhalation anesthetic, fumigant, and an extractant for decaffeinating coffee) (ATSDR, 1997c).

2.4.3. Exposure Standards

Table 2-15 summarizes the federal regulations limiting TCE exposure.

Table 2-15. TCE standards

Standard	Value	Reference
OSHA Permissible Exposure Limit: Table Z-2 8-hr time-weighted average.	100 ppm (538 mg/m^3)	29 CFR 1910.1000 (7/1/2000)
OSHA Permissible Exposure Limit: Table Z-2 Acceptable ceiling concentration (this cannot be exceeded for any time period during an 8-hr shift except as allowed in the maximum peak standard below).	200 ppm (1,076 mg/m^3)	29 CFR 1910.1000 (7/1/2000)
OSHA Permissible Exposure Limit: Table Z-2 Acceptable maximum peak above the acceptable ceiling concentration for an 8-hr shift. Maximum Duration: 5 minutes in any 2 hrs.	300 ppm (1,614 mg/m^3)	29 CFR 1910.1000 (7/1/2000)
Maximum contaminant level under the Safe Drinking Water Act.	5 ppb (5 µg/L)	40 CFR 141.161
FDA Tolerances for decaffeinated ground coffee decaffeinated soluble (instant) coffee extract spice oleoresins.	 25 ppm (25 µg/g) 10 ppm (10 µg/g) 30 ppm (30 µg/g)	21 CFR 173.290 (4/1/2000)

OSHA = Occupational Safety and Health Administration

2.5. EXPOSURE SUMMARY

TCE is a volatile compound with moderate water solubility. Most TCE produced today is used for metal degreasing. The highest environmental releases are to the air. Ambient air monitoring data suggests that levels have remained fairly constant since 1999 at about 0.3 µg/m^3. Indoor levels are commonly three or more times higher than outdoor levels due to releases from

building materials and consumer products. TCE is among the most common groundwater contaminants and the median level based on a large survey by USGS for 1985−2001 is 0.15 μg/L. It has also been detected in a wide variety of foods in the 1−100 μg/kg range. None of the environmental sampling has been done using statistically based national surveys. However, a substantial amount of air and groundwater data have been collected allowing reasonably well supported estimates of typical daily intakes by the general population: inhalation—13 μg/day and water ingestion—0.2 μg/day. The limited food data suggests an intake of about 5 μg/day, but this must be considered preliminary.

Much higher exposures have occurred to various occupational groups. For example, past studies of aircraft workers have shown short term peak exposures in the hundreds of ppm (>540,000 $\mu g/m^3$) and long term exposures in the low tens of ppm (>54,000 $\mu g/m^3$). Occupational exposures have likely decreased in recent years due to better release controls and improvements in worker protection.

Preliminary exposure estimates were presented for a variety of TCE related compounds which include metabolites of TCE and other parent compounds that produce similar metabolites. Exposure to the TCE related compounds can alter or enhance TCE‘s metabolism and toxicity by generating higher internal metabolite concentrations than would result from TCE exposure by itself. The preliminary estimates suggest that exposures to most of the TCE related compounds are comparable to or greater than TCE itself.

3. TOXICOKINETICS

TCE is a lipophilic compound that readily crosses biological membranes. Exposures may occur via the oral, dermal, and inhalation routes, with evidence for systemic availability from each route. TCE is rapidly and nearly completely absorbed from the gut following oral administration, and studies with animals indicate that exposure vehicle may impact the time-course of absorption: oily vehicles may delay absorption, whereas aqueous vehicles result in a more rapid increase in blood concentrations.

Following absorption to the systemic circulation, TCE distributes from blood to solid tissues by each organ‘s solubility. This process is mainly determined by the blood:tissue partition coefficients, which are largely established by tissue lipid content. Adipose partitioning is high, adipose tissue may serve as a reservoir for TCE, and accumulation into adipose tissue may prolong internal exposures. TCE attains high concentrations relative to blood in the brain, kidney, and liver—all of which are important target organs of toxicity. TCE is cleared via metabolism mainly in three organs: the kidney, liver, and lungs.

The metabolism of TCE is an important determinant of its toxicity. Metabolites are generally thought to be responsible for toxicity—especially for the liver and kidney. Initially, TCE may be oxidized via cytochrome P450 (CYP) xenobiotic metabolizing isozymes or conjugated with glutathione (GSH) by glutathione-S-transferase (GST) enzymes. While CYP2E1 is generally accepted to be the CYP form most responsible for TCE oxidation at low concentrations, other forms may also contribute, though their contributions may be more important at higher, rather than lower, environmentally-relevant exposures.

Once absorbed, TCE is excreted primarily either in breath as unchanged TCE or carbon dioxide (CO_2), or in urine as metabolites. Minor routes of elimination include excretion of metabolites in saliva, sweat, and feces. Following oral administration or upon cessation of inhalation exposure, exhalation of unmetabolized TCE is a major elimination pathway. Initially, elimination of TCE upon cessation of inhalation exposure demonstrates a steep concentration-time profile: TCE is rapidly eliminated in the minutes and hours postexposure, and then the rate of elimination via exhalation decreases. Following oral or inhalation exposure, urinary elimination of parent TCE is minimal, with urinary elimination of the metabolites TCA and TCOH accounting for the bulk of the absorbed dose of TCE.

Sections 3.1–3.4 below describe the absorption, distribution, metabolism, and excretion (ADME) of TCE and its metabolites in greater detail. Section 3.5 then discusses PBPK modeling of TCE and its metabolites.

3.1. ABSORPTION

TCE is a low-molecular-weight lipophilic solvent; these properties explain its rapid transfer from environmental media into the systemic circulation after exposure. As discussed below, it is readily absorbed into the bloodstream following exposure via oral ingestion and inhalation, with more limited data indicating dermal penetration.

3.1.1. Oral

Available reports on human exposure to TCE via the oral route are largely restricted to case reports of occupational or intentional (suicidal) ingestions and suggest significant gastric absorption (e.g., Brüning et al., 1998; Yoshida et al., 1996; Perbellini et al., 1991). Clinical symptoms attributable to TCE or metabolites were observed in these individuals within a few hours of ingestion (such as lack of consciousness), indicating absorption of TCE. In addition, TCE and metabolites were measured in blood or urine at the earliest times possible after ingestion, typically upon hospital admission, while urinary excretion of TCE metabolites was followed for several days following exposure. Therefore, based on these reports, it is likely that TCE is readily absorbed in the gastrointestinal (GI) tract; however, the degree of absorption cannot be confidently quantified because the ingested amounts are not known.

Experimental evidence in mice and rats supports rapid and extensive absorption of TCE, although variables such as stomach contents, vehicle, and dose may affect the degree of gastric absorption. D'Souza et al. (1985) reported on bioavailability and blood kinetics in fasted and nonfasted male Sprague-Dawley rats following intragastric administration of TCE at 5–25 mg/kg in 50% polyethylene glycol (PEG 400) in water. TCE rapidly appeared in peripheral blood (at the initial 0.5 minutes sampling) of fasted and nonfasted rats with peak levels being attained shortly thereafter (6–10 minutes), suggesting that absorption is not diffusion limited, especially in fasted animals. The presence of food in the GI tract, however, seems to influence TCE absorption based on findings in the nonfasted animals of lesser bioavailability (60–80 vs. 90% in fasted rats), smaller peak blood levels (two- to threefold lower than nonfasted animals), and a somewhat longer terminal half-life ($t_{1/2}$) (174 vs. 112 minutes in fasted rats).

Studies by Prout et al. (1985) and Dekant et al. (1986b) have shown that up to 98% of administered radiolabel was found in expired air and urine of rats and mice following gavage administration of [^{14}C]-radiolabeled TCE ([^{14}C]-TCE). Prout et al. (1985) and Green and Prout (1985) compared the degree of absorption, metabolites, and routes of elimination among two strains each of male rats (Osborne-Mendel and Park Wistar) and male mice (B6C3F$_1$ and Swiss-Webster) following a single oral administration of 10, 500, or 1,000 [^{14}C]-TCE. Additional dose groups of Osborne-Mendel male rats and B6C3F$_1$ male mice also received a single oral dose of 2,000 mg/kg [^{14}C]-TCE. At the lowest dose of 10 mg/kg, there were no major differences between rats and mice in routes of excretion, with most of the administered radiolabel (nearly 60–70%) being in the urine. At this dose, the expired air from all groups

contained 1–4% of unchanged TCE and 9–14% CO_2. Fecal elimination of the radiolabel ranged from 8.3% in Osborne-Mendel rats to 24.1% in Park Wistar rats. However, at doses between 500 and 2,000 mg/kg, the rat progressively excreted a higher proportion of the radiolabel as unchanged TCE in expired air, such that 78% of the administered high dose was found in expired air (as unchanged TCE) while only 13% was excreted in the urine.

Following exposure to a chemical by the oral route, distribution is determined by delivery to the first organ encountered in the circulatory pathway—the liver (i.e., the first-pass effect), where metabolism and elimination may limit the proportion that may reach extrahepatic organs. Lee et al. (1996) evaluated the efficiency and dose-dependency of presystemic elimination of TCE in male Sprague-Dawley rats following administration into the carotid artery, jugular vein, hepatic portal vein, or the stomach of TCE (0.17, 0.33, 0.71, 2, 8, 16, or 64 mg/kg) in a 5% aqueous Alkamus emulsion (polyethoxylated vegetable oil) in 0.9% saline. The first-pass elimination, decreased from 57.5 to <1% with increasing dose (0.17–16 mg/kg), which implied that hepatic TCE metabolism may be saturated at doses >16 mg/kg in the male rat. At doses of ≥16 mg/kg, hepatic first-pass elimination was almost nonexistent indicating that, at relatively large doses, virtually all of TCE passes through the liver without being extracted (Lee et al., 1996). In addition to the hepatic first-pass elimination findings, pulmonary extraction, which was relatively constant (at nearly 5–8% of dose) over the dose range, also played a role in eliminating TCE.

In addition, oral absorption appears to be affected by both dose and vehicle used. The majority of oral TCE studies have used either aqueous solution or corn oil as the dosing vehicle. Most studies that relied on an aqueous vehicle delivered TCE as an emulsified suspension in Tween 80® or PEG 400 in order to circumvent the water solubility problems. Lee et al. (2000a; 2000b) used Alkamus (a polyethoxylated vegetable oil emulsion) to prepare a 5% aqueous emulsion of TCE that was administered by gavage to male Sprague-Dawley rats. The findings confirmed rapid TCE absorption, but reported decreasing absorption rate constants (i.e., slower absorption) with increasing gavage dose (2–432 mg/kg). The time to reach blood peak concentrations increased with dose and ranged between 2 and 26 minutes postdosing. Other pharmacokinetics data, including area under the blood concentration time curve (AUC) and prolonged elevation of blood TCE levels at the high doses, indicated prolonged GI absorption and delayed elimination due to metabolic saturation occurring at the higher TCE doses.

A study by Withey et al. (1983) evaluated the effect of dosing TCE with corn oil vs. pure water as a vehicle by administering four VOCs separately in each dosing vehicle to male Wistar rats. Based on its limited solubility in pure water, the dose for TCE was selected at 18 mg/kg (administered in 5 mL/kg). Times to peak in blood reported for TCE averaged 5.6 minutes when water was used. In comparison, the time to peak in blood was much longer (approximately 100 minutes) when the oil vehicle was used and the peaks were smaller, below the level of detection, and not reportable.

Time-course studies reporting times to peak in blood or other tissues have been performed using both vehicles (Larson and Bull, 1992a, b; D'Souza et al., 1985; Green and Prout, 1985; Dekant et al., 1984; Withey et al., 1983). Related data for other solvents (Dix et al., 1997; Lilly et al., 1994; Kim et al., 1990a; Kim et al., 1990b; Chieco et al., 1981) confirmed differences in TCE absorption and peak height between the two administered vehicles. One study has also evaluated the absorption of TCE from soil in rats (Kadry et al., 1991) and reported absorption within 16 hours for clay and 24 hours for sandy soil. In summary, these studies confirm that TCE is relatively quickly absorbed from the stomach, and that absorption is dependent on the vehicle used.

3.1.2. Inhalation

TCE is a lipophilic volatile compound that is readily absorbed from inspired air. Uptake from inhalation is rapid and the absorbed dose is proportional to exposure concentration and duration, and pulmonary ventilation rate. Distribution into the body via arterial blood leaving the lungs is determined by the net dose absorbed and eliminated by metabolism in the lungs. Metabolic clearance in the lungs will be further discussed in Section 3.3, below. In addition to metabolism, solubility in blood is the major determinant of the TCE concentration in blood entering the heart and being distributed to the each body organ via the arterial blood. The measure of TCE solubility in each organ is the partition coefficient, or the concentration ratio between both organ phases of interest. The blood-to-air partition coefficient quantifies the resulting concentration in blood leaving the lungs at equilibrium with alveolar air. The value of the blood-to-air partition coefficient is used in PBPK modeling (see Section 3.5). The blood-to-air partition has been measured in vitro using the same principles in different studies and found to range between 8.1 and 11.7 in humans with somewhat higher values in mice and rats (13.3–25.8) (see Tables 3-1–3-2, and references therein).

Table 3-1. Blood:air partition coefficient values for humans

Blood:air partition coefficient	Reference/notes
8.1 ± 1.8	Fiserova-Bergerova et al. (1984); mean ± SD (SD converted from SE based on n = 5)
8.11	Gargas et al. (1989); (n = 3–15)
9.13 ± 1.73 [6.47–11]	Fisher et al. (1998); mean ± SD [range] of females (n = 6)
9.5	Sato and Nakajima (1979); (n = 1)
9.77	Koizumi (1989)
9.92	Sato et al. (1977); (n = 1)
11.15 ± 0.74 [10.1–12.1]	Fisher et al. (1998); mean ± SD [range] of males (n = 7)
11.2 ± 1.8 [7.9–15]	Mahle et al. (2007); mean ± SD; 20 male pediatric patients aged 3–7 yrs (range; USAF, 2004)
11.0 ± 1.6 [6.6–13.5]	Mahle et al. (2007); mean ± SD; 18 female pediatric patients aged 3–17 yrs (range; USAF, 2004)
11.7 ± 1.9 [6.7–16.8]	Mahle et al. (2007); mean ± SD; 32 male patients aged 23–82 yrs (range; USAF, 2004)
10.6 ± 2.3 [3–14.4]	Mahle et al. (2007); mean ± SD; 27 female patients aged 23–82 yrs (range; USAF, 2004)

SE = standard error

Table 3-2. Blood:air partition coefficient values for rats and mice

Blood:air partition coefficient	Reference/notes
Rat	
15 ± 0.5	Fisher et al. (1998); mean ± SD (SD converted from SE based on n = 3)
17.5	Rodriguez et al. (2007)
20.5 ± 2.4	Barton et al. (1995); mean ± SD (SD converted from SE based on n = 4)
20.69 ± 3.3	Simmons et al. (2002); mean ± SD (n = 7–10)
21.9	Gargas et al. (1989) (n = 3–15)
25.8	Koizumi (1989) (pooled n = 3)
25.82 ± 1.7	Sato et al. (1977); mean ± SD (n = 5)
13.3 ± 0.8 [11.6–15]	Mahle et al. (2007); mean ± SD; 10 PND 10 male rat pups (range; USAF, 2004)
13.4 ± 1.8 [11.8–17.2]	Mahle et al. (2007); mean ± SD; 10 PND 10 female rat pups (range; USAF, 2004)
17.5 ± 3.6 [11.7–23.1]	Mahle et al. (2007); mean ± SD; 9 adult male rats (range; USAF, 2004)
21.8 ± 1.9 [16.9–23.5]	Mahle et al. (2007); mean ± SD; 11 aged male rats (range; USAF, 2004)
Mouse	
13.4	Fisher et al. (1991); male
14.3	Fisher et al. (1991); female
15.91	Abbas and Fisher (1997)

PND = postnatal day

TCE enters the human body quickly by inhalation, and, at high concentrations, it may lead to death (Coopman et al., 2003), narcosis, unconsciousness, and acute kidney damage (Carrieri et al., 2007). Controlled exposure studies in humans have shown absorption of TCE to approach a steady state within a few hours after the start of inhalation exposure (Fernandez et al., 1977; Monster et al., 1976; Vesterberg and Astrand, 1976; Vesterberg et al., 1976). Several studies have calculated the net dose absorbed by measuring the difference between the inhaled concentration and the exhaled air concentration. Soucek and Vlachova (1960) reported 58–70% absorption of the amount inhaled for 5-hour exposures of 93–158 ppm. Bartonicek (1962) obtained an average retention value of 58% after 5 hours of exposure to 186 ppm. Monster et al. (1976) also took into account minute ventilation measured for each exposure, and calculated of 37–49% absorption in subjects exposed to 70 and 140 ppm. The impact of exercise, the increase in workload, and its effect on breathing has also been measured in controlled inhalation exposures. Astrand and Ovrum (1976) reported 50–58% uptake at rest and 25–46% uptake during exercise from exposure to 100 or 200 ppm (540 or 1,080 mg/m^3, respectively) of TCE for 30 minutes (see Table 3-3). These authors also monitored heart rate and pulmonary ventilation. In contrast, Jakubowski and Wieczorek (1988) calculated about 40% retention in volunteers exposed to TCE at 9 ppm (mean inspired concentration of 48–49 mg/m^3) for 2 hours at rest, with no change in retention during increased workload due to exercise (see Table 3-4).

Table 3-3. Air and blood concentrations during exposure to TCE in humans

TCE concentration (mg/m^3)	Work load (watt)	Exposure series[a]	TCE concentration in			Uptake as % of amount available	Amount taken up (mg)
			Alveolar air (mg/m^3)	Arterial blood (mg/kg)	Venous blood (mg/kg)		
540	0	I	124 ± 9	1.1 ± 0.1	0.6 ± 0.1	53 ± 2	79 ± 4
540	0	II	127 ± 11	1.3 ± 0.1	0.5 ± 0.1	52 ± 2	81 ± 7
540	50	I	245 ± 12	2.7 ± 0.2	1.7 ± 0.4	40 ± 2	160 ± 5
540	50	II	218 ± 7	2.8 ± 0.1	1.8 ± 0.3	46 ± 1	179 ± 2
540	50	II	234 ± 12	3.1 ± 0.3	2.2 ± 0.4	39 ± 2	157 ± 2
540	50	II	244 ± 16	3.3 ± 0.3	2.2 ± 0.4	37 ± 2	147 ± 9
1,080	0	I	280 ± 18	2.6 ± 0.0	1.4 ± 0.3	50 ± 2	156 ±9
1,080	0	III	212 ± 7	2.1 ± 0.2	1.2 ± 0.1	58 ± 2	186 ± 7
1,080	50	I	459 ± 44	6.0 ± 0.2	3.3 ± 0.8	45 ± 2	702 ± 31
1,080	50	III	407 ± 30	5.2 ± 0.5	2.9 ± 0.7	51 ± 3	378 ± 18
1,080	100	III	542 ± 33	7.5 ± 0.7	4.8 ± 1.1	36 ± 3	418 ± 39
1,080	150	III	651 ± 53	9.0 ± 1.0	7.4 ± 1.1	25 ± 5	419 ± 84

[a]Series I consisted of 30-minute exposure periods of rest, rest, 50 watts, and 50 watts; Series II consisted of 30-minute exposure periods of rest, 50 watts, 50 watts, 50 watts; and Series III consisted of 30-minute exposure periods of rest, 50 watts, 100 watts, 150 watts.

Source: Astrand and Ovrum (1976)

Table 3-4. Retention of inhaled TCE vapor in humans

Workload	Inspired concentration (mg/m^3)	Pulmonary ventilation (m^3/hr)	Retention	Uptake (mg/hr)
Rest	48 ± 3[a]	0.65 ± 0.07	0.40 ± 0.05	12 ± 1.1
25 Watts	49 ± 1.3	1.30 ± 0.14	0.40 ± 0.05	25 ± 2.9
50 Watts	49 ± 1.6	1.53 ± 0.13	0.42 ± 0.06	31 ± 2.8
75 Watts	48 ± 1.9	1.87 ± 0.14	0.41 ± 0.06	37 ± 4.8

[a]Mean ± SD, n = 6 adult males.

Source: Jakubowski and Wieczorek (1988)

Environmental or occupational settings may result from a pattern of repeated exposure to TCE. Monster et al. (1979a) reported 70-ppm TCE exposures in volunteers for 4 hours for 5 consecutive days, averaging a total uptake of 450 mg per 4 hours of exposure (see Table 3-5). In dry-cleaning workers, Skender et al. (1991) reported initial blood concentrations of 0.38 µmol/L, increasing to 3.4 µmol/L 2 days after. Results of these studies support rapid absorption of TCE via inhalation.

Table 3-5. Uptake of TCE in volunteers following 4 hour exposure to 70 ppm

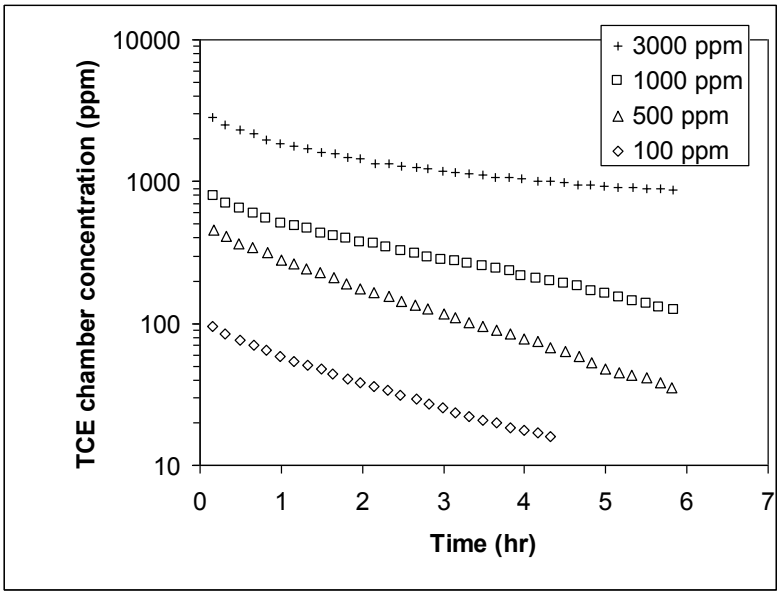
	Body weight (kg)	Minute-volume (L/min)	Percentage retained	Uptake (mg/d)	Uptake (mg/kg-d)
A	80	9.8 ± 0.4	45 ± 0.8	404 ± 23	5.1
B	82	12.0 ± 0.7	44 ± 0.9	485 ± 35	5.9
C	82	10.9 ± 0.8	49 ± 1.2	493 ± 28	6.0
D	67	11.8 ± 0.8	35 ± 2.6	385 ± 38	5.7
E	90	11.0 ± 0.7	46 ± 1.1	481 ± 25	5.3
Mean					5.6 ± 0.4

Source: Monster et al. (1979b).

Direct measurement of retention after inhalation exposure in rodents is more difficult because exhaled breath concentrations are challenging to obtain. The only available data are from Dallas et al. (1991), who designed a nose-only exposure system for rats using a facemask equipped with one-way breathing valves to obtain measurements of TCE in inspired and exhaled air. In addition, indwelling carotid artery cannulae were surgically implanted to facilitate the simultaneous collection of blood. After a 1-hour acclimatization period, rats were exposed to 50 or 500 ppm TCE for 2 hours, and the time course of TCE in blood and expired air was measured during and for 3 hours following exposure. When air concentration data were analyzed to reveal absorbed dose (minute volume multiplied by the concentration difference between inspired and exhaled breath), it was demonstrated that the fractional absorption of either concentration was >90% during the initial 5 minutes of exposure. Fractional absorption then decreased to 69 and 71% at 50 and 500 ppm during the second hour of exposure. Cumulative uptake appeared linear with respect to time over the 2-hour exposure, resulting in absorbed doses of 8.4 and 73.3 mg/kg in rats exposed to 50 and 500 ppm, respectively. Given the 10-fold difference in inspired concentration and the 8.7-fold difference in uptake, the authors interpreted this information to indicate that metabolic saturation occurred at some concentration <500 ppm. In comparing the absorbed doses to those developed for the 70-ppm-exposed human [see Monster et al. (1979a)], Dallas et al. (1991) concluded that on a systemic dose (mg/kg) basis, rats receive a much higher TCE dose from a given inhalation exposure than do humans. In particular, using the results cited above, the absorption per ppm-hour was 0.084 and 0.073 mg/kg-ppm-hour at 50 and 500 ppm in rats (Dallas et al., 1991) and 0.019 mg/kg-ppm-hour at 70 ppm in humans (Monster et al., 1979a)—a difference of around fourfold. However, rats have about a 10-fold higher alveolar ventilation rate per unit body weight than humans (Brown et al., 1997), which more than accounts for the observed increase in absorption.

Other experiments, such as closed-chamber gas uptake experiments or blood concentration measurements following open-chamber (fixed concentration) experiments, measure absorption indirectly but are consistent with significant retention. Closed-chamber gas-

uptake methods (Gargas et al., 1988) place laboratory animals or in vitro preparations into sealed systems in which a known amount of TCE is injected to produce a predetermined chamber concentration. As the animal retains a quantity of TCE inside its body, due to metabolism, the closed-chamber concentration decreases with time when compared to the start of exposure. Many different studies have made use of this technique in both rats and mice to calculate total TCE metabolism (i.e., Simmons et al., 2002; Fisher et al., 1991; Andersen et al., 1987a). This inhalation technique is combined with PBPK modeling to calculate metabolic parameters, and the results of these studies are consistent with rapid absorption of TCE via the respiratory tract. Figure 3-1 shows an example from Simmons et al. (2002), in Long-Evans rats, that demonstrates an immediate decline in chamber concentrations of TCE indicating absorption, with multiple initial concentrations needed for each metabolic calculation. At concentrations below metabolic saturation, a secondary phase of uptake appears, after 1 hour from starting the exposure, indicative of metabolism. At concentrations >1,000 ppm, metabolism appears saturated, with time-course curves having a flat phase after absorption. At intermediate concentrations, between 100 and 1,000 ppm, the secondary phase of uptake appears after distribution as continued decreases in chamber concentration as metabolism proceeds. Using a combination of experiments that include both metabolic linear decline and saturation obtained by using different initial concentrations, both components of metabolism can be estimated from the gas uptake curves, as shown in Figure 3-1.



Symbols represent measured chamber concentrations. Source: Simmons et al. (2002).

Figure 3-1. Gas uptake data from closed-chamber exposure of rats to TCE.

Several other studies in humans and rodents have measured blood concentrations of TCE or metabolites and urinary excretion of metabolites during and after inhalation exposure (e.g., Fisher et al., 1998; 1991; 1990; Filser and Bolt, 1979). While qualitatively indicative of absorption, blood concentrations are also determined by metabolism, distribution, and excretion; thus, comparisons between species may reflect similarities or differences in any of the absorption, distribution, metabolism, and excretion processes.

3.1.3. Dermal

Skin membrane is believed to present a diffusional barrier for entrance of the chemical into the body, and TCE absorption can be quantified using a permeability rate or permeability constant, though not all studies performed such a calculation. Absorption through the skin has been shown to be rapid by both vapor and liquid TCE contact with the skin. Human dermal absorption of TCE vapors was investigated by Kezic et al. (2000). Volunteers were exposed to 3.18×10^4 ppm around each enclosed arm for 20 minutes. Adsorption was found to be rapid (within 5 minutes), reaching a peak in exhaled breath around 30 minutes, with a calculated dermal penetration rate averaging 0.049 cm/hour for TCE vapors.

With respect to dermal penetration of liquid TCE, Nakai et al. (1999) used surgically removed skin samples exposed to TCE in aqueous solution in a chamber designed to measure the difference between incoming and outgoing [^{14}C]-TCE. The in vitro permeability constant calculated by these researchers averaged 0.12 cm/hour. In vivo, Sato and Nakajima (1978) exposed adult male volunteers dermally to liquid TCE for 30 minutes, with exhaled TCE appearing at the initial sampling time of 5 minutes after start of exposure, with a maximum observed at 15 minutes. In Kezic et al. (2001), volunteers were exposed dermally for 3 minutes to neat liquid TCE, with TCE detected in exhaled breath at the first sampling point of 3 minutes, and maximal concentrations observed at 5 minutes. Skin irritancy was reported in all subjects, which may have increased absorption. A dermal flux of 430 ± 295 (mean ± standard error [SE]) nmol/cm^2/minute was reported in these subjects, suggesting high interindividual variability.

Another species where dermal absorption for TCE has been reported is in guinea pigs. Jakobson et al. (1982) applied liquid TCE to the shaved backs of guinea pigs and reported peak blood TCE levels at 20 minutes after initiation of exposure. Bogen et al. (1992) estimated permeability constants for dermal absorption of TCE in hairless guinea pigs of 0.16–0.47 mL/cm^2/hour across a range of concentrations (19–100,000 ppm).

3.2. DISTRIBUTION AND BODY BURDEN

TCE crosses biological membranes and quickly results in rapid systemic distribution to tissues—regardless of the route of exposure. In humans, in vivo studies of tissue distribution are limited to tissues taken from autopsies following accidental poisonings or from surgical patients exposed environmentally, so the level of exposure is typically unknown. Tissue levels reported

after autopsy show wide systemic distribution across all tested tissues, including the brain, muscle, heart, kidney, lung, and liver (Coopman et al., 2003; Dehon et al., 2000; De Baere et al., 1997; Ford et al., 1995). However, the reported levels themselves are difficult to interpret because of the high exposures and differences in sampling protocols. In addition, human populations exposed environmentally show detectable levels of TCE across different tissues, including the liver, brain, kidney, and adipose tissues (Kroneld, 1989; Pellizzari et al., 1982; McConnell et al., 1975).

In addition, TCE vapors have been shown to cross the human placenta during childbirth (Laham, 1970), with experiments in rats confirming this finding (Withey and Karpinski, 1985). In particular, Laham (1970) reported determinations of TCE concentrations in maternal and fetal blood following administration of TCE vapors (concentration unreported) intermittently and at birth (see Table 3-6). TCE was present in all samples of fetal blood, with ratios of concentrations in fetal:maternal blood ranging from approximately 0.5 to approximately 2. The concentration ratio was <1.0 in six pairs, >1 in three pairs, and approximately 1 in one pair; in general, higher ratios were observed at maternal concentrations <2.25 mg/100 mL. Because no details of exposure concentration, duration, or time postexposure were given for samples taken, these results are not suitable for use in PBPK modeling, but they do demonstrate the placental transfer of TCE in humans. Withey and Karpinski (1985) exposed pregnant rats to TCE vapors (302, 1,040, 1,559, or 2,088 ppm for 5 hours) on gestation day (GD) 17 and concentrations of TCE in maternal and fetal blood were determined. At all concentrations, TCE concentration in fetal blood was approximately one-third of the concentration in corresponding maternal blood. Maternal blood concentrations approximated 15, 60, 80, and 110 µg/g blood. When the position along the uterine horn was examined, TCE concentrations in fetal blood decreased toward the tip of the uterine horn. TCE appears to also distribute to mammary tissues and is excreted in milk. Pellizzari et al. (1982) conducted a survey of environmental contaminants in human milk using samples from cities in the northeastern region of the United States and one in the southern region. No details of times postpartum, milk lipid content, or TCE concentration in milk or blood were reported, but TCE was detected in 8 milk samples taken from 42 lactating women. Fisher et al. (1990) exposed lactating rats to 600 ppm TCE for 4 hours and collected milk immediately following the cessation of exposure. TCE was clearly detectable in milk, and, from a visual interpretation of the graphic display of their results, concentrations of TCE in milk approximated 110 µg/mL milk.

Table 3-6. Concentrations of TCE in maternal and fetal blood at birth

TCE concentration in blood (mg/100 mL)		Ratio of concentrations fetal:maternal
Maternal	Fetal	
4.6	2.4	0.52
3.8	2.2	0.58
8	5	0.63
5.4	3.6	0.67
7.6	5.2	0.68
3.8	3.3	0.87
2	1.9	0.95
2.25	3	1.33
0.67	1	1.49
1.05	2	1.90

Source: Laham (1970).

In rodents, detailed tissue distribution experiments have been performed using different routes of administration (Keys et al., 2003; Simmons et al., 2002; Greenberg et al., 1999; Abbas and Fisher, 1997; Pfaffenberger et al., 1980; Savolainen et al., 1977). Savolainen et al. (1977) exposed adult male rats to 200 ppm TCE for 6 hours/day for a total of 5 days. Concentrations of TCE in the blood, brain, liver, lung, and perirenal fat were measured 17 hours after cessation of exposure on the fourth day and after 2, 3, 4, and 6 hours of exposure on the fifth day (see Table 3-7). TCE appeared to be rapidly absorbed into blood and distributed to brain, liver, lungs, and perirenal fat. TCE concentrations in these tissues reached near-maximal values within 2 hours of initiation of exposure on the fifth day. Pfaffenberger et al. (1980) dosed rats by gavage with 1 or 10 mg TCE/kg/day in corn oil for 25 days to evaluate the distribution from serum to adipose tissue. During the exposure period, concentrations of TCE in serum were below the limit of detection (1 µg/L) and were 280 and 20,000 ng/g fat in the 1 and 10 mg/day dose groups, respectively. Abbas and Fisher (1997) and Greenberg et al. (1999) measured tissue concentrations in the liver, lung, kidney, and fat of mice administered TCE by gavage (300–2,000 mg/kg) and by inhalation exposure (100 or 600 ppm for 4 hours). In a study to investigate the effects of TCE on neurological function, Simmons et al. (2002) conducted pharmacokinetic experiments in rats exposed to 200, 2,000, or 4,000 ppm TCE vapors for 1 hour. Time-course data were collected on blood, liver, brain, and fat. The data were used to develop a PBPK model to explore the relationship between internal dose and neurological effect. Keys et al. (2003), exposed groups of rats to TCE vapors of 50 or 500 ppm for 2 hours and sacrificed at different time points during exposure. In addition to inhalation, this study also includes gavage and intra-arterial (i.a.) dosing, with the following time course measured: liver, fat, muscle, blood, GI, brain, kidney, heart, lung, and spleen. These pharmacokinetic data were presented with an updated PBPK model for all routes.

Table 3-7. Distribution of TCE to rat tissues[a] following inhalation exposure

Exposure on 5th d	Tissue (concentration in nmol/g tissue)					
	Cerebrum	Cerebellum	Lung	Liver	Perirenal fat	Blood
0[b]	0	0	0.08	0.04	0.23 ± 0.09	0.35 ± 0.1
2	9.9 ± 2.7	11.7 ± 4.2	4.9 ± 0.3	3.6	65.9 ± 1.2	7.5 ± 1.6
3	7.3 ± 2.2	8.8 ± 2.1	5.5 ± 1.4	5.5 ± 1.7	69.3 ± 3.3	6.6 ± 0.9
4	7.2 ± 1.7	7.6 ± 0.5	5.8 ± 1.1	2.5 ± 1.4	69.5 ± 6.3	6.0 ± 0.2
6	7.4 ± 2.1	9.5 ± 2.5	5.6 ± 0.5	2.4 ± 0.2	75.4 ± 14.9	6.8 ± 1.2

[a]Data presented as mean of two determinations ± range.
[b]Sample taken 17 hours following cessation of exposure on day 4.

Source: Savolainen et al. (1977).

Besides the route of administration, another important factor contributing to body distribution is the individual solubility of the chemical in each organ, as measured by a partition coefficient. For volatile compounds, partition coefficients are measured in vitro using the vial equilibration technique to determine the ratio of concentrations between organ and air at equilibrium. Table 3-8 reports values developed by several investigators from mouse, rat, and human tissues. In humans, partition coefficients in the following tissues have been measured: brain, fat, kidney, liver, lung, and muscle; the organ having the highest TCE partition coefficient is fat (63–70), while the lowest is the lung (0.5–1.7). The adipose tissue also has the highest measured value in rodents, and is one of the considerations needed to be accounted for when extrapolating across species. However, the rat adipose partition coefficient value is smaller (23–36), when compared to humans (i.e., TCE is less lipophilic in rats than humans). For the mouse, the measured fat partition coefficient averages 36, ranging between rats and humans. The value of the partition coefficient plays a role in distribution for each organ and is computationally described in computer simulations using a PBPK model. Due to its high lipophilicity in fat, as compared to blood, the adipose tissue behaves as a storage compartment for this chemical, affecting the slower component of the chemical‘s distribution. For example Monster et al. (1979a) reported that, following repeated inhalation exposures to TCE, TCE concentrations in expired breath postexposure were highest for the subject with the greatest amount of adipose tissue (adipose tissue mass ranged 3.5-fold among subjects). The intersubject range in TCE concentration in exhaled breath increased from approximately 2-fold at 20 hours to approximately 10-fold 140 hours postexposure. Notably, they reported that this difference was not due to differences in uptake, as body weight and lean body mass were most closely associated with TCE retention. Thus, adipose tissue may play an important role in postexposure distribution, but does not affect its rapid absorption.

Table 3-8. Tissue:blood partition coefficient values for TCE

Species/ tissue	TCE partition coefficient		References
	Tissue:blood	Tissue:air	
Human			
Brain	2.62	21.2	Fiserova-Bergerova et al. (1984)
Fat	63.8–70.2	583–674.4	Sato et al. (1977); Fiserova-Bergerova et al. (1984); Fisher et al. (1998)
Kidney	1.3–1.8	12–14.7	Fiserova-Bergerova et al. (1984); Fisher et al. (1998)
Liver	3.6–5.9	29.4–54	Fiserova-Bergerova et al. (1984); Fisher et al. (1998)
Lung	0.48–1.7	4.4–13.6	Fiserova-Bergerova et al. (1984); Fisher et al. (1998)
Muscle	1.7–2.4	15.3–19.2	Fiserova-Bergerova et al. (1984); Fisher et al. (1998)
Rat			
Brain	0.71–1.29	14.6–33.3	Sato et al. (1977); Simmons et al. (2002); Rodriguez et al. (2007)
Fat	22.7–36.1	447–661	Gargas et al. (1989); Sato et al. (1977); Simmons et al. (2002); Rodriguez et al. (2007); Fisher et al. (1989); Koizumi (1989); Barton et al. (1995)
Heart	1.1	28.4	Sato et al. (1977)
Kidney	1.0–1.55	17.7–40	Sato et al., (1977); Barton et al., (1995); Rodriguez et al., (2007)
Liver	1.03–2.43	20.5–62.7	Gargas et al. (1989); Sato et al. (1977); Simmons et al. (2002); Rodriguez et al. (2007); Fisher et al. (1989); Koizumi, (1989); Barton et al. (1995)
Lung	1.03	26.6	Sato et al. (1977)
Muscle	0.46–0.84	6.9–21.6	Gargas et al. (1989); Sato et al. (1977); Simmons et al. (2002); Rodriguez et al. (2007); Fisher et al. (1989); Koizumi, (1989); Barton et al. (1995)
Spleen	1.15	29.7	Sato et al. (1977)
Testis	0.71	18.3	Sato et al. (1977)
Milk	7.10	Not reported	Fisher et al. (1990)
Mouse			
Fat	36.4	578.8	Abbas and Fisher (1997)
Kidney	2.1	32.9	Abbas and Fisher (1997)
Liver	1.62	23.2	Fisher et al. (1991)
Lung	2.6	41.5	Abbas and Fisher (1997)
Muscle	2.36	37.5	Abbas and Fisher (1997)

Mahle et al. (2007) reported age-dependent differences in partition coefficients in rats, (see Table 3-9) that can have implications as to life-stage-dependent differences in tissue TCE distribution. To investigate the potential impact of these differences, Rodriguez et al. (2007) developed models for the postnatal day (PND) 10 rat pup; the adult and the aged rat, including age-specific tissue volumes and blood flows; and age-scaled metabolic constants. The models predict similar uptake profiles for the adult and the aged rat during a 6-hour exposure to 500 ppm; uptake by the PND 10 rat was higher (see Table 3-10). The effect was heavily dependent on age-dependent changes in anatomical and physiological parameters (alveolar

ventilation rates and metabolic rates); age-dependent differences in partition coefficient values had minimal impact on predicted differences in uptake.

Table 3-9. Age-dependence of tissue:air partition coefficients in rats

Age[a]	Liver	Kidney	Fat	Muscle	Brain
PND 10 male	22.1 ± 2.3[b]	15.2 ± 1.3	398.7 ± 89.2	43.9 ± 11.0	11.0 ± 0.6
PND 10 female	21.2 ± 1.7	15.0 ± 1.1	424.5 ± 67.5	48.6 ± 17.3	11.6 ± 1.2
Adult male	20.5 ± 4.0	17.6 ± 3.9[c]	631.4 ± 43.1[c]	12.6 ± 4.3	17.4 ± 2.6
Aged male	34.8 ± 8.7[c,d]	19.9 ± 3.4[c]	757.5 ± 48.3[c,d]	26.4 ± 10.3[c,d]	25.0 ± 2.0[c,d]

[a]n = 10, adult male and pooled male and female litters; n = 11, aged males.
[b]Data are mean ± SD.
[c]Statistically significant ($p \leq 0.05$) difference between either the adult or aged partition coefficient and the PND 10 male partition coefficient.
[d]Statistically significant ($p \leq 0.05$) difference between aged and adult partition coefficient.

Source: Mahle et al. (2007).

Table 3-10. Predicted maximal concentrations of TCE in rat blood following a 6-hour inhalation exposure

	Exposure concentration					
	50 ppm			500 ppm		
	Predicted peak concentration (mg/L) in:[a]		Predicted time to reach 90% of steady state (hr)[b]	Predicted peak concentration (mg/L) in:[a]		Predicted time to reach 90% of steady state (hr)[b]
Age	Venous blood	Brain		Venous blood	Brain	
PND 10	3.0	2.6	4.1	33	28	4.2
Adult	0.8	1.0	3.5	22	23	11.9
Aged	0.8	1.2	6.7	21	26	23.3

[a]During a 6-hour exposure.
[b]Under continuous exposure.

Source: Rodriguez et al. (2007).

Finally, TCE binding to tissues or cellular components within tissues can affect overall pharmacokinetics. The binding of a chemical to plasma proteins, for example, affects the availability of the chemical to other organs and the calculation of the total half-life. However, most studies have evaluated binding using [^{14}C]-TCE, from which one cannot distinguish covalent binding of TCE from that of TCE metabolites. Nonetheless, several studies have demonstrated binding of TCE-derived radiolabel to cellular components (Mazzullo et al., 1992; Moslen et al., 1977). Bolt and Filser (1977) examined the total amount irreversibly bound to tissues following 9-, 100-, and 1,000-ppm exposures via inhalation in closed-chambers. The largest percent of in vivo radioactivity taken up occurred in the liver; albumin is the protein

favored for binding (see Table 3-11). Banerjee and van Duuren (1978) evaluated the in vitro binding of TCE to microsomal proteins from the liver, lung, kidney, and stomachs in rats and mice. In both rats and mice, radioactivity was similar in stomach and lung, but about 30% lower in kidney and liver.

Table 3-11. Tissue distribution of TCE metabolites following inhalation exposure

Tissue[a]	Percent of radioactivity taken up/g tissue					
	TCE = 9 ppm, n = 4[b]		TCE = 100 ppm, n = 4		TCE = 1,000 ppm, n = 3	
	Total metabolites	Irreversibly bound	Total metabolites	Irreversibly bound	Total metabolites	Irreversibly bound
Lung	0.23 ± 0.026[c]	0.06 ± 0.002	0.24 ± 0.025	0.06 ± 0.006	0.22 ± 0.055	0.1 ± 0.003
Liver	0.77 ± 0.059	0.28 ± 0.027	0.68 ± 0.073	0.27 ± 0.019	0.88 ± 0.046	0.48 ± 0.020
Spleen	0.14 ± 0.015	0.05 ± 0.002	0.15 ± 0.001	0.05 ± 0.004	0.15 ± 0.006	0.08 ± 0.003
Kidney	0.37 ± 0.005	0.09 ± 0.007	0.40 ± 0.029	0.09 ± 0.007	0.39 ± 0.045	0.14 ± 0.016
Small intestine	0.41 ± 0.058	0.05 ± 0.010	0.38 ± 0.062	0.07 ± 0.008	0.28 ± 0.015	0.09 ± 0.015
Muscle	0.11 ± 0.005	0.014 ± 0.001	0.11 ± 0.013	0.012 ± 0.001	0.10 ± 0.011	0.027 ± 0.003

[a]Male Wistar rats, 250 g.
[b]n = number of animals.
[c]Values shown are means ± SD.

Source: Bolt and Filser (1977).

Based on studies of the effects of metabolizing enzyme induction on binding, there is some evidence that a major contributor to the observed binding is from TCE metabolites rather than from TCE itself. Dekant et al. (1986b) studied the effect of enzyme modulation on the binding of radiolabel from [^{14}C]-TCE by comparing tissue binding after administration of 200 mg/kg via gavage in corn oil between control (naïve) rats and rats pretreated with phenobarbital (a known inducer of CYP2B family) or Aroclor 1254 (a known inducer of both CYP1A and CYP2B families of isoenzymes) (see Table 3-12). The results indicate that induction of total CYP content by 3–4-fold resulted in nearly 10-fold increase in radioactivity (disintegrations per minute; [DPM]) bound in liver and kidney. By contrast, Mazzullo et al. (1992) reported that phenobarbital pretreatment did not result in consistent or marked alterations of in vivo binding of radiolabel to deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein in rats and mice at 22 hours after an intraperitoneal (i.p.) injection of [^{14}C]-TCE. On the other hand, in vitro experiments by Mazzullo et al. (1992) reported reduction of TCE-radiolabel binding to calf thymus DNA with introduction of a CYP inhibitor into incubations containing rat liver microsomal protein. Moreover, increase/decrease of GSH levels in incubations containing lung cytosolic protein led to a parallel increase/decrease in TCE-radiolabel binding to calf thymus DNA.

Table 3-12. Binding of [^{14}C] from [^{14}C]-TCE in rat liver and kidney at 72 hours after oral administration of 200 mg/kg [^{14}C]-TCE

Tissue	DPM/g tissue		
	Untreated	Phenobarbital	Arochlor 1254
Liver	850 ± 100	9,300 ± 1,100	8,700 ± 1,000
Kidney	680 ± 100	5,700 ± 900	7,300 ± 800

Source: Dekant et al. (1986b).

3.3. METABOLISM

This section focuses on both in vivo and in vitro studies of the biotransformation of TCE, identifying metabolites that are deemed significant for assessing toxicity and carcinogenicity. In addition, metabolism studies may be used to evaluate the flux of parent compound through the known metabolic pathways. Sex-, species-, and interindividual differences in the metabolism of TCE are discussed, as are factors that possibly contribute to this variability. Additional discussion of variability and susceptibility is presented in Section 4.10.

3.3.1. Introduction

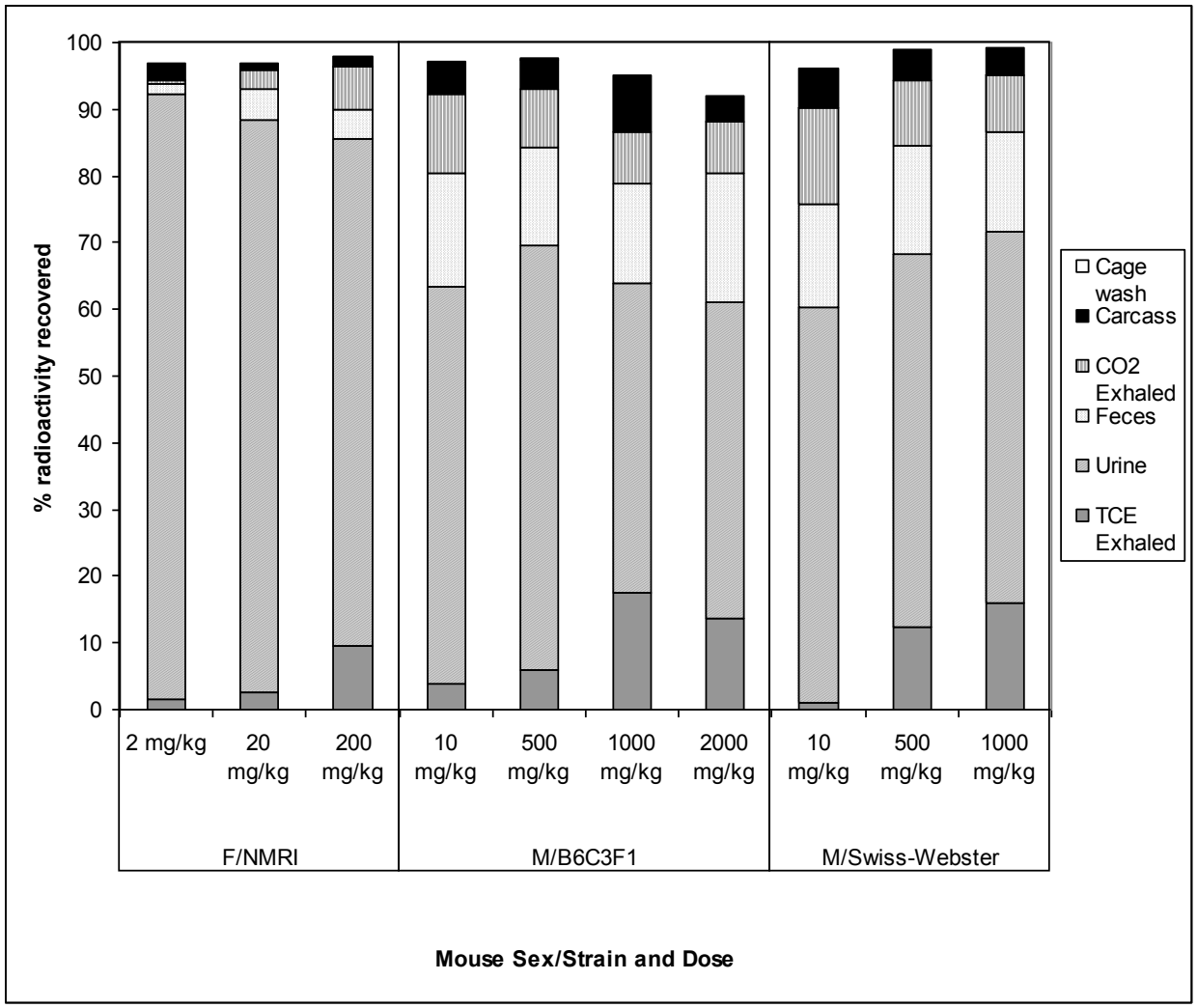
The metabolism of TCE has been studied mostly in mice, rats, and humans and has been extensively reviewed (Lash et al., 2000a; Lash et al., 2000b; IARC, 1995b; US EPA, 1985). It is now well accepted that TCE is metabolized in laboratory animals and in humans through at least two distinct pathways: (1) oxidative metabolism via the CYP mixed-function oxidase system and (2) GSH conjugation followed by subsequent further biotransformation and processing, either through the cysteine conjugate beta lyase pathway or by other enzymes (Lash et al., 2000a; Lash et al., 2000b). While the flux through the conjugative pathway is less, quantitatively, than the flux through oxidation (Bloemen et al., 2001), GSH conjugation is an important route toxicologically, giving rise to relatively potent toxic biotransformation products (Elfarra et al., 1987; Elfarra et al., 1986).

Information about metabolism is important because, as discussed extensively in Chapter 4, certain metabolites are thought to cause one or more of the same acute and chronic toxic effects, including carcinogenicity, as TCE. Thus, in many of these cases, the toxicity of TCE is generally considered to reside primarily in its metabolites rather than in the parent compound itself.

3.3.2. Extent of Metabolism

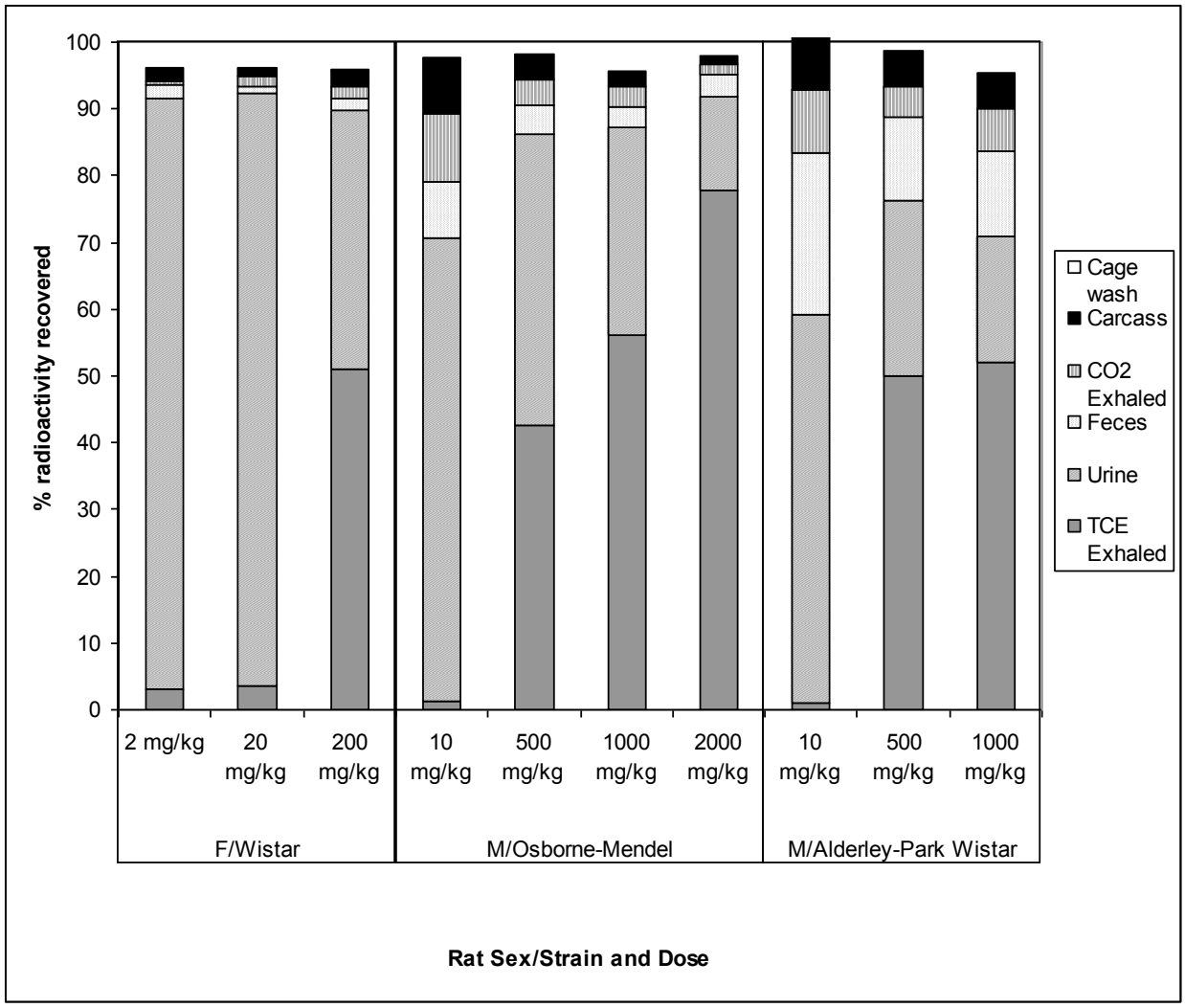
TCE is extensively metabolized in animals and humans. The most comprehensive mass-balance studies are in mice and rats (Dekant et al., 1986a; Dekant et al., 1986b; Green and Prout, 1985; Prout et al., 1985; Dekant et al., 1984) in which [^{14}C]-TCE is administered by gavage at

doses of 2–2,000 mg/kg, the data from which are summarized in Figures 3-2 and 3-3. In both mice and rats, regardless of sex and strain, there is a general trend of increasing exhalation of unchanged TCE with dose, suggesting saturation of a metabolic pathway. The increase is smaller in mice (from 1–6 to 10–18%) than in rats (from 1–3 to 43–78%), suggesting greater overall metabolic capacity in mice. The dose at which apparent saturation occurs appears to be more sex- or strain-dependent in mice than in rats. In particular, the marked increase in exhaled TCE occurred between 20 and 200 mg/kg in female NMRI mice, between 500 and 1,000 mg/kg in $B6C3F_1$ mice, and between 10 and 500 mg/kg in male Swiss-Webster mice. However, because only one study is available in each strain, interlot or interindividual variability might also contribute to the observed differences. In rats, all three strains tested showed marked increase in unchanged TCE exhaled between 20 and 200 mg/kg or between 10 and 500 mg/kg. Recovered urine, the other major source of excretion, had mainly TCA, TCOH, and trichloroethanol-glucuronide conjugate (TCOG), but revealed no detectable TCE. The source of radioactivity in feces was not analyzed, but it is presumed not to include substantial TCE given the complete absorption expected from the corn oil vehicle. Therefore, at all doses tested in mice, and at doses <200 mg/kg in rats, the majority of orally administered TCE is metabolized. Pretreatment of rats with P450 inducers prior to a 200 mg/kg dose did not change the pattern of recovery, but it did increase the amount recovered in urine by 10–15%, with a corresponding decrease in the amount of exhaled unchanged TCE (Dekant et al., 1986b).



Sources: Dekant et al. (1986b; 1984); Green and Prout (1985); Prout et al. (1985).

Figure 3-2. Disposition of [^{14}C]-TCE administered by gavage in mice.



Sources: Dekant et al. (1986b; 1984); Green and Prout (1985); Prout et al. (1985).

Figure 3-3. Disposition of [^{14}C]-TCE administered by gavage in rats.

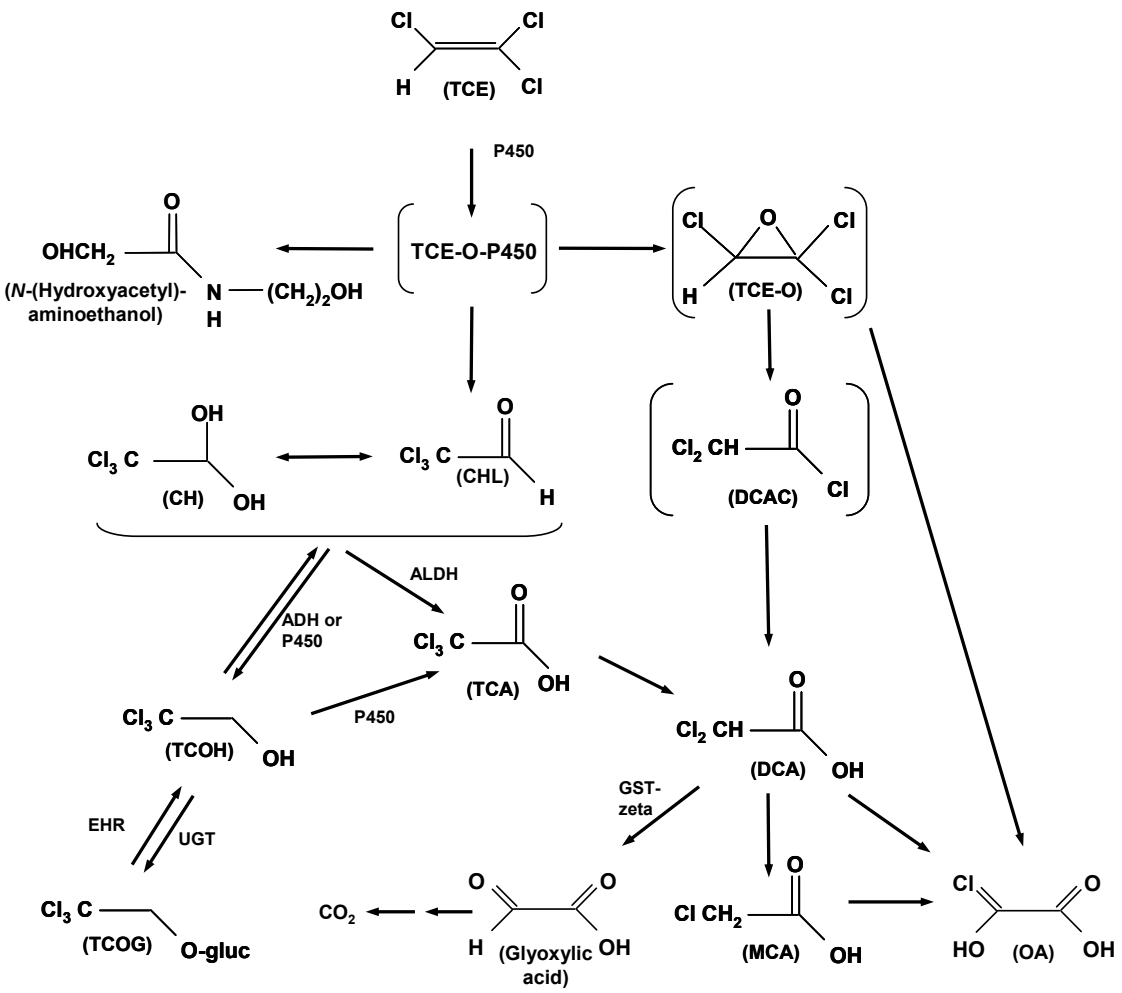
The differences among these studies may reflect a combination of interindividual variability and errors due to the difficulty in precisely estimating dose in inhalation studies, but in all cases, <20% of the retained dose was exhaled unchanged and >50% was excreted in urine as TCA and TCOH. Therefore, it is clear that TCE is extensively metabolized in humans. No saturation was evident in any of these human recovery studies at the exposure levels tested.

3.3.3. Pathways of Metabolism

As mentioned in Section 3.3.1, TCE metabolism in animals and humans has been observed to occur via two major pathways: P450-mediated oxidation and GSH conjugation. Products of the initial oxidation or conjugation step are further metabolized to a number of other metabolites. For P450 oxidation, all steps of metabolism occur primarily in the liver, although limited oxidation of TCE has been observed in the lungs of mice, as discussed below. The GSH conjugation pathway also begins predominantly in the liver, but toxicologically significant metabolic steps occur extrahepatically—particularly in the kidney (Lash et al., 2006; Lash et al., 1999a; Lash et al., 1998b; Lash et al., 1995). The mass-balance studies cited above found that at exposures below the onset of saturation, >50% of TCE intake is excreted in urine as oxidative metabolites (primarily as TCA and TCOH), so TCE oxidation is generally greater than TCE conjugation. This is discussed in detail in Section 3.3.3.3.

3.3.3.1. CYP-Dependent Oxidation

Oxidative metabolism by the CYP, or CYP-dependent, pathway is quantitatively the major route of TCE biotransformation (Lash et al., 2000a; Lash et al., 2000b; US EPA, 1985). The pathway is operative in humans and rodents and leads to several metabolic products, some of which are known to cause toxicity and carcinogenicity (IARC, 1995c; US EPA, 1985). Although several of the metabolites in this pathway have been clearly identified, others are speculative or questionable. Figure 3-4 depicts the overall scheme of TCE P450 metabolism.



Adapted from: Clewell et al. (2000); Cummings et al. (2001); Forkert et al. (2006); Lash et al. (2000a; 2000b); Tong et al. (1998).

Figure 3-4. Scheme for the oxidative metabolism of TCE.

In brief, TCE oxidation via P450, primarily CYP2E1 (Guengerich and Shimada, 1991), yields an oxygenated TCE-P450 intermediate. The TCE-P450 complex is a transition state that goes on to form chloral or TCE oxide. In the presence of water, chloral rapidly equilibrates with chloral hydrate (CH), which undergoes reduction and oxidation by alcohol dehydrogenase and aldehyde dehydrogenase or aldehyde oxidase to form TCOH and TCA, respectively (Dekant et al., 1986b; Green and Prout, 1985; Miller and Guengerich, 1983). TCE oxide can rearrange to DCAC. Table 3-13 summarizes available in vitro measurements of TCE oxidation, as assessed by the formation of CH, TCOH, and TCA. Glucuronidation of TCOH forms TCOG, which is readily excreted in urine. Alternatively, TCOG can be excreted in bile and passed to the small intestine where it is hydrolyzed back to TCOH and reabsorbed (Bull, 2000). TCA is poorly metabolized but may undergo dechlorination to form dichloroacetic acid (DCA). However, TCA is predominantly excreted in urine, albeit at a relatively slow rate as compared to TCOG. Like

the TCE-P450 complex, TCE oxide also seems to be a transient metabolite. Recent data suggest that it is transformed to dichloroactyl chloride, which subsequently decomposes to form DCA (Cai and Guengerich, 1999). As shown in Figure 3-4, several other metabolites, including oxalic acid and *N*-(hydroxyacetyl) aminoethanol, may form from the TCE oxide or the TCE-O-P450 intermediate and have been detected in the urine of rodents and humans following TCE exposure. Pulmonary excretion of CO_2 has been identified in exhaled breath from rodents exposed to [^{14}C]-labeled TCE and is thought to arise from metabolism of DCA. The following sections provide details as to pathways of TCE oxidation, including discussion of inter- and intraspecies differences in metabolism.

Table 3-13. In vitro TCE oxidative metabolism in hepatocytes and microsomal fractions

In vitro system	K_M μM in medium	V_{MAX} nmol TCE oxidized/min/mg MSP or 10^6 hepatocytes	1,000 × V_{MAX}/K_M[a]	Source
Human hepatocytes	210 ± 159[b] (45–403)	0.268 ± 0.215 (0.101–0.691)	2.45 ± 2.28 (0.46–5.57)	Lipscomb et al. (1998b)
Human liver microsomal protein	16.7 ± 2.45 (13.3–19.7)	1.246 ± 0.805 (0.490–3.309)	74.1 ± 44.1 (38.9–176)	Lipscomb et al. (1997) (low K_M)
	30.9 ± 3.3 (27.0–36.3)	1.442 ± 0.464 (0.890–2.353)	47.0 ± 16.0 (30.1–81.4)	Lipscomb et al. (1997) (mid K_M)
	51.1 ± 3.77 (46.7–55.7)	2.773 ± 0.577 (2.078–3.455)	54.9 ± 14.1 (37.3–69.1)	Lipscomb et al. (1997) (high K_M)
	24.6	1.44	58.5	Lipscomb et al. (1998c) (pooled)
	12 ± 3 (9–14)	0.52 ± 0.17 (0.37–0.79)	48 ± 23 (26–79)	Elfarra et al. (1998) (males, high affinity)
	26 ± 17 (13–45)	0.33 ± 0.15 (0.19–0.48)	15 ± 10 (11–29)	Elfarra et al. (1998) (females, high affinity)
Rat liver microsomal protein	55.5	4.826	87.0	Lipscomb et al. (1998c) (pooled)
	72 ± 82	0.96 ± 0.65	24 ± 21	Elfarra et al. (1998) (males, high affinity)
	42 ± 21	2.91 ± 0.71	80 ± 34	Elfarra et al. (1998) (females, high affinity)
Rat kidney microsomal protein	940	0.154	0.164	Cummings et al. (2001)
Mouse liver microsomal protein	35.4	5.425	153	Lipscomb et al. (1998c) (pooled)
	378 ± 414	8.6 ± 4.5	42 ± 29	Elfarra et al. (1998) (males)
	161 ± 29	26.06 ± 7.29	163 ± 37	Elfarra et al. (1998) (females)

[a]K_M for human hepatocytes converted from ppm in headspace to μM in medium using reported hepatocyte:air partition coefficient (Lipscomb et al., 1998b).
[b]Results presented as mean ± SD (minimum–maximum).

MSP = Microsomal protein.

3.3.3.1.1. Formation of TCE oxide

In previous studies of halogenated alkene metabolism, the initial step was the generation of a reactive epoxide (Anders and Jakobson, 1985). Early studies in anesthetized human patients

(Powell, 1945), dogs (Butler, 1949), and later reviews (e.g., Goeptar et al., 1995) suggest that the TCE epoxide may be the initial reaction product of TCE oxidation.

Epoxides can form acyl chlorides or aldehydes, which can then form aldehydes, carboxylic acids, or alcohols, respectively. Thus, earlier studies suggesting the appearance of CH, TCA, and TCOH as the primary metabolites of TCE were considered consistent with the oxidation of TCE to an epoxide intermediate (Butler, 1949; Powell, 1945). Following in vivo exposures to 1,1-DCE, a halocarbon very similar in structure to TCE, mouse liver cytosol and microsomes and lung Clara cells exhibited extensive P450-mediated epoxide formation (Forkert, 1999b; Forkert, 1999a; Forkert et al., 1999; Dowsley et al., 1996). Indeed, TCE oxide inhibits purified CYP2E1 activity (Cai and Guengerich, 2001b) similarly to TCE inhibition of CYP2E1 in human liver microsomes (Lipscomb et al., 1997).

Conversely, cases have been made against TCE oxide as an obligate intermediate to the formation of chloral. Using liver microsomes and reconstituted P450 systems (Miller and Guengerich, 1983, 1982) or isolated rat hepatocytes (Miller and Guengerich, 1983), it has been suggested that chlorine migration and generation of a TCE-O-P450 complex (via the heme oxygen) would better explain the observed destruction of the P450 heme, an outcome not likely to be epoxide-mediated. Miller and Guengerich (1982) found CYP2E1 to generate an epoxide but argued that the subsequent production of chloral was not likely related to the epoxide. Green and Prout (1985) argued against epoxide (free form) formation in vivo in mice and rats, suggesting that the expected predominant metabolites would be carbon monoxide, CO_2, MCA, and DCA, rather than the observed predominant appearance of TCA, TCOH, and TCOG.

It appears likely that both a TCE-O-P450 complex and a TCE oxide are formed, resulting in both CH and DCAC, respectively, though it appears that the former predominates. In particular, it has been shown that DCAC can be generated from TCE oxide, dichloracetyl chloride can be trapped with lysine (Cai and Guengerich, 1999), and dichloracetyl-lysine adducts are formed in vivo (Forkert et al., 2006). Together, these data strongly suggest TCE oxide as an intermediate metabolite, albeit short-lived, from TCE oxidation in vivo.

3.3.3.1.2. Formation of CH, TCOH and TCA

CH (in equilibrium with chloral) is a major oxidative metabolite produced from TCE as has been shown in numerous in vitro systems, including human liver microsomes and purified P450 CYP2E1 (Guengerich et al., 1991) as well as recombinant rat, mouse, and human P450s including CYP2E1 (Forkert et al., 2005). However, in rats and humans, in vivo circulating CH is generally absent from blood following TCE exposure. In mice, CH is detectable in blood and tissues but is rapidly cleared from systemic circulation (Abbas and Fisher, 1997). The low systemic levels of CH are due to its rapid transformation to other metabolites.

CH is further metabolized predominantly to TCOH (Shultz and Weiner, 1979; Sellers et al., 1972) and/or CYP2E1 (Ni et al., 1996). The role for alcohol dehydrogenase was suggested

by the observation that ethanol inhibited CH reduction to TCOH (Larson and Bull, 1989; Muller et al., 1975; Sellers et al., 1972). For instance, Sellers et al. (1972) reported that co-exposure of humans to ethanol and CH resulted in a higher percentage of urinary TCOH (24% of CH metabolites) compared to TCA (19%). When ethanol was absent, 10 and 11% of CH was metabolized to TCOH and TCA, respectively. However, because ethanol can be oxidized by both alcohol dehydrogenase and CYP2E1, there is some ambiguity as to whether these observations involve competition with one or the other of these enzymes. For instance, Ni et al. (1996) reported that CYP2E1 expression was necessary for metabolism of CH to mutagenic metabolites in a human lymphoblastoid cell line, suggesting a role for CYP2E1. Furthermore, Ni et al. (1996) reported that cotreatment of mice with CH and pyrazole, a specific CYP2E1 inducer, resulted in enhanced liver microsomal lipid peroxidation, while treatment with 2,4-dichloro-6-phenoxyethylamine, an inhibitor of CYP2E1, suppressed lipid peroxidation, suggesting CYP2E1 as a primary enzyme for CH metabolism in this system. Lipscomb et al. (1996) suggested that two enzymes are likely responsible for CH reduction to TCOH based on observation of biphasic metabolism for this pathway in mouse liver microsomes. This behavior has also been observed in mouse liver cytosol, but was not observed in rat or human liver microsomes. Moreover, CH metabolism to TCOH increased significantly both in the presence of nicotinamide adenine dinucleotide (NADH) in the 700 × g supernatant of mouse, rat, and human liver homogenate as well as with the addition of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) in human samples, suggesting that two enzymes may be involved (Lipscomb et al., 1996).

TCOH formed from CH is available for oxidation to TCA (see below) or glucuronidation via uridine 5‘-diphospho-glucuronyltransferase to TCOG, which is excreted in urine or in bile (Stenner et al., 1997). Biliary TCOG is hydrolyzed in the gut and available for reabsorption to the liver as TCOH, where it can be glucuronidated again or metabolized to TCA. This enterohepatic circulation appears to play a significant role in the generation of TCA from TCOH and in the observed lengthy residence time of this metabolite, compared to TCE. Using jugular-, duodenal-, and bile duct-cannulated rats, Stenner et al. (1997) showed that enterohepatic circulation of TCOH from the gut back to the liver and subsequent oxidation to TCA was responsible for 76% of TCA measured in the systemic blood.

Oxidation of CH and TCOH to TCA has been demonstrated in vivo in mice (Larson and Bull, 1992a; Dekant et al., 1986b; Green and Prout, 1985), rats (Stenner et al., 1997; Pravecek et al., 1996; Templin et al., 1995b; Larson and Bull, 1992a; Dekant et al., 1986b; Green and Prout, 1985), dogs (Templin et al., 1995b), and humans (Sellers et al., 1978). Urinary metabolite data in mice and rats exposed to 200 mg/kg TCE (Larson and Bull, 1992a; Dekant et al., 1986b); and humans following oral CH exposure (Sellers et al., 1978) show greater TCOH production relative to TCA production. However, because of the much longer urinary half-life in humans of TCA relative to TCOH, the total amount of TCA excreted may be similar to TCOH (Fisher et al.,

1998; Monster et al., 1976). This is thought to be primarily due to conversion of TCOH to TCA, either directly or via "back-conversion" of TCOH to CH, rather than due to the initial formation of TCA from CH (Owens and Marshall, 1955).

In vitro data are also consistent with CH oxidation to TCA being much less than CH reduction to TCOH. For instance, Lipscomb et al. (1996) reported 1,832-fold differences in K_M values and 10–195-fold differences in clearance efficiency (V_{MAX}/K_M) for TCOH and TCA in all three species (see Table 3-14). Clearance efficiency of CH to TCA in mice is very similar to humans but is 13-fold higher than rats. Interestingly, Bronley-DeLancey et al. (2006) recently reported that similar amounts of TCOH and TCA were generated from CH using cryopreserved human hepatocytes. However, the intersample variation was extremely high, with measured V_{MAX} ranging from 8-fold greater TCOH to 5-fold greater TCA and clearance (V_{MAX}/K_M) ranging from 13-fold greater TCOH to 17-fold greater TCA. Moreover, because a comparison with fresh hepatocytes or microsomal protein was not made, it is not clear to what extent these differences are due to population heterogeneity or experimental procedures.

Table 3-14. In vitro kinetics of TCOH and TCA formation from CH in rat, mouse, and human liver homogenates

Species	TCOH			TCA		
	K_M[a]	V_{MAX}[b]	V_{MAX}/K_M[c]	K_M[a]	V_{MAX}[b]	V_{MAX}/K_M[c]
Rat	0.52	24.3	46.7	16.4	4	0.24
Mouse[d]	0.19	11.3	59.5	3.5	10.6	3.0
High affinity	0.12	6.3	52.5	Not applicable	Not applicable	Not applicable
Low affinity	0.51	6.1	12.0	Not applicable	Not applicable	Not applicable
Human	1.34	34.7	25.9	23.9	65.2	2.7

[a]K_M presented as mM CH in solution.
[b]V_{MAX} presented as nmoles/mg supernatant protein/minute.
[c]Clearance efficiency represented by V_{MAX}/K_M.
[d]Mouse kinetic parameters derived for observations over the entire range of CH exposure as well as discrete, bi-phasic regions for CH concentrations below (high affinity) and above (low affinity) 1.0 mM.

Source: Lipscomb et al. (1996).

The metabolism of CH to TCA and TCOH involves several enzymes including CYP2E1, alcohol dehydrogenase, and aldehyde dehydrogenase enzymes (Ni et al., 1996; Wang et al., 1993; Guengerich et al., 1991; Miller and Guengerich, 1983; Shultz and Weiner, 1979). Because these enzymes have preferred cofactors (NADPH, NADH, and NAD^+), cellular cofactor ratio and redox status of the liver may have an impact on the preferred pathway (Lipscomb et al., 1996; Kawamoto et al., 1988a).

3.3.3.1.3. Formation of DCA and other products

As discussed above, DCA could hypothetically be formed via multiple pathways. The work reviewed by Guengerich (2004) suggested that one source of DCA may be through a TCE oxide intermediary. Miller and Guengerich (1983) reported evidence of formation of the epoxide, and Cai and Guengerich (1999) reported that a significant amount (about 35%) of DCA is formed from aqueous decomposition of TCE oxide via hydrolysis in an almost pH-independent manner. Because this reaction forming DCA from TCE oxide is a chemical process rather than a process mediated by enzymes, and because evidence suggests that some epoxide was formed from TCE oxidation, Guengerich (2004) notes that DCA would be an expected product of TCE oxidation (see also Yoshioka et al., 2002). Alternatively, dechlorination of TCA and oxidation of TCOH have been proposed as sources of DCA (Lash et al., 2000a). Merdink et al. (2000) investigated dechlorination of TCA and reported trapping a DCA radical with the spin-trapping agent phenyl-tert-butyl nitroxide, identified by gas chromatography/mass spectroscopy, in both a chemical Fenton system and rodent microsomal incubations with TCA as substrate. Dose-dependent catalysis of TCA to DCA was observed in cultured microflora from B6C3F$_1$ mice (Moghaddam et al., 1996). However, while antibiotic-treated mice lost the ability to produce DCA in the gut, plasma DCA levels were unaffected by antibiotic treatment, suggesting that the primary site of murine DCA production is other than the gut (Moghaddam et al., 1997).

However, direct evidence for DCA formation from TCE exposure remains equivocal. In vitro studies in human and animal systems have demonstrated very little DCA production in the liver (James et al., 1997). In vivo, DCA was detected in the blood of mice (Templin et al., 1993; Larson and Bull, 1992a) and humans (Fisher et al., 1998) and in the urine of rats and mice (Larson and Bull, 1992b) exposed to TCE by aqueous gavage. However, the use of strong acids in the analytical methodology produces ex vivo conversion of TCA to DCA in mouse blood (Ketcha et al., 1996). This method may have resulted in the appearance of DCA as an artifact in human plasma (Fisher et al., 1998) and mouse blood in vivo (Templin et al., 1995b). Evidence for the artifact is suggested by DCA AUCs that were larger than would be expected from the available TCA (Templin et al., 1995b). After the discovery of these analytical issues, Merdink et al. (1998) reevaluated the formation of DCA from TCE, TCOH, and TCA in mice, with particular focus on the hypothesis that DCA is formed from dechlorination of TCA. They were unable to detect blood DCA in naive mice after administration of TCE, TCOH, or TCA. Low levels of DCA were detected in the blood of children administered therapeutic doses of CH (Henderson et al., 1997), suggesting TCA or TCOH as the source of DCA. Oral TCE exposure in rats and dogs failed to produce detectable levels of DCA (Templin et al., 1995b).

Another difficulty in assessing the formation of DCA is its rapid metabolism at low exposure levels. Degradation of DCA is mediated by GST-zeta (Saghir and Schultz, 2002; Tong et al., 1998), apparently occurring primarily in the hepatic cytosol. DCA metabolism results in

suicide inhibition of the enzyme, evidenced by decreased DCA metabolism in DCA-treated animals (Gonzalez-Leon et al., 1999) and humans (Shroads et al., 2008) and loss of DCA metabolic activity and enzymatic protein in liver samples from treated animals (Schultz et al., 2002). This effect has been noted in young mice exposed to DCA in drinking water at doses approximating 120 mg/kg-day (Schultz et al., 2002). The experimental data and pharmacokinetic model simulations of several investigators (Li et al., 2008; Shroads et al., 2008; Jia et al., 2006; Keys et al., 2004; Merdink et al., 1998) suggest that several factors prevent the accumulation of measurable amounts of DCA: (1) its formation as a short-lived intermediate metabolite and (2) its rapid elimination relative to its formation from TCA. While DCA elimination rates appear approximately one order of magnitude higher in rats and mice than in humans (James et al., 1997) (see Table 3-15), they still may be rapid enough so that even if DCA were formed in humans, it would be metabolized too quickly to appear in detectable quantities in blood.

Table 3-15. In vitro kinetics of DCA metabolism in hepatic cytosol of mice, rats, and humans

Species	V_{MAX} (nmol/min/mg protein)	K_M (μM)	V_{MAX}/K_M
Mouse	13.1	350	37.4
Rat	11.6	280	41.4
Human	0.37	71	5.2

Source: James et al. (1997).

A number of other metabolites, such as oxalic acid, MCA, glycolic acid, and glyoxylic acid, are formed from DCA (Saghir and Schultz, 2002; Lash et al., 2000a). Unlike other oxidative metabolites of TCE, DCA appears to be metabolized primarily via hepatic cytosolic proteins. Since P450 activity resides almost exclusively in the microsomal and mitochondrial cell fractions, DCA metabolism appears to be independent of P450. Rodent microsomal and mitochondrial metabolism of DCA was measured to be ≤10% of cytosolic metabolism (Lipscomb et al., 1995). DCA in the liver cytosol from rats and humans is transformed to glyoxylic acid via a GSH-dependent pathway (James et al., 1997). In rats, the K_M for GSH was 0.075 mM with a V_{MAX} for glyoxylic acid formation of 1.7 nmol/mg protein/minute. While this pathway may not involve GST (as evidenced by very low GST activity in this study), Tong et al. (1998) showed GST-zeta, purified from rat liver, to be involved in metabolizing DCA to glyoxylic acid, with a V_{MAX} of 1,334 nmol/mg protein/minute and K_M of 71.4 μM for glyoxylic acid formation and a GSH K_M of 59 μM.

3.3.3.1.4. Tissue distribution of oxidative metabolism and metabolites

Oxidative metabolism of TCE, irrespective of the route of administration, occurs predominantly in the liver, but TCE metabolism via the P450 (CYP) system also occurs at other sites because CYP isoforms are present to some degree in most tissues of the body. For example, both the lung and kidneys exhibit CYP enzyme activities (Forkert et al., 2005; Cummings et al., 2001; 1997a; Green et al., 1997b). Green et al. (1997b) detected TCE oxidation to chloral in microsomal fractions of whole-lung homogenates from mice, rats, and humans, with the activity in mice the greatest and in humans the least. The rates were slower than in the liver (which also has a higher microsomal protein content as well as greater tissue mass) by 1.8-, 10-, and >10-fold in mice, rats, and humans, respectively. While qualitatively informative, these rates were determined at a single concentration of about 1 mM TCE. A full kinetic analysis was not performed, so clearance and maximal rates of metabolism could not be determined. With the kidney, Cummings et al. (2001) performed a full kinetic analysis using kidney microsomes and found that clearance rates (V_{MAX}/K_M) for oxidation were >100-fold smaller than average rates found in the liver (see Table 3-13). In human kidney microsomes, Amet et al.(1997) reported that CYP2E1 activity was weak and near detection limits, with no CYP2E1 detectable using immunoblot analysis. Cummings and Lash (2000) reported detecting oxidation of TCE in only one of four kidney microsome samples, and only at the highest tested concentration of 2 mM, with a rate of 0.13 nmol/minute/mg protein. This rate contrasts with the V_{MAX} values for human liver microsomal protein of 0.19–3.5 nmol/minute/mg protein reported in various experiments (see Table 3-13). Extrahepatic oxidation of TCE may play an important role for generation of toxic metabolites in situ. The roles of local metabolism in kidney and lung toxicity are discussed in detail in Sections 4.4 and 4.7, respectively.

With respect to further metabolism beyond oxidation of TCE, CH has been shown to be metabolized to TCA and TCOH in lysed whole blood of mice and rats and fractionated human blood (Lipscomb et al., 1996) (see Table 3-16). TCOH production is similar in mice and rats and is approximately twofold higher in rodents than in human blood. However, TCA formation in human blood is two- or threefold higher than in mouse or rat blood, respectively. In human blood, TCA is formed only in the erythrocytes. TCOH formation occurs in both plasma and erythrocytes, but fourfold more TCOH is found in plasma than in an equal volume of packed erythrocytes. While blood metabolism of CH may contribute further to its low circulating levels in vivo the metabolic capacity of blood (and kidney) may be substantially lower than liver. Regardless, any CH reaching the blood may be rapidly metabolized to TCA and TCOH. DCA and TCA are known to bind to plasma proteins. Schultz et al. (1999) measured DCA binding in rats at a single concentration of about 100 µM and found a binding fraction of <10%. However, these data are not greatly informative for TCE exposure in which DCA levels are significantly lower than 100 µM. In addition, the limitation to a single concentration in this experiment precludes fitting a binding curve, as can be done for TCA with Templin et al. (1995a; 1995b;

1993), Schultz et al. (1999), Lumpkin et al. (2003), and Yu et al. (2003), all of which measured TCA binding in various species and at various concentration ranges. Of these, Templin et al. (1995a; 1995b) and Lumpkin et al. (2003) measured levels in humans, mice, and rats. Lumpkin et al. (2003) studied the widest concentration range, spanning reported TCA plasma concentrations from experimental studies. Table 3-17 shows derived binding parameters. However, these data are not entirely consistent among researchers; two- to fivefold differences in B_{MAX} and K_d are noted in some cases, although some differences existed in the rodent strains and experimental protocols used. In general, however, at lower concentrations, the bound fraction appears greater in humans than in rats and mice. Typical human TCE exposures, even in controlled experiments with volunteers, lead to TCA blood concentrations well below the reported K_d (see Table 3-17, below), so the TCA binding fraction should be relatively constant. However, in rats and mice, experimental exposures may lead to peak concentrations similar to, or above, the reported K_d (e.g., Yu et al., 2000; Templin et al., 1993), meaning that the bound fraction should temporarily decrease following such exposures.

Table 3-16. TCOH and TCA formed from CH in vitro in lysed whole blood of rats and mice or fractionated blood of humans (nmoles formed in 400 µL samples over 30 minutes)

	Rat	Mouse	Human	
			Erythrocytes	Plasma
TCOH	45.4 ± 4.9	46.7 ± 1.0	15.7 ± 1.4	4.48 ± 0.2
TCA	0.14 ± 0.2	0.21 ± 0.3	0.42 ± 0.0	Not detected

Source: Lipscomb et al. (1996).

Table 3-17. Reported TCA plasma binding parameters[a]

	A	B_{MAX} (µM)	K_d (µM)	A+ B_{MAX}/K_d	Concentration range (µM bound+free)
Human					
Templin et al. (1995b)	–	1,020	190	5.37	3–1,224
Lumpkin et al. (2003)	–	708.9	174.6	4.06	0.06–3,065
Rat					
Templin et al. (1995b)	–	540	400	1.35	3–1,224
Yu et al. (2000)	0.602	312	136	2.90	3.8–1,530
Lumpkin et al. (2003)	–	283.3	383.6	0.739	0.06–3,065
Mouse					
Templin et al. (1993)	–	310	248	1.25	3–1,224
Lumpkin et al. (2003)	–	28.7	46.1	0.623	0.06–1,226

[a]Binding parameters based on the equation $C_{bound} = A \times C_{free} + B_{MAX} \times C_{free}/(K_d + C_{free})$, where C_{bound} is the bound concentration, C_{free} is the free concentration, and A = 0 for Templin et al. (1995b; 1993) and Lumpkin et al. (2003). The quantity A+ B_{MAX}/K_d is the ratio of bound-to-free at low concentrations.

Limited data are available on tissue:blood partitioning of the oxidative metabolites CH, TCA, TCOH, and DCA, as shown in Table 3-18. As these chemicals are all water soluble and not lipophilic, it is not surprising that their partition coefficients are close to one (within about twofold). It should be noted that the TCA tissue:blood partition coefficients reported in Table 3-18 were measured at concentrations 1.6–3.3 M, over 1,000-fold higher than the reported K_d. Therefore, these partition coefficients should reflect the equilibrium between tissue and free blood concentrations. In addition, only one in vitro measurement has been reported of blood:plasma concentration ratios for TCA: Schultz et al. (1999) reported a value of 0.76 in rats.

Table 3-18. Partition coefficients for TCE oxidative metabolites

Species/tissue[a]	Tissue:blood partition coefficient			
	CH	TCA	TCOH	DCA
Human[b]				
Kidney	–	0.66	2.15	-
Liver	–	0.66	0.59	-
Lung	–	0.47	0.66	-
Muscle	–	0.52	0.91	-
Mouse[c]				
Kidney	0.98	0.74	1.02	0.74
Liver	1.42	1.18	1.3	1.08
Lung	1.65	0.54	0.78	1.23
Muscle	1.35	0.88	1.11	0.37

[a]TCA and TCOH partition coefficients have not been reported for rats.
[b]Fisher et al. (1998).
[c]Abbas and Fisher (1997).

3.3.3.1.5. Species-, sex-, and age-dependent differences of oxidative metabolism

The ability to describe species- and sex-dependent variations in TCE metabolism is important for species extrapolation of bioassay data and identification of human populations that are particularly susceptible to TCE toxicity. In particular, information on the variation in the initial oxidative step of CH formation from TCE is desirable, because this is the rate-limiting step in the eventual formation and distribution of the putative toxic metabolites TCA and DCA (Lipscomb et al., 1997).

Inter- and intraspecies differences in TCE oxidation have been investigated in vitro using cellular or subcellular fractions, primarily of the liver. The available in vitro metabolism data on TCE oxidation in the liver (see Table 3-13) show substantial inter- and intraspecies variability. Across species, microsomal data show that mice apparently have greater capacity (V_{MAX}) than rat or humans, but the variability within species can be 2–10-fold. Part of the explanation may be related to CYP2E1 content. Although liver P450 content is similar across species, mice and rats exhibit higher levels of CYP2E1 content (0.85 and 0.89 nmol/mg protein, respectively)

(Davis et al., 2002; Nakajima et al., 1993) than humans (approximately 0.25–0.30 nmol/mg protein) (Davis et al., 2002; Elfarra et al., 1998). Thus, the data suggest that rodents would have a higher capacity than humans to metabolize TCE, but this is difficult to verify in vivo because very high exposure concentrations in humans would be necessary to assess the maximum capacity of TCE oxidation.

With respect to the K_M of liver microsomal TCE oxidative metabolism, where K_M is indicative of affinity (the lower the numerical value of K_M, the higher the affinity), the trend appears to be that mice and rats have higher K_M values (i.e., lower affinity) than humans, but with substantial overlap due to interindividual variability. Note that, as shown in Table 3-13, the ranking of rat and mouse liver microsomal K_M values between the two reports by Lipscomb et al. (1998c) and Elfarra et al. (1998) is not consistent. However, both studies clearly show that K_M is the lowest (i.e., affinity is highest) in humans. Because clearance at lower concentrations is determined by the ratio V_{MAX} to K_M, the lower apparent K_M in humans may partially offset the lower human V_{MAX}, and lead to similar oxidative clearances in the liver at environmentally relevant doses. However, differences in activity measured in vitro may not translate into in vivo differences in metabolite production, as the rate of metabolism in vivo depends also on the rate of delivery to the tissue via blood flow (Lipscomb et al., 2003). The interaction of enzyme activity and blood flow is best investigated using PBPK models and is discussed, along with descriptions of in vivo data, in Section 3.5.

Data on sex- and age-dependence in oxidative TCE metabolism are limited but suggest relatively modest differences in humans and animals. In an extensive evaluation of CYP-dependent activities in human liver microsomal protein and cryopreserved hepatocytes, Parkinson et al. (2004) identified no age- or gender-related differences in CYP2E1 activity. In liver microsomes from 23 humans, the K_M values for females was lower than males, but V_{MAX} values were very similar (Lipscomb et al., 1997). Appearance of total trichloro compounds (TTCs) in urine following i.p. dosing with TCE was 28% higher in female rats than in males (Verma and Rana, 2003). The oxidation of TCE in male and female rat liver microsomes was not significantly different; however, pregnancy resulted in a decrease of 27–39% in the rate of CH production in treated microsomes from females (Nakajima et al., 1992b). Formation of CH in liver microsomes in the presence of 0.2 or 5.9 mM TCE exhibited some dependency on age of rats, with formation rates in both sexes of 1.1–1.7 nmol/mg protein/minute in 3-week-old animals and 0.5–1.0 nmol/mg protein/minute in 18-week-old animals (Nakajima et al., 1992b).

Fisher et al. (1991) reviewed data available at that time on urinary metabolites to characterize species differences in the amount of urinary metabolism accounted for by TCA (see Table 3-19). They concluded that TCA seemed to represent a higher percentage of urinary metabolites in primates than in other mammalian species, indicating a greater proportion of oxidation leading ultimately to TCA relative to TCOG.

Table 3-19. Urinary excretion of TCA by various species exposed to TCE (based on data reviewed in (Fisher et al., 1991)

Species[a]	Percentage of urinary excretion of TCA		Dose route	TCE dose (mg TCE/kg)	References
	Male	Female			
Baboon[b,c]	16	–	Intramuscular injection	50	Mueller et al. (1982)
Chimpanzee[b]	24	22	Intramuscular injection	50	Mueller et al. (1982)
Monkey, Rhesus[b,c]	19	–	Intramuscular injection	50	Mueller et al. (1982)
Mice, NMRI[d]	–	8–20	Oral intubation	2–200	Dekant et al. (1986b)
Mice, B6C3F$_1$[b]	7–12	–	Oral intubation	10–2,000	Green and Prout (1985)
Rabbit, Japanese White[b,c]	0.5	–	i.p. injection	200	Nomiyama and Nomiyama (1979)
Rat, Wistar[d]	–	14–17	Oral intubation	2–200	Dekant et al. (1986b)
Rat, Osborne-Mendel[a]	6–7	–	Oral intubation	10–2,000	Green and Prout (1985)
Rat, Holtzman[a]	7	–	i.p. injection	10 mg TCE/rat	Nomiyama and Nomiyama (1979)

[a]The human data tabulated in Fisher et al. (1991) from Nomiyama and Nomiyama (1971) were not included here because they were relative to urinary excretion of TTCs—not as fraction of intake as was the case for the other data included here.
[b]Percentage urinary excretion determined from accumulated amounts of TCOH and TCA in urine 3–6 days postexposure.
[c]Sex not specified.
[d]Percentage urinary excretion determined from accumulated amounts of TCOH, DCA, oxalic acid, and *N*-(hydroxyacetyl)aminoethanol in urine 3 days postexposure.

3.3.3.1.6. CYP isoforms and genetic polymorphisms

A number of studies have identified multiple P450 isozymes as having a role in the oxidative metabolism of TCE. These isozymes include CYP2E1 (Nakajima et al., 1992a; Guengerich et al., 1991; Guengerich and Shimada, 1991; Nakajima et al., 1990; Nakajima et al., 1988), CYP3A4 (Shimada et al., 1994), CYP1A1/2, CYP2C11/6 (Nakajima et al., 1993; Nakajima et al., 1992a), CYP2F, and CYP2B1 (Forkert et al., 2005). Recent studies in CYP2E1-knockout mice have shown that in the absence of CYP2E1, mice still have substantial capacity for TCE oxidation (Forkert et al., 2006; Kim and Ghanayem, 2006). However, CYP2E1 appears to be the predominant (i.e., higher affinity) isoform involved in oxidizing TCE (Forkert et al., 2005; Nakajima et al., 1992a; Guengerich et al., 1991; Guengerich and Shimada, 1991). In rat liver, CYP2E1 catalyzed TCE oxidation more than CYP2C11/6 (Nakajima et al., 1992a). In rat recombinant-derived P450s, the CYP2E1 had a lower K_M (higher affinity) and higher V_{MAX}/K_M ratio (intrinsic clearance) than CYP2B1 or CYP2F4 (Forkert et al., 2005). Interestingly, there was substantial differences in K_M between rat and human CYP2E1s and between rat CYP2F4

and mouse CYP2F2, suggesting that species-specific isoforms have different kinetic behavior (see Table 3-20).

Table 3-20. P450 isoform kinetics for metabolism of TCE to CH in human, rat, and mouse recombinant P450s

Experiment	K_M µM	V_{MAX} pmol/min/pmol P450	V_{MAX}/K_M
Human rCYP2E1	196 ± 40	4 ± 0.2	0.02
Rat rCYP2E1	14 ± 3	11 ± 0.3	0.79
Rat rCYP2B1	131 ± 36	9 ± 0.5	0.07
Rat rCYP2F4	64 ± 9	17 ± 0.5	0.27
Mouse rCYP2F2	114 ± 17	13 ± 0.4	0.11

Source: Forkert et al. (2005).

The presence of multiple P450 isoforms in human populations affects the variability in individuals' ability to metabolize TCE. Studies using microsomes from human liver or from human lymphoblastoid cell lines expressing CYP2E1, CYP1A1, CYP1A2, or CYP3A4 have shown that CYP2E1 is responsible for >60% of oxidative TCE metabolism (Lipscomb et al., 1997). Similarities between metabolism of chlorzoxazone (a CYP2E1 substrate) in liver microsomes from 28 individuals (Peter et al., 1990) and TCE metabolism helped identify CYP2E1 as the predominant (high affinity) isoform for TCE oxidation. Additionally, Lash et al. (2000a) suggested that, at concentrations above the K_M value for CYP2E1, CYP1A2, and CYP2A4 may also metabolize TCE in humans; however, their contribution to the overall TCE metabolism was considered low compared to that of CYP2E1. Given the difference in expression of known TCE-metabolizing P450 isoforms (see Table 3-21) and the variability in P450-mediated TCE oxidation (Lipscomb et al., 1997), significant variability may exist in individual human susceptibility to TCE toxicity.

Table 3-21. P450 isoform activities in human liver microsomes exhibiting different affinities for TCE

Affinity group	CYP isoform activity (pmol/min/mg protein)[a]		
	CYP2E1	CYP1A2	CYP3A4
Low K_M	520 ± 295	241 ± 146	2.7 ± 2.7
Mid K_M	820 ± 372	545 ± 200	2.9 ± 2.8
High K_M	1,317 ± 592	806 ± 442	1.8 ± 1.1

[a]Activities of CYP1A2, CYP2E1, and CYP3A4 were measured with phenacetin, chlorzoxazone, and testosterone as substrates, respectively. Data are means ± SD from 10, 9, and 4 samples for the low-, mid-, and high-K_M groups, respectively. Only CYP3A4 activities are not significantly different ($p < 0.05$) from one another by Kruskal-Wallis one-way analysis of variance.

Source: Lash et al. (2000a).

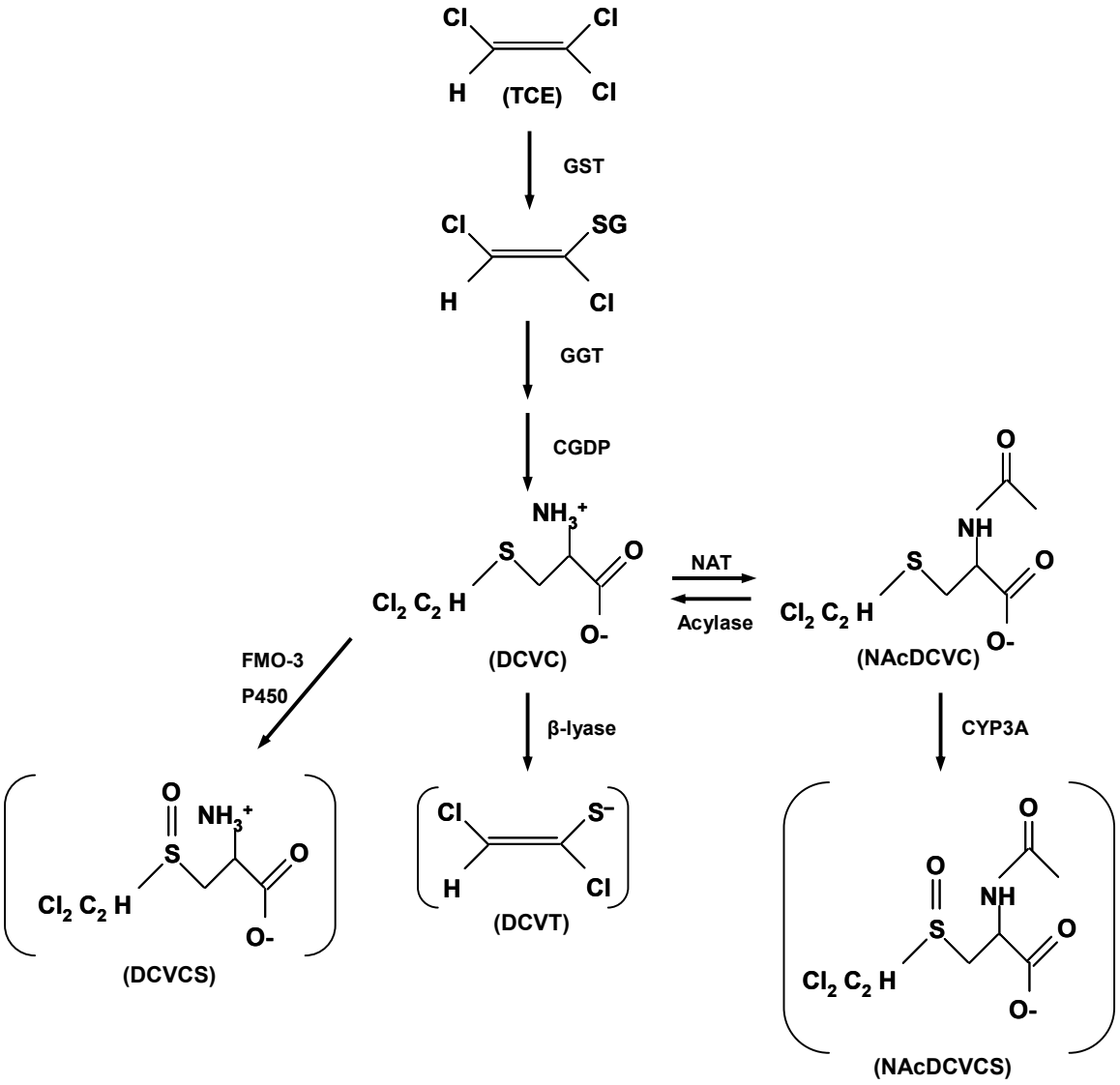
Differences in content and/or intrinsic catalytic properties (K_M, V_{MAX}) of specific enzymes among species, strains, and individuals may play an important role in the observed differences in TCE metabolism and resulting toxicities. Lipscomb et al. (1997) reported observing three statistically distinct groups of K_M values for TCE oxidation using human microsomes. The mean ± standard deviation (SD) (μM TCE) for each of the three groups was 16.7 ± 2.5 (n = 10), 30.9 ± 3.3 (n = 9), and 51.1 ± 3.8 (n = 4). Within each group, there were no significant differences in sex or ethnicity. However, the overall observed K_M values in female microsomes (21.9 ± 3.5 μM, n = 10) were significantly lower than males (33.1 ± 3.5 μM, n = 13). Interestingly, in human liver microsomes, different groups of individuals with different affinities for TCE oxidation appeared to also have different activities for other substrates not only with respect to CYP2E1 but also CYP1A2 (Lash et al., 2000a) (see Table 3-21). Genetic polymorphisms in humans have been identified in the CYP isozymes thought to be responsible for TCE metabolism (Pastino et al., 2000), but no data exist correlating these polymorphisms with enzyme activity. It is relevant to note that repeat polymorphism (Hu et al., 1999) or polymorphism in the regulatory sequence (McCarver et al., 1998) were not involved in the constitutive expression of human CYP2E1; however, it is unknown if these types of polymorphisms may play a role in the inducibility of the respective gene.

Individual susceptibilities to TCE toxicity may also result from variations in enzyme content, either at baseline or due to enzyme induction/inhibition, which can lead to alterations in the amounts of metabolites formed. Certain physiological and pathological conditions or exposure to other chemicals (e.g., ethanol and acetominophen) can induce, inhibit, or compete for enzymatic activity. Given the well-established (or well-characterized) role of the liver to oxidatively metabolize TCE (by CYP2E1), increasing the CYP2E1 content or activity (e.g., by enzyme induction) may not result in further increases in TCE oxidation. Indeed, Kaneko et al. (1994) reported that enzyme induction by ethanol consumption in humans increased TCE metabolism only at high concentrations (500 ppm, 2,687 mg/m^3) in inspired air. However, other

interactions between ethanol and the enzymes that oxidatively metabolize TCE metabolites can result in altered metabolic fate of TCE metabolites. In addition, enzyme inhibition or competition can decrease TCE oxidation and subsequently alter the TCE toxic response via, for instance, increasing the proportion undergoing GSH conjugation Lash et al. (2000a). TCE itself is a competitive inhibitor of CYP2E1 activity (Lipscomb et al., 1997), as shown by reduced *p*-nitrophenol hydroxylase activity in human liver microsomes, and may therefore alter the toxicity of other chemicals metabolized through that pathway. On the other hand, suicidal CYP heme destruction by the TCE-oxygenated CYP intermediate has also been shown (Miller and Guengerich, 1983).

3.3.3.2. GSH Conjugation Pathway

Historically, the conjugative metabolic pathways have been associated with xenobiotic detoxification. This is true for GSH conjugation of many compounds. However, several halogenated alkanes and alkenes, including TCE, are bioactivated to cytotoxic metabolites by the GSH conjugate processing pathway (mercapturic acid) pathways (Elfarra et al., 1987; Elfarra et al., 1986). In the case of TCE, production of reactive species several steps downstream from the initial GSH conjugation is believed to cause cytotoxicity and carcinogenicity, particularly in the kidney. Since the GSH conjugation pathway is in competition with the P450 oxidative pathway for TCE biotransformation, it is important to understand the role of various factors in determining the flux of TCE through each pathway. Figure 3-5 depicts the present understanding of TCE metabolism via GSH conjugation.



Adapted from: Lash et al. (2000a); Cummings and Lash (2000); NRC (2006).

Figure 3-5. Scheme for GSH-dependent metabolism of TCE.

3.3.3.2.1. Formation of S-(1,2-dichlorovinyl)glutathione or S-(2,2-dichlorovinyl)-glutathione (DCVG)

The conjugation of TCE to GSH produces S-(1,2-dichlorovinyl)glutathione or its isomer S-(2,2-dichlorovinyl)glutathione (collectively, S-dichlorovinyl-glutathione, DCVG). There is some uncertainty as to which GST isoforms mediate TCE conjugation. Lash and colleagues studied TCE conjugation in renal tissue preparations, isolated renal tubule cells from male F344 rats and purified GST alpha-class isoforms 1-1, 1-2, and 2-2 (Cummings and Lash, 2000; Cummings et al., 2000b; Lash et al., 2000b). The results demonstrated high conjugative activity in the renal cortex and proximal tubule cells. Although the isoforms studied had similar V_{MAX}

values, the K_M value for GST 2-2 was significantly lower than the other forms, indicating that this form will catalyze TCE conjugation at lower (more physiologically relevant) substrate concentrations. In contrast, using purified rat and human enzymes, Hissink et al. (2002) reported in vitro activity for DCVG formation only for mu- and pi-class GST isoforms, and none towards alpha-class isoforms; however, the rat mu-class GST 3-3 was several-fold more active than the human mu-class GST M1-1. Although GSTs are present in tissues throughout the body, the majority of TCE GSH conjugation is thought to occur in the liver (Lash et al., 2000a). Using in vitro studies with renal preparations, it has been demonstrated that GST catalyzed conjugation of TCE is increased following the inhibition of CYP-mediated oxidation (Cummings and Lash, 2000).

In F344 rats, following gavage doses of 263–1,971 mg/kg TCE in 2 mL corn oil, DCVG was observed in the liver and kidney of females only, in blood of both sexes (Lash et al., 2006), and in bile of males (Dekant, 1990). The data from Lash et al. (2006) are difficult to interpret because the time courses seem extremely erratic, even for the oxidative metabolites TCOH and TCA. Moreover, a comparison of blood levels of TCA and TCOH with other studies in rats at similar doses reveals differences of over 1,000-fold in reported concentrations. For instance, at the lowest dose of 263 mg/kg, the peak blood levels of TCE and TCA in male F344 rats were 10.5 and 1.6 µg/L, respectively (Lash et al., 2006). By contrast, Larson and Bull (1992a) reported peak blood TCE and TCA levels in male Sprague-Dawley rats over 1,000-fold higher—around 10 and 13 mg/L, respectively—following oral doses of 197 mg/kg as a suspension in 1% aqueous Tween 80®. The results of Larson and Bull (1992a) are similar to Lee et al. (2000b), who reported peak blood TCE levels of 20–50 mg/L after male Sprague-Dawley rats received oral doses of 144–432 mg/kg in a 5% aqueous Alkamus emulsion (polyethoxylated vegetable oil), and to Stenner et al. (1997), who reported peak blood levels of TCA in male F344 rats of about 5 mg/L at a slightly lower TCE oral dose of 100 mg/kg administered to fasted animals in 2% Tween 80®. Thus, while useful qualitatively as an indicator of the presence of DCVG in rats, the quantitative reliability of reported concentrations, for metabolites of either oxidation or GSH conjugation, may be questionable.

In humans, DCVG was readily detected at in human blood following onset of a 4-hour TCE inhalation exposure to 50 or 100 ppm (269 or 537 mg/m^3) (Lash et al., 1999b). At 50 ppm, peak blood levels ranged from 2.5 to 30 µM, while at 100 ppm, the mean (± SE, n = 8) peak blood levels were 46.1 ± 14.2 µM in males and 13.4 ± 6.6 µM in females. Although on average, male subjects had threefold higher peak blood levels of DCVG than females, DCVG blood levels in half of the male subjects were similar to or lower than those of female subjects. This suggests a polymorphism in GSH conjugation of TCE rather than a true gender difference (Lash et al., 1999b) as also has been indicated by Hissink et al. (2002) for the human mu-class GST M1-1 enzyme. Interestingly, as shown in Table 3-22, the peak blood levels of DCVG are similar on a

molar basis to peak levels of TCE, TCA, and TCOH in the same subjects, as reported in Fisher et al. (1998).

Table 3-22. Comparison of peak blood concentrations in humans exposed to 100 ppm (537 mg/m^3) TCE for 4 hours

Chemical species	Peak blood concentration (mean ± SD, µM)	
	Males	Females
TCE	23 ± 11	14 ± 4.7
TCA	56 ± 9.8	59 ± 12
TCOH	21 ± 5.0	15 ± 5.6
DCVG	46.1 ± 14.2	13.4 ± 6.6

Sources: Fisher et al. (1998); Lash et al. (1999a).

Tables 3-23–3-25 summarize DCVG formation from TCE conjugation from in vitro studies of liver and kidney cellular and subcellular fractions in mouse, rat, and human (tissue-distribution and species- and gender-differences in DCVG formation are discussed below). As shown by these tables, different investigators have reported considerably different rates for TCE conjugation in human liver and kidney cell fractions. For instance, values in Table 3-23 from Lash et al. (1999b) are between 2 and 5 orders of magnitude higher than those reported by Green et al. (1997a) or Dekant et al. (1990) (see Table 3-25). In addition, Green et al. (1997a) and Dekant et al. (1990) reported a difference in the relative importance of rat liver cytosol and rat liver microsomes for GSH conjugation, with Green et al. (1997a) reporting activity in the cytosol and none in the microsomes and Dekant et al. (1990) reporting the opposite.

Table 3-23. GSH conjugation of TCE (at 1–2 mM) in liver and kidney cellular fractions in humans, male F344 rats, and male B6C3F$_1$ mice from Lash laboratory

Species and cellular/subcellular fraction (TCE concentration)	DCVG formation (nmol/hr/mg protein or 10^6 cells)[a]	
	Male	Female
Human		
Hepatocytes (0.9 mM) (pooled)	11 ± 3	
Liver cytosol (1 mM) (individual samples)	156 ± 16	174 ± 13
Liver cytosol (2 mM) (pooled)	346	
Liver microsomes (1 mM) (individual samples)	108 ± 24	83 ± 11
Liver microsomes (1 mM) (pooled)	146	
Kidney cytosol (2 mM) (pooled)	42	
Kidney microsomes (1 mM) (pooled)	320	
Rat		
Liver cytosol (2 mM)	7.30 ± 2.8	4.86 ± 0.14
Liver microsomes (2 mM)	10.3 ± 2.8	7.24 ± 0.24
Kidney cortical cells (2 mM)	0.48 ± 0.02	0.65 ± 0.15
Kidney cytosol (2 mM)	0.45 ± 0.22	0.32 ± 0.02
Kidney microsomes (2 mM)	Not detected	0.61 ± 0.06
Mouse		
Liver cytosol (2 mM)	24.5 ± 2.4	21.7 ± 0.9
Liver microsomes (2 mM)	40.0 ± 3.1	25.6 ± 0.8
Kidney cytosol (2 mM)	5.6 ± 0.24	3.7 ± 0.48
Kidney microsomes (2 mM)	5.47 ± 1.41	16.7 ± 4.7

[a]Mean ± SE.

Sources: Lash et al. (1999a; 1998a; 1995); Cummings and Lash (2000).

Table 3-24. Kinetics of TCE metabolism via GSH conjugation in male F344 rat kidney and human liver and kidney cellular and subcellular fractions from Lash laboratory

Tissue and cellular fraction	K_M (µM TCE)	V_{MAX} (nmol DCVG/min/mg protein or 10^6 hepatocytes)	1,000 × V_{MAX}/K_M
Rat			
Kidney proximal tubular cells: low affinity	2,910	0.65	0.22
Kidney proximal tubular cells: high affinity	460	0.47	1.0
Human			
Liver hepatocytes[a]	37~106	0.16~0.26	2.4~4.5
Liver cytosol: low affinity	333	8.77	2.6
Liver cytosol: high affinity	22.7	4.27	190
Liver microsomes: low affinity	250	3.1	12
Liver microsomes: high affinity	29.4	1.42	48
Kidney proximal tubular cells: low affinity	29,400	1.35	0.046
Kidney proximal tubular cells: high affinity	580	0.11	0.19
Kidney cytosol	26.3	0.81	31
Kidney microsomes	167	6.29	38

[a]Kinetic analyses of first 6–9 (out of 10) data points from Figure 1 from Lash et al. (1999b) using Lineweaver-Burk or Eadie-Hofstee plots and linear regression (R^2 = 0.50~0.95). Regression with best R^2 used first 6 data points and Eadie-Hofstee plot, with resulting K_M and V_{MAX} of 106 and 0.26, respectively.

Sources: Lash et al. (1999b); Cummings and Lash (2000); (Cummings et al., 2000b).

Table 3-25. GSH conjugation of TCE (at 1.4–4 mM) in liver and kidney cellular fractions in humans, male F344 rats, and male B6C3F$_1$ mice from Green and Dekant laboratories

Species and cellular/subcellular fraction (TCE concentration)	DCVG formation (nmol/hr/mg protein) (substrate concentration in mM)[a]	
	Dekant et al. (1990)	Green et al. (1997a)
Human		
Liver cytosol	-	0.00019 ± 0.00014
Liver microsomes	-	Not determined
Kidney cytosol	-	Not determined
Kidney microsomes	-	Not determined
Rat		
Liver cytosol	<0.002	0.00162 ± 0.00002
Liver microsomes	0.002	Not determined
Kidney cytosol	-	Not determined
Kidney microsomes	-	Not determined
Mouse		
Liver cytosol	-	0.0025
Liver microsomes	-	Not determined
Kidney cytosol	-	Not determined
Kidney microsomes	-	Not determined

[a]Where available, mean ± SD.

Sources: Dekant et al. (1990), Green et al. (1997a).

The reasons for such discrepancies are unclear, but they may be related to different analytical methods (Lash et al., 2000a). In particular, Lash et al. (1999b) employed the ―Reed method," which used ion-exchange high-performance liquid chromatography (HPLC) of derivatized analytes. This HPLC method is characterized by variability and an overall decline in retention times over the life of the HPLC column due to derivatization of amine groups on the column (Lash et al., 1999a). Although data are limited, the GSH pathway metabolite levels reported by methods that utilize [^{14}C]-TCE and radiochemical detection followed by mass spectrometry (MS) identification of the metabolites are lower. In particular, Green et al. (1997a) and Dekant et al. (1990) both used HPLC with radiochemical detection. Peak identity was confirmed by Green et al. (1997a) using liquid chromatography (LC)/MS and by GC/MS following hydrolysis by Dekant et al. (1990). In addition, studies using HPLC-MS/MS techniques with stable isotope-labeled DCVG and dichlorovinyl cysteine (DCVC) standards have also been used to detect GSH pathway metabolite levels Kim et al. (2009). Based on the in vitro work presented in Table 3-23 using the ―Reed method," one would expect mouse serum DCVG levels to be ~4-6 times lower than humans. However, using the HPLC-MS/MS technique of Kim et al. (2009), the peak DCVG serum levels are ~1,000 times lower in mouse

serum than determined by Lash et al. (1999b) in human serum. Although advances in LC technology, and differences in exposure routes (inhalation vs. oral, with different first pass), exposure doses, and the degree of competition with TCE oxidation (greater in mouse than in human) should be considered, this much-larger-than-expected difference is consistent with the suggestion that the "Reed method" provides an overestimation of DCVG levels in humans. This could occur if the "Reed method" identifies nonspecific derivatives as DCVG or other GSH pathway metabolites. However, the degree of overestimation is unclear, and differing results in humans may be attributable to true interindividual variation (especially since GSTs are known to be polymorphic). Overall, there remains significant uncertainty in the quantitative estimation of DCVG formation from TCE both in vivo and in vitro.

3.3.3.2.2. Formation of S-(1,2-dichlorovinyl) cysteine or S-(2,2-dichlorovinyl) cysteine (DCVC)

The cysteine conjugate, isomers S-(1,2-dichlorovinyl) cysteine (1,2-DCVC) or S-(2,2-dichlorovinyl) cysteine (2,2-DCVC) (collectively S-dichlorovinyl-cysteine, DCVC), is formed from DCVG in a two-step sequence. DCVG is first converted to the cysteinylglycine conjugate S-(1,2-dichlorovinyl)-L-cysteinylglycine or its isomer S-(2,2-dichlorovinyl)-L-cysteinylglycine by γ-glutamyl transpeptidase (GGT) in the renal brush border (Lash et al., 1988; Elfarra and Anders, 1984).

Cysteinylglycine dipeptidases in the renal brush border and basolateral membrane convert DCVG to DCVC via glycine cleavage (Goeptar et al., 1995; Lash et al., 1995). This reaction can also occur in the bile or gut, as DCVG excreted into the bile is converted to DCVC and reabsorbed into the liver where it may undergo further acetylation.

3.3.3.2.3. Formation of N-Acetyl-S-(1,2-dichlorovinyl)-L-cysteine or N-Acetyl-S-(2,2-dichlorovinyl)-L-cysteine (NAcDCVC)

N-acetylation of DCVC can either occur in the kidney, as demonstrated in rat kidney microsomes (Duffel and Jakoby, 1982), or in the liver (Birner et al., 1997). Subsequent release of DCVC from the liver to blood may result in distribution to the kidney resulting in increased internal kidney exposure to the acetylated metabolite over and above what the kidney already is capable of generating. In the kidney, N-Acetyl-S-(1,2-dichlorovinyl)-L-cysteine or N-Acetyl-S-(2,2-dichlorovinyl)-L-cysteine (collectively N-Acetyl-S-dichlorovinyl-L-cysteine, NAcDCVC) may undergo deacetylation, which is considered a rate-limiting-step in the production of proximal tubule damage (Wolfgang et al., 1989a; Zhang and Stevens, 1989). As a polar mercapturate, NAcDCVC may be excreted in the urine as evidenced by findings in mice (Birner et al., 1993), rats (Bernauer et al., 1996; Commandeur and Vermeulen, 1990), and humans who were exposed to TCE (Bernauer et al., 1996; Birner et al., 1993), suggesting a common GSH-mediated metabolic pathway for DCVC among species.

3.3.3.2.4. Beta lyase metabolism of DCVC

The enzyme cysteine conjugate β-lyase catalyzes the breakdown of 1,2-DCVC to reactive nephrotoxic metabolites (Goeptar et al., 1995). This reaction involves removal of pyruvate and ammonia and production of S-dichlorovinyl thiol (DCVT), an unstable intermediate, which rearranges to other reactive alkylation metabolites that form covalent bonds with cellular nucleophiles (Goeptar et al., 1995; Dekant et al., 1988). The rearrangement of DCVT to enethiols and their acetylating agents has been described in trapping experiments (Dekant et al., 1988) and proposed to be responsible for nucleophilic adduction and toxicity in the kidney. The quantification of acid-labile adducts was proposed as a metric for TCE flux through the GSH pathway. However, the presence of analytical artifacts precluded such analysis. In fact, measurement of acid-labile adduct products resulted in higher values in mice than in rats (Eyre et al., 1995b, a).

DCVC metabolism to reactive species via a β-lyase pathway has been observed in vitro by Green et al. (1997a), who reported greater β-lyase activity in rats than in mice or humans. However, in vitro DCVC metabolism by the competing enzyme *N*-acetyl transferase was also reported to be greater in rats than mice and humans. In vivo, β-lyase activity in humans and rats (reaction rates were not reported) was demonstrated using a surrogate substrate, 2-(fluoro-methoxy)-1,1,3,3,3-pentafluoro-1-propene (Iyer et al., 1998). β-lyase-mediated reactive adducts have been described in several extrarenal tissues, including rat and human liver and intestinal microflora (Larsen and Stevens, 1986; Tomisawa et al., 1986; Stevens, 1985; Tomisawa et al., 1984; Stevens and Jakoby, 1983; Dohn and Anders, 1982; Tateishi et al., 1978) and rat brain (Alberati-Giani et al., 1995; Malherbe et al., 1995).

In the kidneys, glutamine transaminase K appears to be primarily responsible for β-lyase metabolism of DCVC (Perry et al., 1993; Lash et al., 1990; Jones et al., 1988; Stevens et al., 1988; Lash et al., 1986; Stevens et al., 1986). β-Lyase transformation of DCVC appears to be regulated by 2-keto acids. DCVC toxicity in isolated rat proximal tubular cells was significantly increased with the addition of α-keto-γ-methiolbutyrate or phenylpyruvate (Elfarra et al., 1986). The presence of α-keto acid cofactors is necessary to convert the inactive form of the β-lyase enzyme (containing pyridoxamine phosphate) to the active form (containing pyridoxal phosphate) (Goeptar et al., 1995).

Both low- and high-molecular-weight enzymes with β-lyase activities have been identified in rat kidney cytosol and mitochondria (Abraham et al., 1995a; Abraham et al., 1995b; Stevens et al., 1988; Lash et al., 1986). While glutamine transaminase K and kynureninase-associated β-lyase activities have been identified in rat liver (Alberati-Giani et al., 1995; Stevens, 1985), they are quite low compared to renal glutamine transaminase K activity and do not result in hepatotoxicity in DCVG- or DCVC-treated rats (Elfarra and Anders, 1984). Similar isoforms of β-lyase have also been reported in mitochondrial fractions of brain tissue (Cooper, 2004).

The kidney enzyme, L-α-hydroxy (L-amino) acid oxidase, is capable of forming an iminium intermediate and keto acid analogues (pyruvate or S-(1,2-dichlorovinyl)-2-oxo-3-mercaptopropionate) of DCVC, which decomposes to dichlorovinylthiol (Lash et al., 1990; Stevens et al., 1989). In rat kidney homogenates, this enzyme activity resulted in as much as 35% of GSH pathway-mediated bioactivation. However, this enzyme is not present in humans, an important consideration for extrapolation of renal effects across species.

3.3.3.2.5. DCVC and NAcDCVC

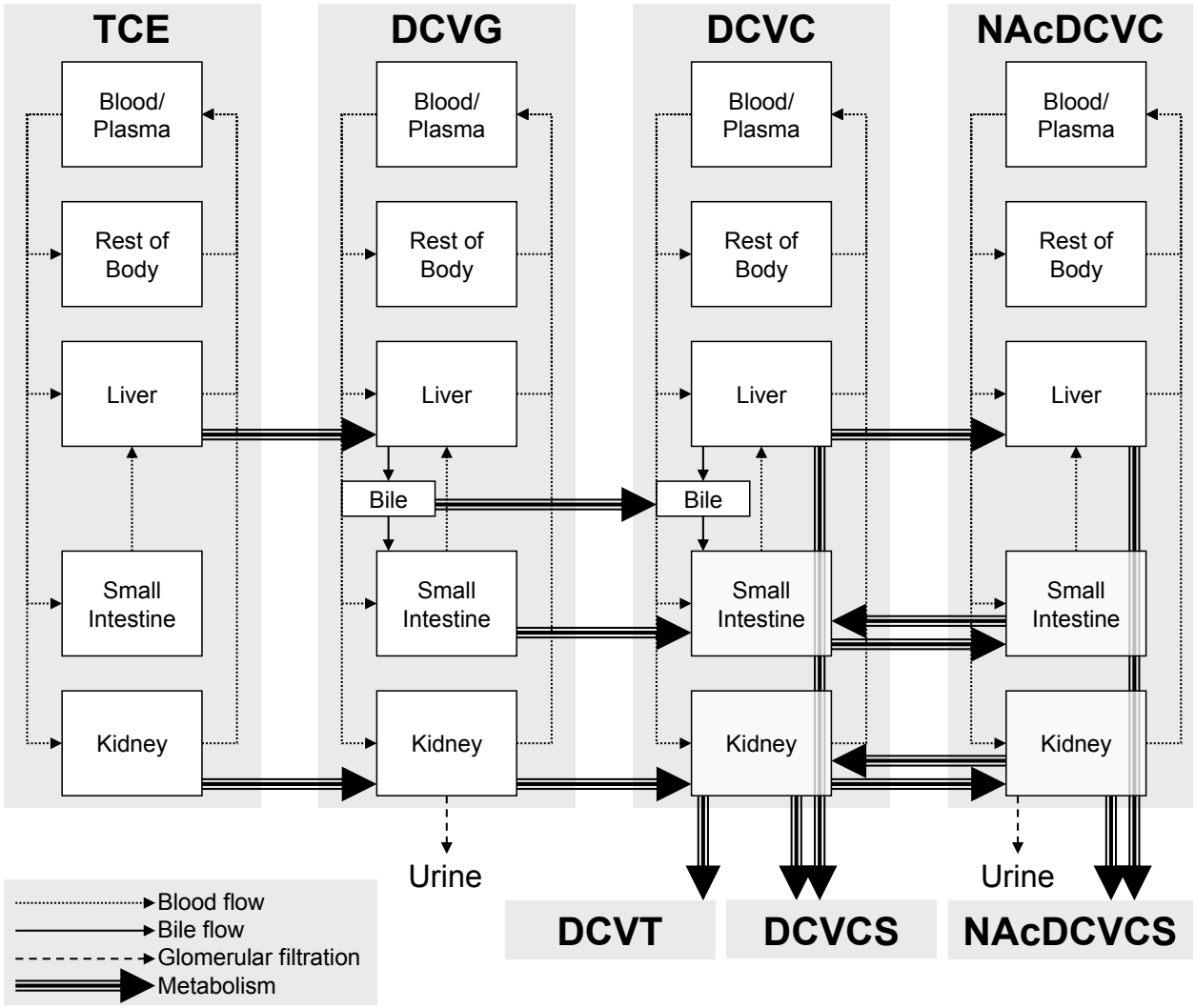
A second pathway for bioactivation of TCE S-conjugates involves sulfoxidation of either the cysteine or mercapturic acid conjugates (Krause et al., 2003; Lash et al., 2003; Birner et al., 1998; Werner et al., 1996, 1995a; Werner et al., 1995b; Lash et al., 1994; Park et al., 1992; Sausen and Elfarra, 1990). Sulfoxidation of DCVC was mediated mainly by flavin monooxygenase 3 (FMO3), rather than CYP, in rabbit liver microsomes (Ripp et al., 1997) and human liver microsomes (Krause et al., 2003). Krause et al. (2003) also reported DCVC sulfoxidation by human cDNA-expressed FMO3, as well as detection of FMO3 protein in human kidney samples. While Krause et al. (2003) were not able to detect sulfoxidation in human kidney microsomes, the authors noted FMO3 expression in the kidney was lower and more variable than that in the liver. However, sulfoxidation products in tissues or urine have not been reported in vivo.

Sulfoxidation of NAcDCVC, by contrast, was found to be catalyzed predominantly, if not exclusively, by CYP3A enzymes (Werner et al., 1996), whose expressions are highly polymorphic in humans. Sulfoxidation of other haloalkyl mercapturic acid conjugates has also been shown to be catalyzed by CYP3A (Altuntas et al., 2004; Werner et al., 1995a; Werner et al., 1995b). While Lash et al. (2000a) suggested that this pathway would be quantitatively minor because of the relatively low CYP3A levels in the kidney, no direct data exist to establish the relative toxicological importance of this pathway relative to bioactivation of DCVC by β-lyase or FMO3. However, the contribution of CYP3A in S-conjugate sulfoxidation to nephrotoxicity in vivo was recently demonstrated by Sheffels et al. (2004) with fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (FDVE). In particular, in vivo production and urinary excretion of FDVE-mercapturic acid sulfoxide metabolites were unambiguously established by mass spectrometry, and CYP inducers/inhibitors increased/decreased nephrotoxicity in vivo while having no effect on urinary excretion of metabolites produced through β-lyase (Sheffels et al., 2004). These data suggest that, by analogy, sulfoxidation of NAcDCVC may be an important bioactivating pathway.

3.3.3.2.6. Tissue distribution of GSH metabolism

The sites of enzymatic metabolism of TCE to the various GSH pathway-mediated metabolites are significant in determining target tissue toxicity along this pathway. Figure 3-6

presents a schematic of interorgan transport and metabolism of TCE along the GSH pathway. TCE is taken up either by the liver or kidney and conjugated to DCVG. The primary factors affecting TCE flux via this pathway include high hepatic GST activity, efficient transport of DCVG from the liver to the plasma or bile, high renal brush border and low hepatic GGT activities, and the capability for GSH conjugate uptake into the renal basolateral membranes with limited or no uptake into liver cell plasma membranes.



See Figure 3-5 for enzymes involved in metabolic steps. Source: Lash et al. (2000a; 2000b); NRC (2006).

Figure 3-6. Interorgan TCE transport and metabolism via the GSH pathway.

As discussed previously, GST activity is present in many different cell types. However, the liver is the major tissue for GSH conjugation. GST activities in rat and mouse cytosolic fractions were measured using 1-chloro-2,4-dinitrobenzene, a GST substrate that is nonspecific

for particular isoforms (Lash et al., 1998b). Specific activities (normalized for protein content) in whole-kidney cytosol were slightly less than those in the liver (0.64 compared to 0.52 mU/mg protein for males and females). However, the much larger mass of the liver compared to the kidney indicates that far more total GST activity resides in the liver. This is consistent with in vitro data on TCE conjugation to DCVG, discussed previously (see Tables 3-23 and 3-24). For instance, in humans, rats, and mice, liver cytosol exhibits greater DCVG production than kidney cytosol. Distinct high- and low-affinity metabolic profiles were observed in the liver but not in the kidney (see Table 3-24). In microsomes, human liver and kidney had similar rates of DCVG production, while for rats and mice, the production in the liver was substantially greater. According to studies by Lash et al. (1998a; 1998b), the activity of GGT, the first step in the conversion of DCVG to DCVC, is much higher in the kidney than the liver of mice, rats, and humans, with most of the activity being concentrated in the microsomal, rather than the cytosolic, fraction of the cell (see Table 3-26). In rats, this activity is quite high in the kidney but is below the level of detection in the liver, while the relative kidney-to-liver levels in humans and mice were higher by 18- and up to 2,300-fold, respectively. Similar qualitative findings were also reported in another study (Hinchman and Ballatori, 1990) when total organ GGT levels were compared in several species (see Table 3-27). Cysteinylglycine dipeptidase was also preferentially higher in the kidney than the liver of all tested species although the interorgan differences in this activity (one–ninefold) seemed to be less dramatic than for GGT (see Table 3-27). High levels of both GGT and dipeptidases have also been reported in the small intestine of rat (Kozak and Tate, 1982) and mouse (Habib et al., 1996), as well as GGT in the human jejunum (Fairman et al., 1977). No specific human intestinal cysteinylglycine dipeptidase has been identified; however, a related enzyme (EC 3.4.13.11) from human kidney microsomes has been purified and studied (Adachi et al., 1989), while several human intestinal dipeptidases have been characterized including a membrane dipeptidase (EC 3.4.13.19), which has a wide dipeptide substrate specificity including cysteinylglycine (Ristoff and Larsson, 2007; Hooper et al., 1994).

Table 3-26. GGT activity in liver and kidney subcellular fractions of mice, rats, and humans

Species	Sex	Tissue	Cellular fraction	Activity (mU/mg)
Mouse	Male	Liver	Cytosol	0.07 ± 0.04
			Microsomes	0.05 ± 0.04
		Kidney	Cytosol	1.63 ± 0.85
			Microsomes	92.6 ± 15.6
	Female	Liver	Cytosol	0.10 ± 0.10
			Microsomes	0.03 ± 0.03
		Kidney	Cytosol	0.79 ± 0.79
			Microsomes	69.3 ± 14.0
Rat	Male	Liver	Cytosol	<0.02
			Microsomes	<0.02
		Kidney	Cytosol	<0.02
			Microsomes	1,570 ± 100
	Female	Liver	Cytosol	<0.02
			Microsomes	<0.02
		Kidney	Cytosol	<0.02
			Microsomes	1,840 ± 40
Human	Male	Liver	Cytosol	8.89 ± 3.58
			Microsomes	29
		Kidney	Cytosol	13.2 ± 1.0
			Microsomes	960 ± 77

Sources: Lash et al. (1999a; 1998a)

Table 3-27. Multispecies comparison of whole-organ activity levels of GGT and dipeptidase

Species	Whole organ enzyme activity (µmol substrate/organ)			
	Kidney		Liver	
	GGT	Dipeptidase	GGT	Dipeptidase
Rat	1,010 ± 41	20.2 ± 1.1	7.1 ± 1.4	6.1 ± 0.4
Mouse	60.0 ± 4.2	3.0 ± 0.3	0.47 ± 0.05	1.7 ± 0.2
Rabbit	1,119 ± 186	112 ± 17	71.0 ± 9.1	12.6 ± 1.0
Guinea pig	148 ± 13	77 ± 10	46.5 ± 4.2	13.2 ± 1.5
Pig	3,800 ± 769	2,428 ± 203	1,600 ± 255	2,178 ± 490
Macaque	988	136	181	71

Source: Hinchman and Ballatori (1990).

3.3.3.2.7. Sex- and species-dependent differences in GSH metabolism

Diverse sex and species differences appear to exist in TCE metabolism via the GSH pathway. In rodents, rates of TCE conjugation to GSH in male rats and mice are higher than

females (see Table 3-23). Verma and Rana (2003) reported twofold higher GST activity values in liver cytosol of female rats, compared to males, given 15 i.p. injections of TCE over 30 days period. This effect may be due to sex-dependent variation in induction, as GST activities in male and female controls were similar. DCVG formation rates by liver and kidney subcellular fractions were much higher in both sexes of mice than in rats and, except for mouse kidney microsomes, the rates were generally higher in males than in females of the same species (see Table 3-23).

In terms of species differences, comparisons at 1–2 mM TCE concentrations (see Table 3-23) suggest that, in liver and kidney cytosol, the greatest DCVG production rate was in humans, followed by mice and then rats. However, different investigators have reported considerably different rates for TCE conjugation in human liver and kidney cell fractions. For instance, values in Table 3-23 from Lash et al. (1999b) are between 2 and 5 orders of magnitude higher than those reported by Green et al. (1997a). The rates of DCVG formation by liver cytosol from male F344 rat, male B6C3F$_1$ mouse, and human were 1.62, 2.5, and 0.19 pmol/minute/mg protein, respectively, while there was no measurable activity in liver microsomes or subcellular kidney fractions (Green et al., 1997a). The reasons for such discrepancies are unclear but may be related to different analytical methods employed such as detection of radiolabled substrate vs. derivatized analytes (Lash et al., 2000a).

Expression of GGT activity does not appear to be influenced by sex (see Table 3-26); but species differences in kidney GGT activity are notable with rat subcellular fractions exhibiting the highest levels and mice and humans exhibiting about 4–6 and 50%, respectively, of rat levels (Lash et al., 1999a; Lash et al., 1998a). Table 3-27 shows measures of whole-organ GGT and dipeptidase activities in rats, mice, guinea pigs, rabbits, pigs, and monkeys. These data show that the whole kidney possesses higher activities than liver for these enzymes, despite the relatively larger mass of the liver.

As discussed above, the three potential bioactivating pathways subsequent to the formation of DCVC are catalyzed by β-lyase, FMO3, or CYP3A. Lash et al. (2000a) compared in vitro β-lyase activities and kinetic constants (when available) for kidney of rats, mice, and humans. They reported that variability of these values spans up to two orders of magnitude depending on substrate, analytical method used, and research group. Measurements of rat, mouse, and human β-lyase activities collected by the same researchers following tetrachloroethylene exposure (Green et al., 1990) resulted in higher K_M and lower V_{MAX} values for mice and humans than rats. Further, female rats exhibited higher K_M and lower V_{MAX} values than males.

With respect to FMO3, Ripp et al. (1999) found that this enzyme appeared catalytically similar across multiple species, including humans, rats, dogs, and rabbits, with respect to several substrates, including DCVC, but that there were species differences in expression. Specifically, in male liver microsomes, rabbits had 3-fold higher methionine S-oxidase activity than mice and dogs had 1.5-fold higher activity than humans and rats. Species differences were also noted in

male and female kidney microsomes; rats exhibited two- to sixfold higher methionine S-oxidase activity than the other species. Krause et al. (2003) detected DCVC sulfoxidation in incubations with human liver microsomes but did not in an incubation with a single sample of human kidney microsomes. However, FMO3 expression in the 26 human kidney samples was found to be highly variable, with a range of five- to sixfold (Krause et al., 2003).

No data on species differences in CYP3A-mediated sulfoxidation of NAcDCVC are available. However, Altuntas et al. (2004) examined sulfoxidation of cysteine and mercapturic acid conjugates of fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (FDVE) in rat and human liver and kidney microsomes. They reported that the formation of sulfoxides from the mercapturates *N*-Ac-FFVC and (Z)-*N*-Ac-FFVC (FFVC is (*E*,*Z*)-S-(1-fluoro-2-fluoromethoxy-2-(trifluoromethyl)vinyl-Lcysteine) were greatest in rat liver microsomes, and 2–30-fold higher than in human liver microsomes (which had high variability). Sulfoxidation of *N*-Ac-FFVC could not be detected in either rat or human kidney microsomes, but sulfoxidation of (Z)-*N*-Ac-FFVC was detected in both rat and human kidney microsomes at rates comparable to human liver microsomes. Using human- and rat-expressed CYP3A, Altuntas et al. (2004) reported that rates of sulfoxidation of (Z)-*N*-Ac-FFVC were comparable in human CYP3A4 and rat CYP3A1 and CYP3A2, but that only rat CYP3A1 and A2 catalyzed sulfoxidation of *N*-Ac-FFVC. As the presence or absence of the species differences in mercapturate sulfoxidation appears to be highly chemical-specific, no clear inferences can be made as to whether species differences exist for sulfoxidation of NAcDCVC

Also relevant to assess the flux through the various pathways are the rates of *N*-acetylation and de-acetylation of DCVC. This is demonstrated by the results of Elfarra and Hwang (1990) using S-(2-benzothiazolyl)-L-cysteine as a marker for β-lyase metabolism in rats, mice, hamsters, and guinea pigs. Guinea pigs exhibited about twofold greater flux through the β-lyase pathway, but this was not attributable to higher β-lyase activity. Rather, guinea pigs have relatively low *N*-acetylation and high deacetylation activities, leading to a high level of substrate recirculation (Lau et al., 1995). Thus, a high *N*-deacetylase:*N*-acetylase activity ratio may favor DCVC recirculation and subsequent metabolism to reactive species. In human, Wistar rat, Fischer rat, and mouse cytosol, deacetylation rates for NAcDCVC varied less than threefold (0.35, 0.41, 0.61, and 0.94 nmol DCVC formed/minute/mg protein in humans, rats, and mice) (Birner et al., 1993). However, similar experiments have not been carried out for *N*-acetylation of DCVC, so the balance between its *N*-acetylation and de-acetylation has not been established.

3.3.3.2.8. Human variability and susceptibility in GSH conjugation

Knowledge of human variability in metabolizing TCE through the GSH pathway is limited to in vitro comparisons of variance in GST activity rates. Unlike CYP-mediated oxidation, quantitative differences in the polymorphic distribution or activity levels of GST isoforms in humans are not presently known. However, the available data (Lash et al., 1999a;

Lash et al., 1999b) do suggest that significant variation in GST-mediated conjugation of TCE exists in humans. In particular, at a single substrate concentration of 1 mM, the rate of GSH conjugation of TCE in human liver cytosol from 9 male and 11 females spanned a range of 2.4-fold (34.7–83.6 nmol DCVG formed/20-minute/mg protein) (Lash et al., 1999a). In liver microsomes from 5 males and 15 females, the variation in activity was 6.5-fold (9.9–64.6 nmol DCVG formed/20 minute/mg protein). No sex-dependent variation was identified. Despite being less pronounced than the known variability in human CYP-mediated oxidation, the impact on risk assessment of the variability in GSH conjugation to TCE is currently unknown especially in the absence of data on variability for *N*-acetylation and bioactivation via β-lyase, FMO3, or CYP3A in the human kidney.

3.3.3.3. Relative roles of the CYP and GSH pathways

In vivo mass balance studies in rats and mice, discussed above, have shown unequivocally that in these species, CYP oxidation of TCE predominates over GSH conjugation. In these species, at doses of 2–2,000 mg/kg of [^{14}C]-TCE, the sum of radioactivity in exhaled TCE, urine, and exhaled CO_2 constitutes 69–94% of the dose, with the vast majority of the radioactivity in urine (95–99%) attributable to oxidative metabolites (Dekant et al., 1986b; Green and Prout, 1985; Prout et al., 1985; Dekant et al., 1984). The rest of the radioactivity was found mostly in feces and the carcass. More rigorous quantitative limits on the amount of GSH conjugation based on in vivo data such as these can be obtained using PBPK models, discussed in Section 3.5.

Comprehensive mass-balance studies are unavailable in humans. DCVG and DCVC in urine have not been detected in any species, while the amount of urinary NAcDCVC from human exposures is either below detection limits or very small from a total mass balance point of view (Bloemen et al., 2001; Lash et al., 1999b; Bernauer et al., 1996; Birner et al., 1993). For instance, the ratio of primary oxidative metabolites (TCA + TCOH) to NAcDCVC in urine of rats and humans exposed to 40–160 ppm (215–860 mg/m^3) TCE heavily favored oxidation, resulting in ratios of 986–2,562:1 in rats and 3,292–7,163:1 in humans (Bernauer et al., 1996). Bloemen et al. (2001) reported that, at most, 0.05% of an inhaled TCE dose would be excreted as NAcDCVC, and concluded that this suggested that TCE metabolism by GSH conjugation was of minor importance. While it is a useful biomarker of exposure and an indicator of GSH conjugation, NAcDCVC may capture only a small fraction of TCE flux through the GSH conjugation pathway due to the dominance of bioactivating pathways (Lash et al., 2000a).

A number of lines of evidence suggest that the amount of TCE conjugation to GSH in humans, while likely smaller than the amount of oxidation, may be much more substantial than analysis of urinary mercapturates would suggest. In Table 3-28, in vitro estimates of the V_{MAX}, K_M, and clearance (V_{MAX}/K_M) for hepatic oxidation and conjugation of TCE are compared in a manner that accounts for differences in cytosolic and microsomal partitioning and protein

content. Surprisingly, the range of in vitro kinetic estimates for oxidation and conjugation of TCE substantially overlap, suggesting similar flux through each pathway, though with high interindividual variation. The microsomal and cytosolic protein measurements of GSH conjugation should be caveated by the observation by Lash et al. (1999b) that GSH conjugation of TCE was inhibited by ~50% in the presence of oxidation. Note that this comparison cannot be made in rats and mice because in vitro kinetic parameters for GSH conjugation in the liver are not available in those species (only activity at 1 or 2 mM have been measured).

Table 3-28. Comparison of hepatic in vitro oxidation and conjugation of TCE[a]

Cellular or subcellular fraction	V_{MAX}[b] (nmol TCE metabolized/min/g tissue)		K_M[c] (µM in blood)		V_{MAX}/K_M (mL/min/g tissue)	
	Oxidation	GSH conjugation	Oxidation	GSH conjugation	Oxidation	GSH conjugation
Hepatocytes	10.0–68.4	16~25	22.1–198	16~47	0.087–1.12	0.55~1.0
Liver microsomes	6.1–111	45	2.66–11.1*	5.9*	1.71–28.2*	7.6*
			71.0–297**	157**	0.064–1.06**	0.29**
Liver cytosol	–	380	–	4.5*	–	84*
	–		–	22.7**	–	16.7**

[a]When biphasic metabolism was reported, only high affinity pathway is shown here.
[b]Conversion assumptions for V_{MAX}: hepatocellularity of 99 million cells/g liver (Barter et al., 2007); liver microsomal protein content of 32 mg protein/g tissue (Barter et al., 2007); and liver cytosolic protein content of 89 mg protein/g tissue (based on rats: Prasanna et al. (1989); van Bree et al. (1990).
[c]Conversion assumptions for K_M:
For hepatocytes, K_M in headspace converted to K_M in blood using blood:air partition coefficient of 9.5 (reported range of measured values 6.5–12.1, Table 3-1);
For microsomal protein, option (*) assumes K_M in medium is equal to K_M in tissue, and converts to K_M in blood by using a liver:blood partition coefficient of 5 (reported ranges of measured values 3.6–5.9, Table 3-8), and option (**) converts K_M in medium to K_M in air using the measured microsomal protein:air partition coefficient of 1.78 (Lipscomb et al., 1997), and then converts to K_M in blood by using the blood:air partition coefficient of 9.5; and
For cytosolic protein, option (*) assumes K_M in medium is equal to K_M in tissue, and converts to K_M in blood by using a liver:blood partition coefficient of 5 (reported ranges of measured values 3.6–5.9, Table 3-8), and option (**) assumes K_M in medium is equal to K_M in blood, so no conversion is necessary.

Furthermore, as shown earlier in Table 3-22, the human in vivo data of Lash et al. (1999b) show blood concentrations of DCVG similar, on a molar basis, to those of TCE, TCA, or TCOH, suggesting substantial conjugation of TCE. In addition, these data give a lower limit as to the amount of TCE conjugated. In particular, by multiplying the peak blood concentration of DCVG by the blood volume, a minimum amount of DCVG in the body at that time can be derived (i.e., assuming the minimal empirical distribution volume equal to the blood volume). As shown in Table 3-29, this lower limit amounts to about 0.4–3.7% of the inhaled TCE dose. Since this is the minimum amount of DCVG in the body at a single time point, the total amount of DCVG formed is likely to be substantially greater, owing to possible distribution outside of

the blood as well as the metabolism and/or excretion of DCVG. Lash et al. (1999b) found that levels of urinary mercapturates were near or below the level of detection of 0.19 μM, results that are consistent with those of Bloemen et al. (2001), who reported urinary concentrations below 0.04 μM at two- to fourfold lower cumulative exposures. Taken together, these results confirm the suggestion by Lash et al. (2000a) that NAcDCVC is a poor quantitative marker for the flux through the GSH pathway.

Table 3-29. Estimates of DCVG in blood relative to inhaled TCE dose in humans exposed to 50 and 100 ppm (269 and 537 mg/m^3) (Lash et al., 1999b)

Sex exposure	Estimated inhaled TCE dose (mmol)[a]	Estimated peak amount of DCVG in blood (mmol)[b]
Males		
50 ppm × 4 hrs	3.53	0.11 ± 0.08
100 ppm × 4 hrs	7.07	0.26 ± 0.08
Females		
50 ppm × 4 hrs	2.36	0.010 ± 0
100 ppm × 4 hrs	4.71	0.055 ± 0.027

[a]Inhaled dose estimated by (50 or 100 ppm)/(24,450 ppm/mM) × (240 minutes) × Q_P, where alveolar ventilation rate Q_P is 7.2 L/minute for males and 4.8 L/minute for females. Q_P is calculated as $(V_T - V_D) \times f_R$ with the following respiratory parameters: tidal volume V_T (0.75 L for males, 0.46 L for females), dead space V_D (0.15 L for males, 0.12 L for females), and respiration frequency f_R (12 minutes^{-1} for males, 14 minutes^{-1} for females) [assumed sitting, awake from The International Commission on Radiological Protection (ICRP, 2003)].
[b]Peak amount of DCVG in blood estimated by multiplying the peak blood concentration by the estimated blood volume: 5.6 L in males and 4.1 L in females (ICRP, 2003).

Sources: Fisher et al. (1998); Lash et al. (1999b).

However, as discussed in Section 3.3.3.2.1, data from other laboratories have reported substantially lower amounts of GSH conjugation in vitro. The reasons for such discrepancies are unclear, but they may be related to different analytical methods (Lash et al., 2000a). More recent in vivo data from Kim et al. (2009) in mice reported ~1,000 times lower DCVG in mouse serum as compared to the levels of DCVG reported by Lash et al. (1999b) in human blood. These data are consistent with the suggestion that the ―Reed method" employed by Lash et al. (1999b) overestimated DCVG levels in humans. However, the degree of overestimation is unclear, as is the degree to which differences may be attributable to true inter-species or inter-individual variability.

In summary, TCE oxidation is likely to be greater quantitatively than conjugation with GSH in mice, rats, and humans. Some evidence suggests that the flux through the GSH pathway, particularly in humans, may be greater by an order of magnitude or more than the <0.1% typically excreted of NAcDCVC in urine. This is evidenced both by a direct comparison of in vitro rates of oxidation and conjugation, as well as by in vivo data on the amount of DCVG in

blood. PBPK models can be used to more quantitatively synthesize these data and put more rigorous limits on the relative amounts of TCE oxidation and conjugation with GSH. Such analyses are discussed in Section 3.5. However, these data are not consistent with studies in other laboratories using different analytical methods, which report 2–5 orders of magnitude lower estimates of GSH conjugation. Because the reason for these differences have not been fully determined, substantial uncertainty remains in the degree of GSH conjugation, particularly in humans.

3.4. TCE EXCRETION

This section discusses the major routes of excretion of TCE and its metabolites in exhaled air, urine, and feces. Unmetabolized TCE is eliminated primarily via exhaled air. As discussed in Section 3.3, the majority of TCE absorbed into the body is eliminated by metabolism. With the exception of CO_2, which is eliminated solely via exhalation, most TCE metabolites have low volatility and, therefore, are excreted primarily in urine and feces. Although trace amounts of TCE metabolites have also been detected in sweat and saliva (Bartonicek, 1962), these excretion routes are likely to be relatively minor.

3.4.1. Exhaled Air

In humans, pulmonary elimination of unchanged TCE and other volatile compounds is related to ventilation rate, cardiac output, and the solubility of the compound in blood and tissue, which contribute to final exhaled air concentration of TCE. In their study of the impact of workload on TCE absorption and elimination, Astrand and Ovrum (1976) characterized the postexposure elimination of TCE in expired breath. TCE exposure (540 or 1,080 mg/m^3; 100 or 200 ppm) was for a total of 2 hours, at workloads of 0−150 watts. Elimination profiles were roughly equivalent among groups, demonstrating a rapid decline in TCE concentrations in expired breath postexposure (see Table 3-30).

Table 3-30. Concentrations of TCE in expired breath from inhalation-exposed humans (Astrand, 1982)

Time postexposure	Alveolar air		
	I[a]	II	III
0 min	459 ± 44	244 ± 16	651 ± 53
30 min	70 ± 5	51 ± 3	105 ± 18
60 min	40 ± 4	28 ± 2	69 ± 8
90 min	35 ± 9	21 ± 1	55 ± 2
120 min	31 ± 8	16 ± 1	45 ± 1
300 min	8 ± 1	9 ± 2	14 ± 2
420 min	5 ± 0.5	4 ± 0.5	8 ± 1.3
19 hrs	2 ± 0.3	2 ± 0.2	4 ± 0.5

[a]Roman numerals refer to groups assigned different workloads; concentrations are in mg/m^3 for expired air.

The lung clearance of TCE represents the volume of air from which all TCE can be removed per unit time, and is a measure of the rate of excretion via the lungs. Monster et al. (1976) reported lung clearances ranging from 3.8 to 4.9 L/minute in four adults exposed at rest to 70 and 140 ppm of TCE for 4 hours. Pulmonary ventilation rates in these individuals at rest ranged from 7.7 to 12.3 L/minute. During exercise, when ventilation rates increased to 29–30 L/minute, lung clearance was correspondingly higher, 7.7–12.3 L/minute. Under single and repeated exposure conditions, Monster et al. (1979; 1976) reported that 7−17% of absorbed TCE was excreted in exhaled breath. Pulmonary elimination of unchanged TCE at the end of exposure is a first-order diffusion process across the lungs from blood into alveolar air, and it can be thought of as the reversed equivalent of its uptake from the lungs. Exhaled pulmonary excretion occurs in several distinct (delayed) phases corresponding to release from different tissue groups, at different times. Sato et al. (1977) detected three first-order phases of pulmonary excretion in the first 10 hours after exposure to 100 ppm for 4 hours, with fitted half-times of pulmonary elimination of 0.04, 0.67, and 5.6 hours, respectively. Opdam (1989) sampled alveolar air up to 20–310 hours after 29–62-minute exposures to 6–38 ppm, and reported terminal half-lives of 8–44 hours at rest. Chiu et al. (2007) sampled alveolar air up to 100 hours after 6-hour exposures to 1 ppm and reported terminal half-lives of 14–23 hours. The long terminal half-time of TCE pulmonary excretion indicates that considerable time is necessary to completely eliminate the compound, primarily due to the high partitioning to adipose tissues (see Section 3.2).

As discussed above, several studies (Green and Prout, 1985; Prout et al., 1985; Dekant et al., 1984) have investigated the disposition of [^{14}C]-TCE in rats and mice following gavage administrations (see Section 3.3.2). These studies have reported CO_2 as an exhalation excretion product in addition to unchanged TCE. With low doses, the amount of TCE excreted unchanged in exhaled breath is relatively low. With increasing dose in rats, a disproportionately increased amount of radiolabel is expired as unchanged TCE. This may indicate saturation of metabolic activities in rats at doses ≥200 mg/kg, which is perhaps only minimally apparent in the data from mice. In addition, exhaled air TCE concentration has been measured after constant inhalation exposure for 2 hours to 50 or 500 ppm in rats (Dallas et al., 1991), and after dermal exposure in rats and humans (Poet et al., 2000). Exhaled TCE data from rodents and humans have been integrated into the PBPK model presented in Section 3.5.

Finally, TCOH is also excreted in exhaled breath, though at a rate about 10,000-fold lower than unmetabolized TCE (Monster, 1979; Monster et al., 1976).

3.4.2. Urine

Urinary excretion after TCE exposure consists predominantly of the metabolites, TCA and TCOH, with minor contributions from other oxidative metabolites and GSH conjugates.

Measurements of unchanged TCE in urine have been at or below detection limits (e.g., Chiu et al., 2007; Fisher et al., 1998). The recovery of urinary oxidative metabolites in mice, rats, and humans was addressed earlier (see Section 3.3.2) and will not be discussed here. Because of their relatively long elimination half-life, urinary oxidative metabolites have been used as an occupational biomarker of TCE exposure for many decades (Carrieri et al., 2007; Ikeda and Imamura, 1973). Ikeda and Imamura (1973) measured TTCs, TCOH, and TCA in urine over 3 consecutive postexposure days for four exposure groups totaling 24 adult males and one exposure group comprising 6 adult females. The elimination half-lives for TTC were 26.1–48.8 hours in males and 50.7 hours in females. The elimination half-lives for TCOH were 15.3 hours in the only group of males studied and 42.7 hours in females. The elimination half-lives for TCA were 39.7 hours in the only group of males studied and 57.6 hours in females. These authors compared their results to previously published elimination half-lives for TTC, TCOH, and TCA. Following experimental exposures of groups of two–five adults, elimination half-lives were 31−50 hours for TTC, 19–29 hours for TCOH, and 36–55 hours for TCA (Nomiyama and Nomiyama, 1971; Ogata et al., 1971; Stewart et al., 1970; Bartonicek, 1962). The urinary elimination half-lives of TCE metabolites in a subject who worked with and was addicted to sniffing TCE for 6–8 years approximated 49.7 hours for TCOH, 72.6 hours for TCA, and 72.6 hours for TTC (Ikeda et al., 1971).

The quantitative relationship between urinary concentrations of oxidative metabolites and exposure in an occupational setting was investigated by Ikeda (1977). This study examined the urinary elimination of TCE and metabolites in urine of 51 workers from 10 workshops. The concentration of TCA and TCOH in urine demonstrated a marked concentration-dependence, with concentrations of TCOH being approximately twice as high as those for TCA. Urinary half-life values were calculated for six males and six females from five workshops; males were intermittently exposed to 200 ppm and females were intermittently exposed to 50 ppm (269 mg/m^3). Urinary elimination half-lives for TTC, TCOH, and TCA were 26.1, 15.3, and 39.7 hours in males, respectively, and 50.7, 42.7 and 57.6 hours in females, respectively, which were similar to the range of values previously reported. These authors estimated that urinary elimination of parent TCE during exposure might account for one-third of the systemically absorbed dose. Importantly, urinary TCA exhibited marked saturation at exposures >50 ppm. Because neither TTC nor urinary TCOH (in the form of the glucuronide TCOG) showed such an effect, this saturation cannot be due to TCE oxidation itself, but must rather be from one of the metabolic processes forming TCA from TCOH. Unfortunately, since biological monitoring programs usually measure only urinary TCA, rather than TTC, urinary TCA levels above around 150 mg/L cannot distinguish between exposures at 50 ppm and at much higher concentrations.

It is interesting to attempt to extrapolate on a cumulative exposure basis the Ikeda (1977) results for urinary metabolites obtained after occupational exposures at 50 ppm to the controlled exposure study by Chiu et al. (2007) at 1.2 ppm for 6 hours (the only controlled exposure study

for which urinary concentrations, rather than only cumulative excretion, are available). Ikeda (1977) reported that measurements were made during the second half of the week, so one can postulate a cumulative exposure duration of 20~40 hours. At 50 ppm, Ikeda (1977) report a urinary TCOH concentration of about 290 mg/L, so that per ppm-hour, the expected urinary concentration would be 290/(50 × 20 ~ 40) = 0.145 ~ 0.29 mg/L-ppm-hour. The cumulative exposure in Chiu et al. (2007) is 1.2 × 6 = 7.2 ppm-hour, so the expected urinary TCOH concentration would be 7.2 × (0.145 ~ 0.29) = 1.0 ~ 2.1 mg/L. This estimate is somewhat surprisingly consistent with the actual measurements of Chiu et al. (2007) during the first day postexposure, which ranged from 0.8 to ~1.2 mg/L TCOH in urine.

On the other hand, extrapolation of TCA concentrations was less consistent. At 50 ppm, Ikeda (1977) report a urinary TCA concentration of about 140 mg/L, so that per ppm-hour, the expected urinary concentration would be 140/(50 × 20 ~ 40) = 0.07 ~ 0.14 mg/L-ppm-hour. The cumulative exposure in Chiu et al. (2007) is 1.2 × 6 = 7.2 ppm-hour, so the expected urinary TCA concentration would be 7.2 × (0.07 ~ 0.14) = 0.5 ~ 1.0 mg/L, whereas Chiu et al. (2007) reported urinary TCA concentrations on the first day after exposure of 0.03~0.12 mg/L. However, as noted in Chiu et al. (2007), relative urinary excretion of TCA was 3–10-fold lower in Chiu et al. (2007) than other studies at exposures of 50~140 ppm, which may explain part of the discrepancies. However, this may be due, in part, to saturation of many urinary TCA measurements, and, furthermore, interindividual variance, observed to be substantial in Fisher et al. (1998), cannot be ruled out.

Urinary elimination kinetics have been reported to be much faster in rodents than in humans. For instance, adult rats were exposed to 50, 100, or 250 ppm (269, 537, or 1,344 mg/m^3) via inhalation for 8 hours or were administered an i.p. injection (1.47 g/kg) and the urinary elimination of TTCs was followed for several days (Ikeda and Imamura, 1973). These authors calculated urinary elimination half-lives of 14.3–15.6 hours for female rats and 15.5–16.6 hours for male rats; the route of administration did not appear to influence half-life value. In other rodent experiments using orally administered radiolabeled TCE, urinary elimination was complete within 1 or 2 days after exposure (Green and Prout, 1985; Prout et al., 1985; Dekant et al., 1984).

3.4.3. Feces

Fecal elimination accounts for a small percentage of TCE as shown by limited information in the available literature. Bartonicek (1962) exposed seven volunteers to 1.042 mg TCE/L air for 5 hours and examined TCOH and TCA in feces on the 3rd and 7th day following exposure. The mean amount of TCE retained during exposure was 1,107 mg, representing 51–64% (mean 58%) of administered dose. On the 3rd day following TCE exposure, TCOH and TCA in feces demonstrated mean concentrations of 17.1 and 18.5 mg/100 g feces, similar to concentrations in urine. However, because of the 10-fold smaller daily rate of excretion of feces

relative to urine, this indicates fecal excretion of these metabolites is much less significant than urinary excretion. Neither TCOH nor TCA was detected in feces on the 7th day following exposure.

In rats and mice, total radioactivity has been used to measure excretion in feces after gavage TCE administration in corn oil, but since the radiolabel was not characterized, it is not possible to determine whether the radiolabel in feces represented unabsorbed parent compound, excreted parent compound, and/or excreted metabolites. Dekant et al. (1984) reported that mice eliminated 5% of the total administered TCE, while rats eliminated 2% after gavage. Dekant et al. (1986b) reported a dose-response-related increase in fecal elimination with dose, ranging between 0.8 and 1.9% in rats and between 1.6 and 5% in mice after gavage in corn oil. Due to the relevant role of CYP2E1 in the metabolism of TCE (see Section 3.3.3.1.6), Kim and Ghanayem (2006) compared fecal elimination in both wild-type and CYP2E1 knockout mice and reported fecal elimination ranging between 4.1 and 5.2% in wild-type and between 2.1 and 3.8% in knockout mice exposed by gavage in aqueous solution.

3.5. PBPK MODELING OF TCE AND ITS METABOLITES

3.5.1. Introduction

PBPK models are extremely useful tools for quantifying the relationship between external measures of exposure and internal measures of toxicologically relevant dose. In particular, for the purposes of this assessment, PBPK models are evaluated for the following: (1) providing additional quantitative insights into the ADME of TCE and metabolites described in the sections above; (2) cross-species pharmacokinetic extrapolation of rodent studies of both cancer and noncancer effects; (3) exposure-route extrapolation; and (4) characterization of human pharmacokinetic variability. The following sections first describe and evaluate previous and current TCE PBPK modeling efforts, then discuss the insights into ADME (1, above), and finally present conclusions as to the utility of the model to predict internal doses for use in dose-response assessment (2–4, above).

3.5.2. Previous PBPK Modeling of TCE for Risk Assessment Application

TCE has an extensive number of both in vivo pharmacokinetic and PBPK modeling studies [see Chiu et al. (2006b) supplementary material, for a review]. Models previously developed for occupational or industrial hygiene applications are not discussed here but are reviewed briefly in Clewell et al. (2000). Models designed for risk assessment applications have focused on descriptions of TCE and its major oxidative metabolites, TCA, TCOH, and TCOG. Most of these models were extensions of the ―first generation‖ of models developed by Fisher and coworkers (Allen and Fisher, 1993; Fisher et al., 1991) in rats, mice, and humans. These models, in turn, are based on a Ramsey and Andersen (1984) structure with flow-limited tissue compartments and equilibrium gas exchange, saturable Michaelis-Menten kinetics for oxidative

metabolism, and lumped volumes for the major circulating oxidative metabolites TCA and TCOH. Fisher and coworkers updated their models with new in vivo and in vitro experiments performed in mice (Greenberg et al., 1999; Abbas and Fisher, 1997) and volunteers (Fisher et al., 1998) and summarized their findings in Fisher (2000). Clewell et al. (2000) added enterohepatic recirculation of TCOG and pathways for local oxidative metabolism in the lung and GST metabolism in the liver. While Clewell et al. (2000) does not include the updated Fisher (2000) data, they have used a wider set of in vivo and in vitro mouse, rat, and human data than previous models. Finally, Bois (2000a, b) performed reestimations of PBPK model parameters for the Fisher and Clewell models using a Bayesian population approach [Gelman (1996), and discussed further below].

As discussed in Rhomberg (2000), the choice as to whether to use the Fisher, Clewell, and/or Bois models for cross-species extrapolation of rodent cancer bioassays led to quantitative results that differed by as much as an order of magnitude. There are a number of differences in modeling approaches that can explain their differing results. First, the Clewell et al. (2000) model differed structurally in its use of single-compartment volume-of-distribution models for metabolites as opposed to the Fisher (Fisher, 2000) models, which use multiple physiologic compartments. Also, the Clewell et al. (2000) model, but not the Fisher models, includes enterohepatic recirculation of TCOH/TCOG (although reabsorption was set to zero in some cases). In addition to structural differences in the models, the input parameter values for these various models were calibrated using different subsets of the overall in vivo database [see Chiu et al. (2006b), supplementary material, for a review]. The Clewell et al. (2000) model is based primarily on a variety of data published before 1995; the Fisher (2000) models were based primarily on new studies conducted by Fisher and coworkers (after 1997); and the Bois (2000a, b) reestimations of the parameters for the Clewell et al. (2000) and Fisher (2000) models used slightly different data sets than the original authors. The Bois (2000a, b) reanalyses also led to somewhat different parameter estimates than the original authors, both because of the different data sets used as well as because the methodology used by Bois allowed many more parameters to be estimated simultaneously than were estimated in the original analyses.

Given all of these methodological differences, it is not altogether surprising that the different models led to different quantitative results. Even among the Fisher models themselves, Fisher (2000) noted some inconsistencies, including differing estimates for metabolic parameters between mouse gavage and inhalation experiments. These authors included possible explanations for these inconsistencies: the impact of corn oil vehicle use during gavage (Staats et al., 1991) and the impact of a decrease in ventilation rate in mice due to sensory irritation during the inhalation of solvents [e.g., Stadler and Kennedy (1996)].

As discussed in a report by the National Research Council (NRC, 2006), several additional PBPK models relevant to TCE pharmacokinetics have been published since 2000 and are reviewed briefly here. Poet et al. (2000) incorporated dermal exposure to TCE in PBPK

models in rats and humans, and published in vivo data in both species from dermal exposure (Poet et al., 2000; Thrall and Poet, 2000). Albanese et al. (2002) published a series of models with more complex descriptions of TCE distribution in adipose tissue but did not show comparisons with experimental data. Simmons et al. (2002) developed a PBPK model for TCE in the Long-Evans rat that focused on neurotoxicity endpoints and compared model predictions with experimentally determined TCE concentrations in several tissues, including the brain. Keys et al. (2003) investigated the lumping and unlumping of various tissue compartments in a series of PBPK models in the rat and compared model predictions with TCE tissue concentrations in a multitude of tissues. Although none of these TCE models included metabolite descriptions, the experimental data were available for either model or evaluation. Finally, Keys et al. (2004) developed a model for DCA in the mouse that included a description of suicide inhibition of GST-zeta, but this model was not been linked to TCE.

3.5.3. Development and Evaluation of an Interim "Harmonized" TCE PBPK Model

Throughout 2004, EPA and the U.S. Air Force jointly sponsored an integration of the Fisher, Clewell, and Bois modeling efforts (Hack et al., 2006). In brief, a single interim PBPK model structure combining features from both the Fisher and Clewell models was developed and used for all three species of interest (mice, rats, and humans). An effort was made to combine structures in as simple a manner as possible; the evaluation of most alternative structures was left for future work. The one level of increased complexity introduced was inclusion of species- and dose-dependent TCA plasma binding, although only a single in vitro study of Lumpkin et al. (2003) was used as parameter inputs. As part of this joint effort, a hierarchical Bayesian population analysis using Markov chain Monte Carlo (MCMC) sampling [similar to the Bois (2000a, b) analyses] was performed on the revised model with a cross-section of the combined database of kinetic data to provide estimates of parameter uncertainty and variability (Hack et al., 2006). Particular attention was given to using data from each of the different efforts, but owing to time and resource constraints, a combined analysis of all data was not performed. The results from this effort suggested that a single model structure could provide reasonable fits to a variety of data evaluated for TCE and its major oxidative metabolites TCA, TCOH, and TCOG. However, in many cases, different parameter values—particularly for metabolism—were required for different studies, indicating significant interindividual or interexperimental variability. In addition, these authors concluded that dosimetry of DCA, conjugative metabolites, and metabolism in the lung remained highly uncertain (Hack et al., 2006).

Subsequently, EPA conducted a detailed evaluation of the Hack et al. (2006) model that included: (1) additional model runs to improve convergence; (2) evaluation of posterior distributions for population parameters; and (3) comparison of model predictions both with the data used in the Hack et al. (2006) analysis as well as with additional data sets identified in the

literature. Appendix A provides the details and conclusions of this evaluation, briefly summarized in Table 3-31, along with their pharmacokinetic implications.

3.5.4. PBPK Model for TCE and Metabolites Used for This Assessment

3.5.4.1. Introduction

Based on the recommendations of the NRC (2006) as well as additional analysis and evaluation of the Hack et al. (2006) PBPK model, an updated PBPK model for TCE and metabolites was developed for use in this risk assessment. The updated model is reported in Evans et al. (2009) and Chiu et al. (2009), and the discussion below provides some details in additional to the information in the published articles.

This updated model included modification of some aspects of the Hack et al. (2006) PBPK model structure, incorporation of additional in vitro and in vivo data for estimating model parameters, and an updated hierarchical Bayesian population analysis of PBPK model uncertainty and variability. In the subsections below, the updated PBPK model and baseline parameter values are described, as well as the approach and results of the analysis of PBPK model uncertainty and variability. Appendix A provides more detailed descriptions of the model and parameters, including background on hierarchical Bayesian analyses, model equations, statistical distributions for parameter uncertainty and variability, data sources for these parameter values, and the PBPK model code. Additional computer codes containing input files to the MCSim program are available electronically.

Table 3-31. Conclusions from evaluation of Hack et al. (2006), and implications for PBPK model development

Conclusion from evaluation of Hack et al. (2006) model	Implications for PBPK model parameters, structure, or data
For some model parameters, posterior distributions were somewhat inconsistent with the prior distributions. • For parameters with strongly informative priors (e.g., tissue volumes and flows), this may indicate errors in the model. • For many parameters, the prior distributions were based on visual fits to the same data. If the posteriors are inconsistent, then the priors were "inappropriately" informative, and, thus, the same data were used twice.	Reevaluation of all prior distributions. • Update priors for parameters with independent data (physiological parameters, partition coefficients, in vitro metabolism), looking across all available data sets. • For priors without independent data (e.g., many metabolism parameters), use less informative priors (e.g., log-uniform distributions with wide bounds) to prevent bias. Evaluate modifications to the model structure, as discussed below.
A number of data sets involve TCE (i.a., portal vein), TCA (oral, i.v.), and TCOH (oral, i.v.) dosing routes that are not currently in the model, but could be useful for calibration.	• Additional dosing routes can be added easily.
TCE concentrations in blood, air, and tissues well-predicted only in rats, not in mice and humans. Specifically: • In mice, the oral uptake model could not account for the time-course of several data sets. Blood TCE concentrations after inhalation were consistently overpredicted. • In rats, tissue concentrations measured in data not used for calibration were accurately predicted. • In humans, blood and air TCE concentrations were consistently overpredicted in the majority of (but not all) data sets.	• In mice, uptake from the stomach compartment (currently zero), but previously included in Abbas and Fisher (1997), may improve the model fit. • In mice and humans, additional extrahepatic metabolism, either presystemic (e.g., in the lung) or postsystemic (e.g., in the kidney) and/or a wash-in/wash-out effect may improve the model fit.
Total metabolism appears well-predicted in rats and mice based on closed-chamber data, but required significantly different V_{MAX} values between dose groups. Total recovery in humans (60–70%) is less than the model would predict. In all three species, the ultimate disposition of metabolism is uncertain. In particular, there are uncertainties in attributing the "missing" metabolism to • GSH pathway (e.g., urinary mercapturates may only capture a fraction of the total flux; moreover, in Bernauer et al. (1996), excretion was still on-going at end of collection period; model does not accurately depict time-course of mercapturate excretion). • Other hepatic oxidation (currently attributed to DCA). • Extrahepatic systemic metabolism (e.g., kidney). • Presystemic metabolism in the lung. • Additional metabolism of TCOH or TCA (see below).	• Calibration of GSH pathway may be improved by utilizing in vitro data on liver and kidney GSH metabolism, adding a DCVG compartment to improve the prediction of the time-course for mercapturate excretion, and/or using the Lash et al. (1999b) blood DCVG in humans (necessitating the addition of a DCVG compartment). • Presystemic lung metabolism can only be evaluated if added to the model (in vitro data exist to estimate the V_{MAX} for such metabolism). In addition, a wash-in/wash-out effect (e.g., suggested by Greenberg et al., (1999) can be evaluated using a continuous breathing model that separately tracks inhaled and exhaled air, with adsorption/desorption in the respiratory tract. • Additional elimination pathways for TCOH and TCA can be added for evaluation.

Table 3-31. Conclusions from evaluation of Hack et al. (2006), and implications for PBPK model development (continued)

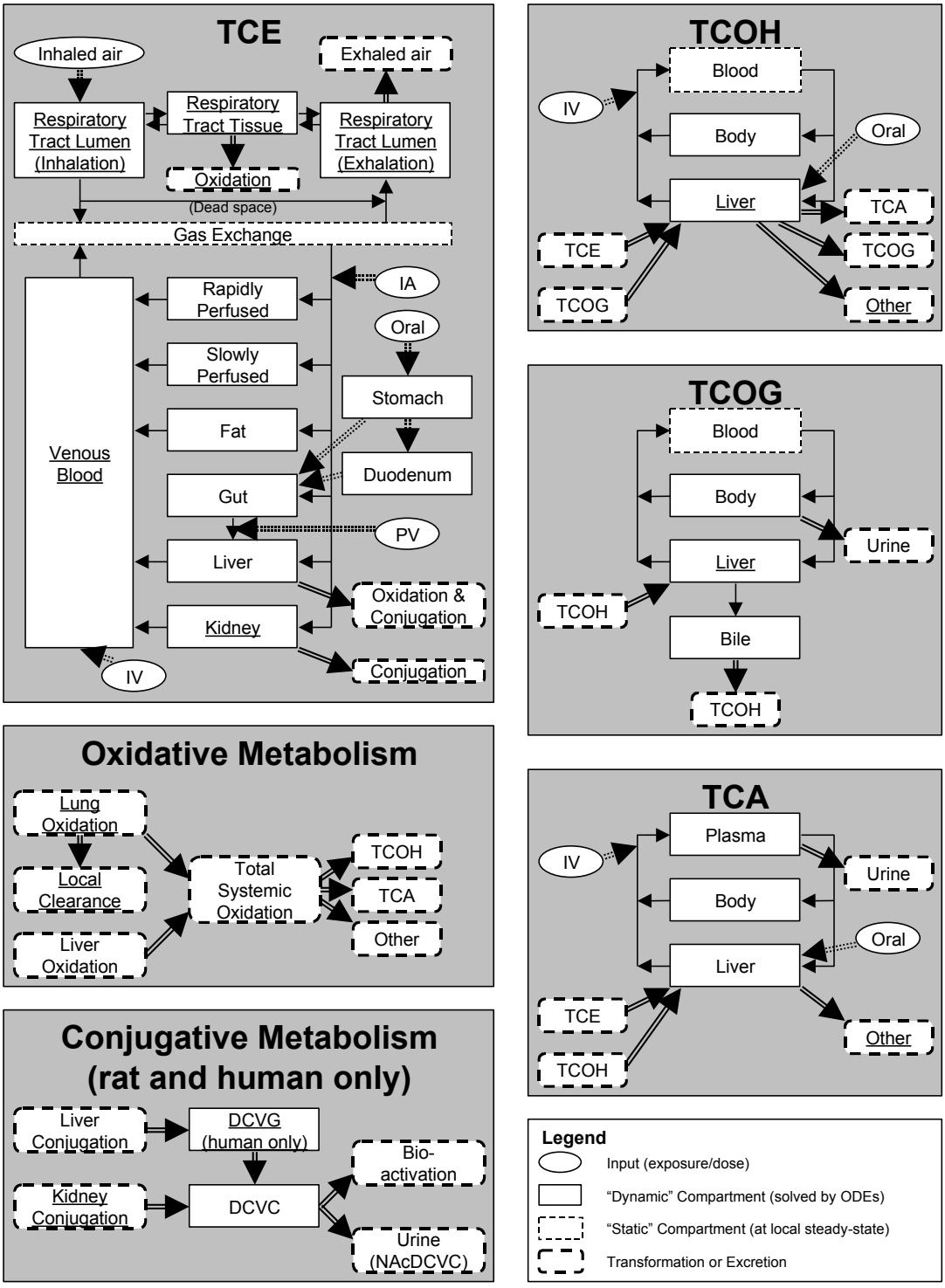
Conclusion from evaluation of Hack et al. (2006) model	Implications for PBPK model parameters, structure, or data
TCA blood/plasma concentrations were well-predicted following TCE exposures in all species. However, there may be inaccuracies in the total flux of TCA production, as well as its disposition. • In TCA dosing studies, the majority (>50%), but substantially <100%, was recovered in urine, suggesting significant metabolism of TCA. Although urinary TCA was well-predicted in mice and humans (but not in rats), if TCA metabolism is significant, then the current model underestimates the flux of TCE metabolism to TCA. • An improved TCOH/TCOG model may also provide better estimates of TCA kinetics (see below). TCOH/TCOG concentrations and excretion were inconsistently predicted, particularly after TCOH dosing. • In mice and rats, first-order clearance for TCOH glucuronidation was predicted to be greater than hepatic blood flow, which is consistent with a first-pass effect that is not currently accounted for. • In humans, the estimated clearance rate for TCOH glucuronidation was substantially smaller than hepatic blood flow. However, the presence of substantial TCOG in blood (as opposed to free TCOH) in the Chiu et al. (2007) data are consistent with greater glucuronidation than predicted by the model. • In TCOH dosing studies, substantially <100% was recovered in urine as TCOG and TCA, suggesting another metabolism or elimination pathway.	• Additional elimination pathways for TCOH and TCA can be added for evaluation. • The addition of a liver compartment for TCOH and TCOG would permit hepatic first-pass effects to be accounted for, as appears necessary for mice and rats.

i.v. = intravenous

3.5.4.2. Updated PBPK Model Structure

The updated TCE PBPK model is illustrated in Figure 3-7, with detailed descriptions of the model structure, equations, and parameters found in Appendix A (see Section A.4), and the major changes from the Hack et al. (2006) model described here. The TCE submodel was augmented by the addition of kidney and venous blood compartments, and an updated respiratory tract model that included both metabolism and the possibility of local storage in the respiratory tissue. In particular, in the updated lung, separate processes describing inhalation and exhalation allowed for adsorption and desorption from tracheobronchial epithelium (wash-in/ wash-out), with the possibility of local metabolism as well. In addition, conjugative metabolism in the kidney was added, motivated by the in vitro data on TCE conjugation described in Sections 3.3.3.2–3.3.3.3. With respect to oxidation, a portion of the lung metabolism was assumed to produce systemically available oxidative metabolites, including TCOH and TCA, with the remaining fraction assumed to be locally cleared. This is clearly a lumping of a multistep process, but the lack of data precludes the development of a more sequential model. TCE oxidation in the kidney was not included because it was not likely to constitute a substantial flux of total TCE oxidation given the much lower CYP activity in the kidney relative to the liver (Cummings and Lash, 2000; Cummings et al., 1999) and the greater tissue mass of the liver.[2] In addition, liver compartments were added to the TCOH and TCOG submodels to account properly for first-pass hepatic metabolism, which is important for consistency across routes of exposure. Furthermore, additional clearance pathways of TCOH and TCA were added to their respective submodels. With respect to TCE conjugation, in humans, an additional DCVG compartment was added between TCE conjugation and production of DCVC. In addition, it should be noted that the urinary clearance of DCVC represents a lumping of *N*-acetylation of DCVC, deacetylation of NAcDCVC, and urinary excretion NAcDCVC, and that the bioactivation of DCVC represents a lumping of thiol production from DCVC by beta-lyase, sulfoxidation of DCVC by FMO3, and sulfoxidation of NAcDCVC by CYP3A. Such lumping was used because these processes are not individually identifiable given the available data.

[2]The extraction ratio for kidney oxidation is likely to be very low, as shown by the following calculation in rats and humans. In rats, the in vitro kidney oxidative clearance (V_{MAX}/K_M) rate (see Table 3-13, converting units) is 1.64×10^{-7} L/minutes/mg microsomal protein. Converting units using 16 mg microsomal protein to g tissue (Bong et al., 1985) gives a clearance rate per unit tissue mass of 2.6×10^{-6} L/minutes/g kidney. This is more than 1,000-fold smaller than the kidney specific blood flow rate of 6.3×10^{-3} L/minutes/g kidney (Brown et al., 1997). In humans, an in vitro clearance rate of 6.5×10^{-8} L/minutes/mg microsomal protein is derived from the only detectable in vitro oxidation rate from Cummings and Lash (2000) of 0.13 nmol/minutes/mg protein at 2 mM. Using the same conversion from microsomal protein to tissue mass gives a clearance rate of 1.0×10^{-6} L/minutes/g kidney, more than 1,000-fold smaller than the kidney specific blood flow of 3.25×10^{-3} L/minutes/g kidney (Brown et al., 1997). No data on kidney metabolism are available in mice, but the results are likely to be similar. Therefore, even accounting for uncertainties of up to an order of magnitude in the in-vitro-to-in-vivo conversion, kidney oxidation should contribute negligibly to total metabolism of TCE.



Boxes with underlined labels are additions or modifications of the Hack et al. (2006) model, which are discussed in Table 3-32.

Figure 3-7. Overall structure of PBPK model for TCE and metabolites used in this assessment.

Table 3-32. Discussion of changes to the Hack et al. (2006) PBPK model implemented for this assessment

Change to Hack et al. (2006) PBPK model	Discussion
TCE respiratory tract compartments and metabolism	In vitro data indicate that the lung (at least in the mouse) has a significant capacity for oxidizing TCE. However, in the Hack et al. (2006) model, respiratory metabolism was blood flow-limited. The model structure used was inconsistent with other PBPK models in which the same mechanism for respiratory metabolism is assumed (e.g., styrene, Sarangapani et al. (2003). In these models, the main source of exposure in the respiratory tract tissue is from the respiratory lumen—not from the tracheobronchial blood flow. In addition, a wash-in/wash-out effect has also been postulated. The current structure, which invokes a —continuous breathing" model with separate —inhaled" and —exhaled" respiratory lumens, can accommodate both respiratory metabolism due to exposure from the respiratory lumen as well as a wash-in/wash-out effect in which there is temporary storage in the respiratory tract tissue. Moreover, preliminary analyses indicated that these changes to the model structure allowed for a substantially better fit to mouse closed-chamber data under the requirement that all of the dose levels are modeled using the same set of parameters.
TCE kidney compartment	In vitro data indicate that the kidney has a significant capacity for conjugating TCE with GSH.
TCE venous blood compartment	Many PBPK models have used a separate blood compartment. It was believed to be potentially important and feasible to implement here because: (1) TCE blood concentrations were often not well-predicted by the Hack et al. (2006) model; (2) the TCA submodel has a plasma compartment, which is a fraction of the blood volume based on the blood volume; (3) adequate independent information on blood volume is available; and (4) the updated model was to include the i.v. route of exposure.
TCOH and TCOG liver compartments	In mice and rats, the Hack et al. (2006) model estimated a rate of TCOH glucuronidation that exceeded hepatic blood flow (all glucuronidation is assumed to occur in the liver), which indicated a significant first-pass effect. Therefore, a separate liver compartment is necessary to account properly for hepatic first-pass.
TCOH and TCA —other" elimination pathways	Mass-balance studies with TCOH and TCA dosing indicated that, although the majority of TCOH and TCA are excreted in urine, the amount is still substantially <100%. Therefore, additional elimination of TCOH and TCA must exist and should be accounted for.
DCVG compartment (human model only)	Blood DCVG data in humans exist as part of the Fisher et al. (1998) experiments, reported in Lash et al. (1999b), and a DCVG compartment is necessary in order to utilize those data.

3.5.4.3. Specification of Baseline PBPK Model Parameter

Point estimates for PBPK model parameters (—baseline values"), used as central estimates in the prior distributions for population mean parameters in the hierarchical Bayesian statistical model (see Appendix A), were developed using standard methodologies to ensure biological plausibility, and were a refinement of those used in Hack et al. (2006). Because the Bayesian parameter estimation methodology utilizes the majority of the useable in vivo data on TCE pharmacokinetics, all baseline parameter estimates were based solely on measurements independent of the in vivo data. This avoids using the same data in both the prior and the likelihood. These parameters were, in turn, given truncated normal or lognormal distributions for the uncertainty in the population mean. If no independent data were available, as is the case for many —downstream" metabolism parameters, then no baseline value was specified, and a

noninformative prior was used. Section 3.5.5.4, below, discusses the updating of these noninformative priors using interspecies scaling.

In keeping with standard practice, many of the PBPK model parameters were "scaled" by body or organ weights, cardiac output, or allometrically by an assumed (fixed) power of body weight. Metabolic capacity and cardiac output were scaled by the ¾ power of body weight and rate coefficients were scaled by the -¼ power of body weight, in keeping with general expectations as to the relationship between metabolic rates and body size (West et al., 2002; U.S. EPA, 1992). So as to ensure a consistent model structure across species as well as improve the performance of the MCMC algorithm, parameters were further scaled to the baseline point-estimates where available, as was done by Hack et al. (2006). For example, to obtain the actual liver volume (VLivC) in L, a point estimate is first obtained by multiplying the fixed, species-specific baseline point estimate for the fractional liver volume by a fixed body weight (measured or species-specific default) with density of 1 kg/L assumed to convert from kg to L. Then, any deviation from this point estimate is represented by multiplying by a separate "scaled" parameter VLivC that has a value of 1 if there is no deviation from the point estimate. These "scaled" parameters are those estimated by the MCMC algorithm, and for which population means and variances are estimated.

Baseline physiological parameters were reestimated based on the updated tissue lumping (e.g., separate blood and kidney compartments) using the standard references, International Commission on Radiological Protection (ICRP, 2003) and Brown et al. (1997). For a few of these parameters, such as hematocrit and respiratory tract volumes in rodents, additional published sources were used as available, but no attempt was made to compile a comprehensive review of available measurements. In addition, a few parameters, such as the slowly perfused volume, were calculated rather than sampled in order to preserve total mass or flow balances.

For chemical-specific distribution and metabolism parameters, in vitro data from various sources were used. Where multiple measurements had been made, as was the case for many partition coefficients, TCA plasma protein binding parameters, and TCE metabolism, different results were pooled together, with their uncertainty reflected appropriately in the prior distribution. Such in vitro measurements were available for most chemical partition coefficients, except for those for TCOG (TCOH used as a proxy) and DCVG. There were also such data to develop baseline values for the oxidative metabolism of TCE in the liver (V_{MAX} and K_M), the relative split in TCE oxidation between formation of TCA and TCOH, and the V_{MAX} for TCE oxidation in the lung. For GSH conjugation, the geometric means of the in vitro data from Lash et al. (1999a)and Green et al. (1997a) were used as central estimates, with a wide enough uncertainty range to encompass both (widely disparate) estimates. Thus, the prior distribution for these parameters was only mildly informative, and the results are primarily determined by the available in vivo data. All other metabolism parameters were not given baseline values and needed to be estimated from the in vivo data.

3.5.4.4. Dose-Metric Predictions

The purpose of this PBPK model is to make predictions of internal dose in rodents used in toxicity studies or in humans in the general population, and not in the groups or individuals for which pharmacokinetic data exist. Therefore, to evaluate its predictive utility for risk assessment, a number of dose-metrics were selected for simulation in a "generic" mouse, rat, or human, summarized in Table 3-33. The parent dose-metric was AUC in blood. TCE metabolism dose-metrics (i.e., related to the amount metabolized) included both total metabolism, metabolism splits between oxidation vs. conjugation, oxidation in the liver vs. the lung, the amount of oxidation in the liver to products *other* than TCOH and TCA, and the amount of TCA produced. These metabolism rate dose-metrics are scaled by body weight in the case of TCA produced, by the metabolizing tissue volume and by body weight to the ¾ power in the cases of the lung and "other" oxidation in the liver, and by body weight to the ¾ power only in other cases. With respect to the oxidative metabolites, liver concentrations of TCA and blood concentrations of free TCOH were used. With respect to conjugative metabolites, the dose-metrics considered were total GSH metabolism scaled by body weight to the ¾ power, and the amount of DCVC bioactivated (rather than excreted in urine) per unit body weight to the ¾ power and per unit kidney mass.

Table 3-33. PBPK model-based dose-metrics

Abbreviation	Description
ABioactDCVCBW34	Amount of DCVC bioactivated in the kidney (mg) per unit body weight$^{3/4}$ (kg$^{3/4}$)
ABioactDCVCKid	Amount of DCVC bioactivated in the kidney (mg) per unit kidney mass (kg)
AMetGSHBW34	Amount of TCE conjugated with GSH (mg) per unit body weight$^{3/4}$ (kg$^{3/4}$)
AMetLiv1BW34	Amount of TCE oxidized in the liver per unit body weight$^{3/4}$ (kg$^{3/4}$)
AMetLivOtherBW34	Amount of TCE oxidized to metabolites other than TCA and TCOH in the liver (mg) per unit body weight$^{3/4}$ (kg$^{3/4}$)
AMetLivOtherLiv	Amount of TCE oxidized to metabolites other than TCA and TCOH in the liver (mg) per unit liver mass (kg)
AMetLngBW34	Amount of TCE oxidized in the respiratory tract (mg) per unit body weight$^{3/4}$ (kg$^{3/4}$)
AMetLngResp	Amount of TCE oxidized in the respiratory tract (mg) per unit respiratory tract tissue mass (kg)
AUCCBld	Area under the curve of the venous blood concentration of TCE (mg-hr/L)
AUCCTCOH	Area under the curve of the blood concentration of TCOH (mg-hr/L)
AUCLivTCA	Area under the curve of the liver concentration of TCA (mg-hr/L)
TotMetabBW34	Total amount of TCE metabolized (mg) per unit body weight$^{3/4}$ (kg$^{3/4}$)
TotOxMetabBW34	Total amount of TCE oxidized (mg) per unit body weight$^{3/4}$ (kg$^{3/4}$)
TotTCAInBW	Total amount of TCA produced (mg) per unit body weight (kg)

All dose-metrics are converted to daily or weekly averages based on simulations lasting 10 weeks for rats and mice and 100 weeks for humans. These simulation times were the shortest

for which additional simulation length did not add substantially to the average (i.e., less than a few percent change with a doubling of simulation time).

3.5.5. Bayesian Estimation of PBPK Model Parameters, and Their Uncertainty and Variability

3.5.5.1. Updated Pharmacokinetic Database

An extensive search was made for data not previously considered in the PBPK modeling of TCE and metabolites, with a few studies identified or published subsequent to the review by Chiu et al. (2006b). The studies considered for analysis are listed in Tables 3-34 and 3-35, along with an indication of whether and how they were used.[3]

The least amount of data was available for mice, so an effort was made to include as many studies as feasible for use in calibrating the PBPK model parameters. Exceptions include mouse studies with CH or DCA dosing, since those metabolites are not included in the PBPK model. In addition, the Birner et al. (1993) data only reported urine concentrations, not the amount excreted in urine. Because there is uncertainty as to total volume of urine excreted, and over what time period, these data were not used. Moreover, many other studies had urinary excretion data, so this exclusion should have minimal impact. Several data sets not included by Hack et al. (2006) were used here. Of particular importance was the inclusion of TCA and TCOH dosing data from Abbas et al. (1997), Green and Prout (1985), Larson and Bull (1992a), and Templin et al. (1993). A substantial amount of data is available in rats, so some data that appeared to be redundant were excluded from the calibration set and saved for comparison with posterior predictions (a "validation" set). In particular, those used for "validation" are one closed-chamber experiment (Andersen et al., 1987b), several data sets with only TCE blood data (Lee et al., 1996; Jakobson et al., 1986; D'Souza et al., 1985), and selected time courses from Fisher et al. (1991) and Lee et al. (2000a; 2000b), and one unpublished data set (Bruckner et al., unpublished). The Andersen et al. (1987b) data were selected randomly from the available closed-chamber data, while the other data sets were selected because they were unpublished or because they were more limited in scope (e.g., TCE blood only) and so were not as efficient for use in the computationally-intensive calibration stage. As with the mouse analyses, TCA and TCOH dosing data were incorporated to better calibrate those pathways.

[3]Additional in vivo data on TCE or metabolites published after the PBPK modeling was completed (Kim et al., 2009; Liu et al., 2009; Sweeney et al., 2009) were evaluated separately, and discussed in Appendix A.

Table 3-34. Rodent studies with pharmacokinetic data considered for analysis

Reference	Species (strain)	Sex	TCE exposures	Other exposures	Calibration	Validation	Not used	Comments
Mouse studies								
Abbas et al. (1996)	Mouse ($B6C3F_1$)	M	–	CH i.v.			√	CH not in model.
Abbas and Fisher (1997)	Mouse ($B6C3F_1$)	M	Oral (corn oil)	–	√[a]			
Abbas et al. (1997)	Mouse ($B6C3F_1$)	M	–	TCOH, TCA i.v.	√			
Barton et al. (1999)	Mouse ($B6C3F_1$)	M	–	DCA i.v. and oral (aqueous)			√	DCA not in model.
Birner et al. (1993)	Mouse (NMRI)	M+F	Gavage	–			√	Only urine concentrations available, not amount.
Fisher and Allen, (1993)	Mouse ($B6C3F_1$)	M+F	Gavage (corn oil)	–	√			
Fisher et al. (1991)	Mouse ($B6C3F_1$)	M+F	Inhalation	–	√[a]			
Green and Prout (1985)	Mouse ($B6C3F_1$)	M	Gavage (corn oil)	TCA i.v.	√			
Greenberg et al. (1999)	Mouse ($B6C3F_1$)	M	Inhalation	–	√[a]			
Larson and Bull (1992b)	Mouse ($B6C3F_1$)	M	–	DCA, TCA oral (aqueous)	√			Only data on TCA dosing was used, since DCA is not in the model.
Larson and Bull (1992a)	Mouse ($B6C3F_1$)	M	Oral (aqueous)	–	√			
Merdink et al. (1998)	Mouse ($B6C3F_1$)	M	i.v.	CH i.v.	√			Only data on TCE dosing was used, since CH is not in the model.

Table 3-34. Rodent studies with pharmacokinetic data considered for analysis (continued)

Reference	Species (strain)	Sex	TCE exposures	Other exposures	Calibration	Validation	Not used	Comments
Prout et al. (1985)	Mouse ($B6C3F_1$, Swiss)	M	Gavage (corn oil)	–	√[a]			
Templin et al. (1993)	Mouse ($B6C3F_1$)	M	Oral (aqueous)	TCA oral	√[a]			
Rat studies								
Andersen et al. (1997)	Rat (F344)	M	Inhalation	–		√[a]		
Barton et al. (1995)	Rat (Sprague-Dawley)	M	Inhalation	–			√	Initial chamber concentrations unavailable, so not used.
Bernauer et al. (1996)	Rat (Wistar)	M	Inhalation	–	√[a]			
Birner et al. (1993)	Rat (Wistar, F344)	M+F	Gavage (ns)	–			√	Only urine concentrations available, not amount.
Birner et al. (1997)	Rat (Wistar)	M+F	–	DCVC i.v.			√	Single dose, route does not recapitulate how DCVC is formed from TCE, excreted NAcDCVC ~100-fold greater than that from relevant TCE exposures (Bernauer et al., 1996).
Bruckner et al. unpublished	Rat (Sprague-Dawley)	M	Inhalation	–		√		Not published, so not used for calibration. Similar to Keys et al. (2003) data.
Dallas et al. (1991)	Rat (Sprague-Dawley)	M	Inhalation	–	√			
D'Souza et al. (1985)	Rat (Sprague-Dawley)	M	i.v., oral (aqueous)	–			√	Only TCE blood measurements, and ≥10-fold greater than other similar studies.
Fisher et al. (1989)	Rat (F344)	F	Inhalation	–	√			
Fisher et al. (1991)	Rat (F344)	M+F	Inhalation	–	√[a]	√		Experiment with blood only data not used for calibration.

Table 3-34. Rodent studies with pharmacokinetic data considered for analysis (continued)

Reference	Species (strain)	Sex	TCE exposures	Other exposures	Calibration	Validation	Not used	Comments
Green and Prout (1985)	Rat (Osborne-Mendel)	M	Gavage (corn oil)	TCA gavage (aqueous)	√			
Hissink et al. (2002)	Rat (Wistar)	M	Gavage (corn oil), i.v.	–	√			
Jakobson et al. (1986)	Rat (Sprague-Dawley)	F	Inhalation	Various pretreatments (oral)		√		Pretreatments not included. Only blood TCE data available.
Kaneko et al. (1994)	Rat (Wistar)	M	Inhalation	Ethanol pretreatment (oral)	√			Pretreatments not included.
Keys et al. (2003)	Rat (Sprague-Dawley)	M	Inhalation, oral (aqueous), i.a.	–	√			
Kimmerle and Eben (1973b)	Rat (Wistar)	M	Inhalation	–	√			
Larson and Bull (1992b)	Rat (F344)	M	–	DCA, TCA oral (aqueous)	√			Only TCA dosing data used, since DCA is not in the model.
Larson and Bull (1992a)	Rat (Sprague-Dawley)	M	Oral (aqueous)	–	√[a]			
Lash et al. (2006)	Rat (F344)	M+F	Gavage (corn oil)	–			√	Highly inconsistent with other studies.
Lee et al. (1996)	Rat (Sprague-Dawley)	M	Arterial, venous, portal, stomach injections	–		√		Only blood TCE data available.
Lee et al. (2000a; 2000b)	Rat (Sprague-Dawley)	M	Stomach injection, i.v., p.v.	p-Nitrophenol pretreatment (i.a.)	√	√		Pretreatments not included. Only experiments with blood and liver data used for calibration.
Merdink et al. (1999)	Rat (F344)	M	–	CH, TCOH i.v.	√			TCOH dosing used; CH not in model.
Poet et al. (2000)	Rat (F344)	M	Dermal	–			√	Dermal exposure not in model.
Prout et al. (1985)	Rat (Osborne-Mendel, Wistar)	M	Gavage (corn oil)	–	√[a]			

Table 3-34. Rodent studies with pharmacokinetic data considered for analysis (continued)

Reference	Species (strain)	Sex	TCE exposures	Other exposures	Calibration	Validation	Not used	Comments
Saghir et al. (2002)	Rat (F344)	M	–	DCA i.v., oral (aqueous)			√	DCA not in model.
Simmons et al. (2002)	Rat (Long-Evans)	M	Inhalation	–	√			
Stenner et al. (1997)	Rat (F344)	M	intraduodenal	TCOH, TCA i.v.	√			
Templin et al. (1995b)	Rat (F344)	M	Oral (aqueous)	–	√[a]			
Thrall et al. (2000)	Rat (F344)	M	i.v., i.p.	With toluene			√	Only exhaled breath data available from i.v. study; i.p. dosing not in model.
Yu et al. (2000)	Rat (F344)	M	–	TCA i.v.	√			

[a]Part or all of the data in the study was used for calibration in Hack et al. (2006).

p.v. = intraperivenous

Table 3-35. Human studies with pharmacokinetic data considered for analysis

Reference	Species (number of individuals)	Sex	TCE exposures	Other exposures	Calibration	Validation	Not used	Comments
Bartonicek (1962)	Human (n = 8)	M+F	Inhalation	–		√		Sparse data, so not included for calibration to conserve computational resources.
Bernauer et al. (1996)	Human	M	Inhalation	–	√[a]			Grouped data, but unique in that includes NAcDCVC urine data.
Bloemen et al. (2001)	Human (n = 4)	M	Inhalation	–		√		Sparse data, so not included for calibration to conserve computational resources.
Chiu et al. (2007)	Human (n = 6)	M	Inhalation	–	√			
Ertle et al. (1972)	Human	M	Inhalation	CH oral			√	Very similar to Muller data.
Fernandez et al. (1977)	Human	M	Inhalation	–		√		
Fisher et al. (1998)	Human (n = 17)	M+F	Inhalation	–	√[a]			
Kimmerle and Eben (1973a)	Human (n = 12)	M+F	Inhalation	–	√			
Lapare et al. (1995)	Human (n = 4)	M+F	Inhalation	–		√[b]		Complex exposure patterns, and only grouped data available for urine, so used for validation.
Lash et al. (1999b)	Human	M+F	Inhalation	–	√			Grouped only, but unique in that DCVG blood data available (same individuals as Fisher et al. (1998)].
Monster et al. (1976)	Human (n = 4)	M	Inhalation	–	√[b]			Experiments with exercise not included.
Monster et al. (1979)	Human	M	Inhalation	–		√[a]		Grouped data only.
Muller et al. (1972)	Human	ns	Inhalation	–			√	Same data also included in Muller et al. (1975).

Table 3-35. Human studies with pharmacokinetic data considered for analysis (continued)

Reference	Species (number of individuals)	Sex	TCE exposures	Other exposures	Calibration	Validation	Not used	Comments
Muller et al. (1974)	Human	M	Inhalation	CH, TCA, TCOH oral	√	√[a]		TCA and TCOH dosing data used for calibration, since it is rare to have metabolite dosing data. TCE dosing data used for validation, since only grouped data available. CH not in model.
Muller et al. (1975)	Human	M	Inhalation	Ethanol oral		√[a]		Grouped data only.
Paykoc et al. (1945)	Human (n = 3)	ns	–	TCA i.v.	√			
Poet et al. (2000)	Human	M+F	Dermal	–				Dermal exposure not in model.
Sato et al. (1977)	Human	M	Inhalation	–		√		
Stewart et al. (1970)	Human	ns	Inhalation	–		√[a]		
Treibig et al. (1976)	Human	ns	Inhalation	–		√[a]		
Vesterberg and Astrand (1976)	Human	M	Inhalation	–			√	All experiments included exercise, so were not included.

[a]Part or all of the data in the study was used for calibration in Hack et al. (2006).
[b]Grouped data from this study was used for calibration in Hack et al. (2006), but individual data were used here.

The human pharmacokinetic database of controlled exposure studies is extensive, but also more complicated. For the majority of the studies, only grouped or aggregated data were available, and most of those data were saved for "validation" since there remained a large number of studies for which individual data were available. However, some data that may be uniquely informative are only available in grouped form, in particular DCVG blood concentrations, NAcDCVC urinary excretion, and data from TCA and TCOH dosing. While there are analytic uncertainties as to the DCVG blood measurements, discussed above in Section 3.3.3.2.1, they were nonetheless included here because they are the only in vivo data available on this measurement in humans. The uncertainty associated with their use is discussed below (see Section 3.5.7.3.2).

In addition, several human data sets, while having individual data, involved sparse collection at only one or a few time points per exposure (Bloemen et al., 2001; Bartonicek, 1962) and were subsequently excluded to conserve computational resources. Lapare et al. (1995), which involved multiple, complex exposure patterns over the course of a month and was missing the individual urine data, was also excluded due to the relatively low amount of data given the large computational effort required to simulate the data. Several studies also investigated the effects of exercise during exposure on human TCE toxicokinetics. The additional parameters in a model including exercise would include those for characterizing the changes in cardiac output, alveolar ventilation, and regional blood flow as well as their interindividual variability, and would have further increased the computational burden. Therefore, it was decided that such data would be excluded from this analysis. Even with these exclusions, data on a total of 42 individuals, some involving multiple exposures, were included in the calibration.

3.5.5.2. Updated Hierarchical Population Statistical Model and Prior Distributions

While the individual animals of a common strain and sex within a study are likely to vary to some extent, this variability was not included as part of the hierarchical population model for several reasons. First, generally, only aggregated pharmacokinetic data (arithmetic mean and SD or SE) are available from rodent studies. While methods exist for addressing between-animal variability with aggregated data (e.g., Chiu and Bois, 2007), they require a higher level of computational intensity. Second, dose-response data are generally also only separated by sex and strain, and otherwise aggregated. Thus, in analyzing dose-response data (see Chapter 5), one usually has no choice but to treat all of the animals in a particular study of a particular strain and sex as identical units. In the Hack et al. (2006) model, each simulation was treated as a separate observational unit, so different dosing levels within the same study were treated separately and assigned different PBPK model parameters. However, the animals within a study are generally inbred and kept under similarly controlled conditions, whereas animals in different studies—even if of the same strain and sex—likely have differences in genetic lineage, diet, and handling. Thus, animals *within* a study are likely to be much more homogeneous than animals *between*

studies. As a consequence, in the revised model, for rodents, different animals of the same sex and strain in the same study (or series of studies conducted simultaneously) were treated as identical, and grouped together as a single "subject." Thus, the predictions from the population model in rodents simulate "average" pharmacokinetics for a particular "lot" of rodents of a particular species, strain, and sex. Between-animal variability is not explicitly modeled, but it is incorporated in a "residual" error term as part of the likelihood function (see Appendix A, Section A.4.3.4). Therefore, a high degree of within-study variability would be reflected in a high posterior value in the variance of the residual-error.

In humans, however, interindividual variability is of interest, and, furthermore, substantial individual data are available in humans. However, in some studies, the same individual was exposed more than once, so those data should be grouped together [in the Hack et al. (2006) model, they were treated as different "individuals"]. Because the primary interest here is chronic exposure, and because it would add substantially to the computational burden, interoccasion variability—changes in pharmacokinetic parameters in a single individual over time—is not addressed. Therefore, each individual is considered a single "subject," and the predictions from the population model in humans are the "average" across different occasions for a particular individual (adult). Between-occasion variability is not explicitly modeled, but it is incorporated in a "residual" error term as part of the likelihood function (see Appendix A, Section A.4.3.4). Therefore, a high degree of between-occasion variability would be reflected in a high posterior value in the variance of the residual-error.

As discussed in Section 3.3.3.1, sex and (in rodents) strain differences in oxidative metabolism were modest or minimal. While some sex-differences have been noted in GSH metabolism (see Sections 3.3.3.2.7 and 3.3.3.2.8), almost all of the available in vivo data are in males, making it more difficult to statistically characterize that difference with PBPK modeling. Therefore, within a species, different sexes and (in rodents) strains were considered to be drawn from a single, species-level population. For humans, each individual was considered to be drawn from a single (adult) human population.

Thus, from here forward, the term "subject" will be used to refer to both a particular "lot" of a particular rodents' species, strain, and sex for, and a particular human individual. The term "population" will, therefore, refer to the collection of rodent "lots" of the same species and the collection of human individuals.

Figure A-1 in Appendix A illustrates the hierarchical structure. Informative prior distributions reflecting the uncertainty in the population mean and variance, detailed in Appendix A, were updated from those used in Hack et al. (2006) based on an extensive analysis of the available literature. The population variability of the scaling parameter across subjects is assumed to be distributed as a truncated normal distribution, a standard assumption in the absence of specific data suggesting otherwise. Because of the truncation of extreme values, the sensitivity to this choice is expected to be small as long as the true underlying distribution is uni-

modal and symmetric. In addition, most scaling parameters, being strictly positive in their original units, were log-transformed—so these parameters have lognormal distributions in their original units. The uncertainty distribution for the population parameters was assumed to be a truncated normal distribution for population mean parameters and an inverse gamma distribution for population variance parameters—both standard choices in hierarchical models. Section 3.5.5.3, next, discusses specification of prior distributions in the case where no data independent of the calibration data exist.

3.5.5.3. Use of Interspecies Scaling to Update Prior Distributions in the Absence of Other Data

For many metabolic parameters, little or no in vitro or other prior information is available to develop prior distributions. Initially, for such parameters, noninformative priors in the form of log-uniform distributions with a range spanning at least 10^4 were specified. However, in the time available for analysis (up to about 100,000 iterations), only for the mouse did all of these parameters achieve adequate convergence. This suggests that some of these parameters are poorly identified for the rat and human. Additional preliminary runs indicated replacing the log-uniform priors with lognormal priors and/or requiring more consistency between species could improve identifiability sufficiently for adequate convergence. However, an objective method of "centering" the lognormal distributions that did not rely on the in vivo data (e.g., via visual fitting or limited optimization) being calibrated against was necessary in order to minimize potential bias.

Therefore, the approach taken was to consider three species sequentially, from mouse to rat to human, and to use interspecies scaling to update the prior distributions across species. This sequence was chosen because the models are essentially "nested" in this order, the rat model adds to the mouse model the "downstream" GSH conjugation pathways, and the human model adds to the rat model the intermediary DCVG compartment. Therefore, for those parameters with little or no independent data *only*, the mouse posteriors were used to update the rat priors, and both the mouse and rat posteriors were used to update the human priors. Table 3-36 contains a list of the parameters for which this scaling was used to update prior distributions. The scaling relationship is defined by the "scaled parameters" listed in Appendix A (see Section A.4.1, Table A-4), and generally follows standard practice. For instance, V_{MAX} and clearance rates scale by body weight to the ¾ power, whereas K_M values are assumed to not scale, and rate constants (inverse time units) scale by body weight to the -¼ power.

Table 3-36. Parameters for which scaling from mouse to rat, or from mouse and rat to human, was used to update the prior distributions

Parameter with no or highly uncertain a priori data	Mouse → rat	Rat → human	Mouse+ rat → human	Comments
Respiratory lumen→tissue diffusion flow rate	√		√	No a priori information
TCOG body/blood partition coefficient	√		√	Prior centered on TCOH data, but highly uncertain
TCOG liver/body partition coefficient	√		√	Prior centered on TCOH data, but highly uncertain
Fraction of hepatic TCE oxidation not to TCA+TCOH	√		√	No a priori information
V_{MAX} for hepatic TCE GSH conjugation	√			Rat data on at 1 and 2 mM. Human data at more concentrations, so V_{MAX} and K_M can be estimated
K_M for hepatic TCE GSH conjugation	√			
V_{MAX} for renal TCE GSH conjugation	√			Rat data on at 1 and 2 mM. Human data at more concentrations, so V_{MAX} and K_M can be estimated
K_M for renal TCE GSH conjugation	√			
V_{MAX} for Tracheo-bronchial TCE oxidation	√		√	Prior based on activity at a single concentration
K_M for Tracheo-bronchial TCE oxidation	√		√	No a priori information
Fraction of respiratory oxidation entering systemic circulation	√		√	No a priori information
V_{MAX} for hepatic TCOH→TCA	√		√	No a priori information
K_M for hepatic TCOH→TCA	√		√	No a priori information
V_{MAX} for hepatic TCOH→TCOG	√		√	No a priori information
K_M for hepatic TCOH→TCOG	√		√	No a priori information
Rate constant for hepatic TCOH→other	√		√	No a priori information
Rate constant for TCA plasma→urine	√		√	Prior centered at glomerular filtration rate, but highly uncertain
Rate constant for hepatic TCA→other	√		√	No a priori information
Rate constant for TCOG liver→bile	√		√	No a priori information
Lumped rate constant for TCOG bile→TCOH liver	√		√	No a priori information
Rate constant for TCOG→urine	√		√	Prior centered at glomerular filtration rate, but highly uncertain
Lumped rate constant for DCVC→Urinary NAcDCVC		√		Not included in mouse model
Rate constant for DCVC bioactivation		√		Not included in mouse model

[a]See Appendix A, Table A-4 for scaling relationships.

The scaling model is given explicitly as follows. If θ_i are the "scaled" parameters (usually also natural-log-transformed) that are actually estimated, and A is the "universal" (species-independent) parameter, then $\theta_i = A + \varepsilon_i$, where ε_i is the species-specific "departure" from the scaling relationship, assumed to be normally distributed with variance ζ_ε^2. Therefore, the mouse model gives an initial estimate of "A," which is used to update the prior distribution for $\theta_r = A + \varepsilon_r$ in the rat. The rat and mouse together then give a "better" estimate of A, which is used to update the prior distribution for $\theta_h = A + \varepsilon_h$ in the human, with the assumed distribution for ε_h. The mathematical details are given in Appendix A, but three key points in this model are worth noting here:

- It is known that interspecies scaling is not an exact relationship, and that, therefore, in any *particular* case, it may either over- or underestimate. Therefore, the variance in the new priors reflect a combination of (1) the uncertainty in the "previous" species' posteriors as well as (2) a "prediction error" that is distributed lognormally with geometric standard deviation (GSD) of 3.16-fold, so that the 95% confidence range about the central estimate spans 100-fold. This choice was dictated partially by practicality, as larger values of the GSD used in preliminary runs did not lead to adequate convergence within the time available for analysis.

- The rat posterior is a product of its prior (which is based on the mouse posterior) and its likelihood. Therefore, using the rat and mouse posteriors together to update the human priors would use the mouse posterior "twice." Therefore, the rat posterior is disaggregated into its prior and its likelihood using a lognormal approximation (since the prior is lognormal), and only the (approximate) likelihood is used along with the mouse posterior to develop the human prior.

- The model transfers the marginal distributions for each parameter across species, so correlations between parameters are not retained. This is a restriction on the software used for conducting MCMC analyses. However, assuming independence will lead to a "broader" joint distribution, given the same marginal distributions. Therefore, this assumption tends to reduce the weight of the interspecies scaling as compared to the species-specific calibration data.

To summarize, in order to improve rate of the convergence of the MCMC analyses in rats and humans, a sequential approach was used for fitting scaling parameters without strong prior species-specific information. In particular, an additional assumption was made that *across species*, these scaling parameters were, in absence of other information, expected to have a common underlying value. These assumptions are generally based on allometric scaling principles—with partition coefficients and concentrations scaling directly and rate constants scaling by body weight to the -¼ power (so clearances and maximum metabolic capacities would scale by body weight to the ¾ power). These assumptions are used consistently throughout the parameter calibration process. Therefore, after running the mouse model, the posterior distribution for these parameters was used, with an additional error term, as priors for the rat

model. Subsequently, after the mouse and rat model were run, their posterior distributions were combined, with an additional error term, to use a priors for the human model. With this methodology for updating the prior distributions, adequate convergence was achieved for the rat and human after 110,000~140,000 iterations (discussed further below).

3.5.5.4. Implementation

The PBPK model was coded in for use in the MCSim software (version 5.0.0), which was developed particularly for implementing MCMC simulations. As a quality control check, results were checked against the original Hack et al. (2006) model, with the original structures restored and parameter values made equivalent, and the results were within the error tolerances of the ordinary differential equation (ODE) solver after correcting an error in the Hack et al. (2006) model for calculating the TCA liver plasma flow. In addition, the model was translated to MatLab (version 7.2.0.232) with simulation results checked and found to be within the error tolerances of the ODE solver used ("ode15s"). Mass balances were also checked using the baseline parameters, as well as parameters from preliminary MCMC simulations, and found to be within the error tolerances of the ODE solver. Appendix A contains the MCSim model code.

3.5.6. Evaluation of Updated PBPK Model

3.5.6.1. Convergence

As in previous similar analyses (David et al., 2006; Hack et al., 2006; Bois, 2000b, a; Gelman et al., 1996), the potential scale reduction factor "*R*" is used to determine whether different independent MCMC chains have converged to a common distribution. The *R* diagnostic is calculated for each parameter in the model, and represents the factor by which the SD or other measure of scale of the posterior distribution (such as a confidence interval [CI]) may potentially be reduced with additional samples (Gelman et al., 2003). This convergence diagnostic declines to 1 as the number of simulation iterations approaches infinity, so values close to 1 indicate approximate convergence, with values of ≤1.1 commonly considered adequate (Gelman et al., 2003). However, as an additional diagnostic, the convergence of model dose-metric predictions was also assessed. Specifically, dose-metrics for a number of generic exposure scenarios similar to those used in long-term bioassays were generated, and their natural log (due to their approximate lognormal posterior distributions) was assessed for convergence using the potential scale reduction factor "*R*." This is akin to the idea of utilizing sensitivity analysis so that effort is concentrated on calibrating the most sensitive parameters for the purpose of interest. In addition, predictions of interest that do not adequately converge can be flagged as such, so that the statistical uncertainty associated with the limited sample size can be considered.

The mouse model had the most rapid reduction in potential scale reduction factors. Initially, four chains of 42,500 iterations each were run, with the first 12,500 discarded as "burn-in" iterations. The initial decision for determining "burn-in" was determined by visual

inspection. At this point, evaluating the 30,000 remaining iterations, all of the population parameters except for the V_{MAX} for DCVG formation had $R < 1.2$, with only the first-order clearance rate for DCVG formation and the V_{MAX} and K_M for TCOH glucuronidation having $R > 1.1$. For the samples used for inference, all of these initial iterations were treated as "burn-in" iterations, and each chain was then restarted and run for an additional 68,700–71,400 iterations (chains were terminated at the same time, so the number of iterations per chains was slightly different). For these iterations, all values of R were <1.03. Dose-metric predictions calculated for exposure scenarios of 10–600 ppm either continuously or 7 hours/day, 5 days/week and 10–3,000 mg/kg-day either continuously or by gavage 5 days/week. These predictions were all adequately converged, with all values of $R < 1.03$.

As discussed above, for parameters with little or no a priori information, the posterior distributions from the mouse model were used to update prior distributions for the rat model, accounting for both the uncertainty reflected in the mouse posteriors as well as the uncertainty in interspecies extrapolation. Four chains were run to 111,960–128,000 iterations each (chains were terminated at the same time and run on computers with slightly different processing speeds, so the number of iterations per chains was slightly different). As is standard, about the first "half" of the chains (i.e., the first 64,000 iterations) were discarded as "burn-in" iterations, and the remaining iterations were used for inferences. For these remaining iterations, the diagnostic R was <1.1 for all population parameters except the fraction of oxidation not producing TCA or TCOH ($R = 1.44$ for population mean, $R = 1.35$ for population variance), the K_M for TCOH → TCA ($R = 1.19$ for population mean), the V_{MAX} and K_M for TCOH glucuronidation ($R = 1.23$ and 1.12, respectively for population mean, and $R = 1.13$ for both population variances), and the rate of "other" metabolism of TCOH ($R = 1.29$ for population mean and $R = 1.18$ for population variance). Due to resource constraints, chains needed to be stopped at this point. However, these are similar to the degree of convergence reported in Hack et al. (2006). Dose-metric predictions calculated for two inhalation exposure scenarios (10–600 ppm continuously or 7 hours/day, 5 days/week) and two oral exposure scenarios (10–3,000 mg/kg-day continuously or by gavage 5 days/week).

All dose-metric predictions had $R < 1.04$, except for the amount of "other" oxidative metabolism (i.e., not producing TCA or TCOH), which had $R = 1.12$–1.16, depending on the exposure scenario. The poorer convergence of this dose-metric is expected given that a key determining parameter, the fraction of oxidation not producing TCA or TCOH, had the poorest convergence among the population parameters.

For the human model, a set of four chains was run for 74,160–84,690 iterations using "preliminary" updated prior distributions based on the mouse posteriors and preliminary runs of the rat model. Once the rat chains were completed, final updated prior distributions were calculated and the last iteration of the preliminary runs were used as starting points for the final runs. The center of the final updated priors shifted by <25% of the SD of either the preliminary

or revised priors, so that the revised median was between the 40th and 60th percentile of the preliminary median, and vice versa. The SDs changed by <5%. Therefore, the use of the preliminary chains as a starting point should introduce no bias, as long as an appropriate burn-in period is used for the final runs.

The final chains were run for an additional 59,140–61,780 iterations, at which point, due to resource constraints, chains needed to be stopped. After the first 20,000 iterations, visual inspection revealed the chains were no longer dependent on the starting point. These iterations were therefore discarded as "burn-in" iterations, and for the remaining ~40,000 iterations used for inferences. All population mean parameters had $R < 1.1$ except for the respiratory tract diffusion constant ($R = 1.20$), the liver:blood partition coefficient for TCOG ($R = 1.23$), the rate of TCE clearance in the kidney producing DCVG ($R = 1.20$), and the rate of elimination of TCOG in bile ($R = 1.46$). All population variances also had $R < 1.1$ except for the variance for the fraction of oxidation not producing TCOH or TCA ($R = 1.10$). Dose-metric predictions were assessed for continuous exposure scenarios at 1–60 ppm in air or 1–300 mg/kg-day orally. These predictions were all adequately converged with all values of $R < 1.02$.

3.5.6.2. Evaluation of Posterior Parameter Distributions

Posterior distributions of the population parameters need to be checked as to whether they appear reasonable given the prior distributions. Inconsistency between the prior and posterior distributions may indicate insufficiently broad (i.e., due to overconfidence) or otherwise incorrectly specified priors, a misspecification of the model structure (e.g., leading to pathological parameter estimates), or an error in the data. As was done with the evaluation of Hack et al. (2006) in Appendix A, parameters were flagged if the interquartile regions of their prior and posterior distributions did not overlap.

Appendix A contains detailed tables of the "sampled" parameters, and their prior and posterior distributions. Because these parameters are generally scaled one or more times to obtain a physically meaningful parameter, they are difficult to interpret. Therefore, in Tables 3-37–3-39, the prior and posterior population distributions for the PBPK model parameters obtained *after* scaling are summarized. Since it is desirable to characterize the contributions from both uncertainty in population parameters and variability within the population, the following procedure is adopted. First, 500 sets of population parameters (i.e., population mean and variance for each scaling parameter) are either generated from the prior distributions via Monte Carlo or extracted from the posterior MCMC samples—these represent the uncertainty in the population parameters. To minimize autocorrelation, for the posteriors, the samples were obtained by "thinning" the chains to the appropriate degree. From each of these sets of population parameters, 100 sets of "subject"-level parameters were generated by Monte Carlo—each of these represents the population variability, given a *particular* set of population parameters. Thus, a total of 50,000 subjects, representing 100 (variability) each for 500 different

populations (uncertainty), were generated. For each of the 500 populations, the scaling parameters are converted to PBPK model parameters, and the population median and GSD is calculated—representing the central tendency and variability for that population. Then, the median and the 95% CIs for the population median and GSD are calculated, and presented in the tables that follow. Thus, these tables summarize separately the uncertainty in population distribution as well as the variability in the population, while also accounting for correlations among the population-level parameters. Finally, Table 3-40 shows the change in the CI in the population median for the PBPK model parameters between the prior and posterior distributions, as well as the shift in the central estimate (median) of the population median PBPK model parameter.

The prior and posterior distributions for most physiological parameters were similar. The posterior distribution was substantially narrower (i.e., less uncertainty) than the prior distribution only in the case of the diffusion rate from the respiratory lumen to the respiratory tissue, which also was to be expected given the very wide, noninformative prior for that parameter.

For distribution parameters, there were only relatively minor changes between prior and posterior distributions for TCE and TCOH partition coefficients. The posterior distributions for several TCA partition coefficients and plasma binding parameters were substantially narrower than their corresponding priors, but the central estimates were similar, meaning that values at the high and low extremes were not likely. For TCOG as well, partition coefficient posterior distributions were substantially narrower, which was expected given the greater uncertainty in the prior distributions (TCOH partition coefficients were used as a proxy).

Again, posterior distributions indicated that the high and low extremes were not likely. Finally, posterior distribution for the distribution volume for DCVG was substantially narrower than the prior distribution, which only provided a lower bound given by the blood volume. In this case, the upper bounds were substantially lower in the posterior.

Posterior distributions for oral absorption parameters in mice and rats (there were no oral studies in humans) were also informed by the data, as reflected in their being substantially more narrow than the corresponding priors. Finally, with a few exceptions, TCE and metabolite kinetic parameters showed substantially narrower posterior distributions than prior distributions, indicating that they were fairly well specified by the in vivo data. The exceptions were the V_{MAX} for hepatic oxidation in humans (for which there was substantial in vitro data) and the V_{MAX} for respiratory metabolism in mice and rats (although the posterior distribution for the K_M for this pathway was substantially narrower than the corresponding prior).

Table 3-37. Prior and posterior uncertainty and variability in mouse PBPK model parameters

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Cardiac output (L/hr)	QC	0.84 (0.59, 1.2)	1 (0.79, 1.3)	1.17 (1.1, 1.4)	1.35 (1.15, 1.54)
Alveolar ventilation (L/hr)	QP	2.1 (1.3, 3.5)	2.1 (1.5, 2.7)	1.27 (1.17, 1.54)	1.45 (1.28, 1.66)
Scaled fat blood flow	QFatC	0.07 (0.03, 0.11)	0.072 (0.044, 0.1)	1.65 (1.22, 2.03)	1.64 (1.3, 1.99)
Scaled gut blood flow	QGutC	0.14 (0.11, 0.17)	0.16 (0.14, 0.17)	1.15 (1.09, 1.19)	1.12 (1.07, 1.19)
Scaled liver blood flow	QLivC	0.02 (0.016, 0.024)	0.021 (0.017, 0.024)	1.15 (1.09, 1.19)	1.15 (1.09, 1.19)
Scaled slowly perfused blood flow	QSlwC	0.22 (0.14, 0.29)	0.21 (0.15, 0.28)	1.3 (1.15, 1.38)	1.3 (1.17, 1.39)
Scaled rapidly perfused blood flow	QRapC	0.46 (0.37, 0.56)	0.45 (0.37, 0.52)	1.15 (1.11, 1.2)	1.17 (1.12, 1.2)
Scaled kidney blood flow	QKidC	0.092 (0.054, 0.13)	0.091 (0.064, 0.12)	1.34 (1.14, 1.45)	1.34 (1.18, 1.44)
Respiratory lumen:tissue diffusive clearance rate (L/hr)	DResp	0.017 (0.000032, 15)	2.5 (1.4, 5.1)	1.37 (1.25, 1.62)	1.53 (1.37, 1.73)
Fat fractional compartment volume	VFatC	0.071 (0.032, 0.11)	0.089 (0.061, 0.11)	1.59 (1.19, 1.93)	1.4 (1.19, 1.78)
Gut fractional compartment volume	VGutC	0.049 (0.041, 0.057)	0.048 (0.042, 0.055)	1.11 (1.07, 1.14)	1.11 (1.08, 1.14)
Liver fractional compartment volume	VLivC	0.054 (0.038, 0.071)	0.047 (0.037, 0.06)	1.22 (1.12, 1.29)	1.23 (1.17, 1.3)
Rapidly perfused fractional compartment volume	VRapC	0.1 (0.087, 0.11)	0.099 (0.09, 0.11)	1.08 (1.05, 1.11)	1.09 (1.06, 1.11)
Fractional volume of respiratory lumen	VRespLumC	0.0047 (0.004, 0.0053)	0.0047 (0.0041, 0.0052)	1.09 (1.06, 1.12)	1.09 (1.07, 1.12)
Fractional volume of respiratory tissue	VRespEffC	0.0007 (0.0006, 0.00079)	7e-04 (0.00062, 0.00078)	1.09 (1.06, 1.12)	1.1 (1.07, 1.12)
Kidney fractional compartment volume	VKidC	0.017 (0.015, 0.019)	0.017 (0.015, 0.019)	1.08 (1.05, 1.11)	1.09 (1.06, 1.11)
Blood fractional compartment volume	VBldC	0.049 (0.042, 0.056)	0.048 (0.043, 0.054)	1.1 (1.06, 1.13)	1.1 (1.08, 1.13)

Table 3-37. Prior and posterior uncertainty and variability in mouse PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Slowly perfused fractional compartment volume	VSlwC	0.55 (0.5, 0.59)	0.54 (0.51, 0.57)	1.05 (1.04, 1.07)	1.05 (1.04, 1.07)
Plasma fractional compartment volume	VPlasC	0.026 (0.016, 0.036)	0.022 (0.016, 0.029)	1.24 (1.15, 1.35)	1.27 (1.19, 1.36)
TCA body fractional compartment volume [not incl. blood+liver]	VBodC	0.79 (0.77, 0.8)	0.79 (0.78, 0.81)	1.01 (1.01, 1.02)	1.01 (1.01, 1.02)
TCOH/G body fractional compartment volume [not incl. liver]	VBodTCOHC	0.84 (0.82, 0.85)	0.84 (0.83, 0.85)	1.01 (1.01, 1.02)	1.01 (1.01, 1.02)
TCE blood:air partition coefficient	PB	15 (10, 23)	14 (11, 17)	1.22 (1.12, 1.42)	1.44 (1.28, 1.53)
TCE fat:blood partition coefficient	PFat	36 (21, 62)	36 (26, 49)	1.26 (1.14, 1.52)	1.32 (1.16, 1.56)
TCE gut:blood partition coefficient	PGut	1.9 (0.89, 3.8)	1.5 (0.94, 2.6)	1.36 (1.2, 1.75)	1.36 (1.2, 1.79)
TCE liver:blood partition coefficient	PLiv	1.7 (0.89, 3.5)	2.2 (1.3, 3.3)	1.37 (1.2, 1.75)	1.39 (1.21, 1.84)
TCE rapidly perfused:blood partition coefficient	PRap	1.8 (0.98, 3.7)	1.8 (1.1, 3)	1.37 (1.2, 1.76)	1.37 (1.2, 1.77)
TCE respiratory tissue:air partition coefficient	PResp	2.7 (1.2, 5)	2.5 (1.5, 4.2)	1.36 (1.19, 1.78)	1.37 (1.19, 1.74)
TCE kidney:blood partition coefficient	PKid	2.2 (0.96, 4.6)	2.6 (1.7, 4)	1.36 (1.2, 1.77)	1.51 (1.25, 1.88)
TCE slowly perfused:blood partition coefficient	PSlw	2.4 (1.2, 4.9)	2.2 (1.4, 3.5)	1.38 (1.2, 1.78)	1.39 (1.21, 1.8)
TCA blood:plasma concentration ratio	TCAPlas	0.76 (0.4, 16)	1.1 (0.75, 1.8)	1.21 (1.09, 1.58)	1.23 (1.1, 1.73)
Free TCA body:blood plasma partition coefficient	PBodTCA	0.77 (0.27, 17)	0.87 (0.59, 1.5)	1.41 (1.23, 1.8)	1.39 (1.24, 1.9)
Free TCA liver:blood plasma partition coefficient	PLivTCA	1.1 (0.36, 21)	1.1 (0.64, 1.9)	1.41 (1.23, 1.8)	1.4 (1.24, 1.87)
Protein:TCA dissociation constant (μmole/L)	kDissoc	100 (13, 790)	130 (24, 520)	2.44 (1.73, 5.42)	2.64 (1.75, 5.45)

Table 3-37. Prior and posterior uncertainty and variability in mouse PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Maximum binding concentration (μmole/L)	B_{MAX}	87 (9.6, 790)	140 (28, 690)	2.72 (1.92, 5.78)	2.88 (1.93, 5.89)
TCOH body:blood partition coefficient	PBodTCOH	1.1 (0.61, 2.1)	0.89 (0.65, 1.3)	1.29 (1.16, 1.66)	1.31 (1.17, 1.61)
TCOH liver:body partition coefficient	PLivTCOH	1.3 (0.73, 2.3)	1.9 (1.2, 2.6)	1.3 (1.16, 1.61)	1.35 (1.18, 1.68)
TCOG body:blood partition coefficient	PBodTCOG	0.95 (0.016, 77)	0.48 (0.18, 1.1)	1.36 (1.19, 2.05)	1.41 (1.22, 2.19)
TCOG liver:body partition coefficient	PLivTCOG	1.3 (0.019, 92)	1.3 (0.64, 2.6)	1.36 (1.18, 2.13)	1.56 (1.28, 2.52)
DCVG effective volume of distribution	VDCVG	0.033 (0.0015, 15)	0.027 (0.0016, 4.1)	1.28 (1.08, 1.97)	1.31 (1.1, 2.19)
TCE stomach absorption coefficient (/hr)	kAS	1.7 (0.0049, 450)	1.7 (0.37, 13)	4.74 (2.29, 23.4)	4.28 (2.39, 13.4)
TCE stomach-duodenum transfer coefficient (/hr)	kTSD	1.4 (0.043, 51)	4.5 (0.51, 26)	3.84 (2.09, 10.6)	4.79 (2.53, 10.9)
TCE duodenum absorption coefficient (/hr)	kAD	1.2 (0.0024, 200)	0.27 (0.067, 1.6)	4.33 (2.14, 26)	4.17 (2.34, 14.4)
TCA stomach absorption coefficient (/hr)	kASTCA	0.63 (0.0027, 240)	4 (0.2, 74)	4.26 (2.27, 23.4)	5.15 (2.56, 22)
V_{MAX} for hepatic TCE oxidation (mg/hr)	V_{MAX}	3.9 (1.4, 15)	2.5 (1.6, 4.2)	2.02 (1.56, 2.85)	1.86 (1.59, 2.47)
K_M for hepatic TCE oxidation (mg/L)	K_M	34 (1.6, 620)	2.7 (1.4, 8)	1.25 (1.15, 1.61)	2.08 (1.48, 3.49)
Fraction of hepatic TCE oxidation not to TCA+TCOH	FracOther	0.43 (0.0018, 1)	0.023 (0.0037, 0.15)	1.23 (1, 2.13)	1.49 (1.25, 2.83)
Fraction of hepatic TCE oxidation to TCA	FracTCA	0.086 (0.00022, 0.66)	0.13 (0.084, 0.21)	1.48 (1.12, 2.56)	1.4 (1.21, 1.96)
V_{MAX} for hepatic TCE GSH conjugation (mg/hr)	V_{MAX}DCVG	3.7 (0.0071, 2,800)	0.6 (0.01, 480)	1.55 (1.33, 2.52)	1.61 (1.37, 2.91)
K_M for hepatic TCE GSH conjugation (mg/L)	K_MDCVG	250 (0.0029, 6,500,000)	2200 (0.17, 2,300,000)	1.81 (1.47, 3.62)	1.93 (1.49, 3.68)

Table 3-37. Prior and posterior uncertainty and variability in mouse PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
V_{MAX} for renal TCE GSH conjugation (mg/hr)	V_{MAX}KidDCVG	0.34 (0.00051, 180)	0.027 (0.0012, 13)	1.49 (1.26, 2.49)	1.54 (1.28, 2.72)
K_M for renal TCE GSH conjugation (mg/L)	K_MKidDCVG	150 (0.0053, 6,200,000)	160 (0.078, 280,000)	1.79 (1.43, 3.45)	1.91 (1.5, 3.91)
V_{MAX} for tracheo-bronchial TCE oxidation (mg/hr)	V_{MAX}Clara	0.24 (0.03, 3.9)	0.42 (0.1, 1.5)	2.32 (1.74, 3.66)	4.13 (2.27, 6.79)
K_M for tracheo-bronchial TCE oxidation (mg/L)	K_MClara	1.5 (0.0018, 630)	0.011 (0.0024, 0.09)	1.47 (1.25, 2.58)	1.63 (1.28, 5.02)
Fraction of respiratory metabolism to systemic circ.	FracLungSys	0.34 (0.0016, 1)	0.78 (0.18, 0.99)	1.24 (1, 2.1)	1.11 (1, 1.72)
V_{MAX} for hepatic TCOH→TCA (mg/hr)	V_{MAX}TCOH	0.064 (0.000014, 380)	0.12 (0.048, 0.28)	1.5 (1.24, 2.61)	1.6 (1.28, 2.92)
K_M for hepatic TCOH→TCA (mg/L)	K_MTCOH	1.4 (0.00018, 5,300)	0.92 (0.26, 2.7)	1.48 (1.24, 2.41)	1.49 (1.26, 2.4)
V_{MAX} for hepatic TCOH→TCOG (mg/hr)	V_{MAX}Gluc	0.11 (0.000013, 310)	4.6 (1.9, 16)	1.48 (1.26, 2.53)	1.47 (1.26, 2.14)
K_M for hepatic TCOH→TCOG (mg/L)	K_MGluc	1.8 (0.0018, 610)	30 (5.3, 130)	1.48 (1.25, 2.48)	1.8 (1.3, 4.72)
Rate constant for hepatic TCOH→other (/hr)	kMetTCOH	0.19 (0.000039, 1,400)	8.8 (1.9, 23)	1.47 (1.25, 2.36)	1.54 (1.26, 2.92)
Rate constant for TCA plasma→urine (/hr)	kUrnTCA	32 (0.38, 1700)	3.2 (1.2, 7.1)	1.57 (1.34, 2.61)	1.84 (1.44, 2.94)
Rate constant for hepatic TCA→other (/hr)	kMetTCA	0.12 (0.0004, 130)	1.5 (0.63, 2.9)	1.48 (1.25, 2.32)	1.51 (1.26, 2.27)
Rate constant for TCOG liver→bile (/hr)	kBile	0.3 (0.0004, 160)	2.4 (0.74, 8.4)	1.48 (1.24, 2.29)	1.51 (1.26, 2.39)
Lumped rate constant for TCOG bile→TCOH liver (/hr)	kEHR	0.21 (0.00036, 150)	0.039 (0.0026, 0.11)	1.47 (1.23, 2.29)	1.53 (1.28, 2.94)
Rate constant for TCOG→urine (/hr)	kUrnTCOG	1 (0.00015, 6,200)	12 (2.6, 77)	1.71 (1.4, 3.13)	3.44 (1.89, 9.49)
Rate constant for hepatic DCVG→DCVC (/hr)	kDCVG	0.24 (0.0004, 160)	0.81 (0.0033, 46)	1.48 (1.25, 2.39)	1.52 (1.25, 2.5)

Table 3-37. Prior and posterior uncertainty and variability in mouse PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Lumped rate constant for DCVC→urinary NAcDCVC (/hr)	kNAT	0.29 (0.0004, 160)	0.37 (0.0021, 34)	1.5 (1.25, 2.49)	1.53 (1.25, 2.77)
Rate constant for DCVC bioactivation (/hr)	kKidBioact	0.18 (0.0004, 150)	0.23 (0.0024, 33)	1.48 (1.25, 2.51)	1.53 (1.25, 3.03)

Table 3-38. Prior and posterior uncertainty and variability in rat PBPK model parameters

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Cardiac output (L/hr)	QC	5.3 (4.2, 6.9)	6.1 (5.2, 7.4)	1.12 (1.07, 1.28)	1.26 (1.12, 1.36)
Alveolar ventilation (L/hr)	QP	10 (5.1, 18)	7.5 (5.8, 10)	1.32 (1.18, 1.71)	1.52 (1.33, 1.84)
Scaled fat blood flow	QFatC	0.071 (0.032, 0.11)	0.081 (0.06, 0.1)	1.66 (1.21, 2.02)	1.5 (1.3, 1.86)
Scaled gut blood flow	QGutC	0.15 (0.12, 0.18)	0.17 (0.15, 0.19)	1.15 (1.09, 1.19)	1.13 (1.08, 1.18)
Scaled liver blood flow	QLivC	0.021 (0.017, 0.026)	0.022 (0.018, 0.025)	1.15 (1.09, 1.2)	1.15 (1.1, 1.19)
Scaled slowly perfused blood flow	QSlwC	0.33 (0.21, 0.46)	0.31 (0.23, 0.4)	1.31 (1.15, 1.4)	1.32 (1.22, 1.41)
Scaled rapidly perfused blood flow	QRapC	0.28 (0.15, 0.42)	0.28 (0.18, 0.36)	1.38 (0.0777, 1.72)	1.42 (0.0856, 1.75)
Scaled kidney blood flow	QKidC	0.14 (0.12, 0.16)	0.14 (0.12, 0.16)	1.11 (1.07, 1.14)	1.11 (1.08, 1.14)
Respiratory lumen:tissue diffusive clearance rate (L/hr)	DResp	9.9 (0.48, 85)	21 (9.5, 46)	1.41 (1.26, 1.77)	1.59 (1.41, 1.9)
Fat fractional compartment volume	VFatC	0.069 (0.031, 0.11)	0.069 (0.046, 0.091)	1.61 (1.2, 1.93)	1.59 (1.34, 1.88)
Gut fractional compartment volume	VGutC	0.032 (0.027, 0.037)	0.032 (0.028, 0.036)	1.11 (1.07, 1.14)	1.11 (1.08, 1.14)
Liver fractional compartment volume	VLivC	0.034 (0.026, 0.042)	0.033 (0.028, 0.039)	1.16 (1.09, 1.21)	1.17 (1.12, 1.21)
Rapidly perfused fractional compartment volume	VRapC	0.087 (0.076, 0.1)	0.088 (0.079, 0.097)	1.1 (1.06, 1.13)	1.1 (1.07, 1.13)
Fractional volume of respiratory lumen	VRespLumC	0.0046 (0.0037, 0.0057)	0.0047 (0.0039, 0.0055)	1.16 (1.1, 1.21)	1.16 (1.11, 1.21)
Fractional volume of respiratory tissue	VRespEffC	0.0005 (0.00039, 0.00061)	5e-04 (0.00041, 0.00058)	1.16 (1.09, 1.21)	1.16 (1.11, 1.2)
Kidney fractional compartment volume	VKidC	0.0069 (0.0056, 0.0082)	0.007 (0.006, 0.008)	1.13 (1.08, 1.17)	1.13 (1.09, 1.17)

Table 3-38. Prior and posterior uncertainty and variability in rat PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Blood fractional compartment volume	VBldC	0.073 (0.063, 0.085)	0.074 (0.066, 0.082)	1.1 (1.06, 1.13)	1.1 (1.07, 1.13)
Slowly perfused fractional compartment volume	VSlwC	0.6 (0.55, 0.63)	0.6 (0.57, 0.62)	1.05 (1.04, 1.06)	1.05 (1.04, 1.06)
Plasma fractional compartment volume	VPlasC	0.039 (0.025, 0.054)	0.04 (0.032, 0.049)	1.24 (1.15, 1.35)	1.22 (1.16, 1.33)
TCA body fractional compartment volume [not incl. blood+liver]	VBodC	0.79 (0.78, 0.81)	0.79 (0.78, 0.8)	1.01 (1.01, 1.01)	1.01 (1.01, 1.01)
TCOH/G body fractional compartment volume [not incl. liver]	VBodTCOHC	0.87 (0.86, 0.87)	0.87 (0.86, 0.87)	1.01 (1, 1.01)	1.01 (1, 1.01)
TCE blood:air partition coefficient	PB	22 (14, 33)	19 (16, 24)	1.26 (1.19, 1.35)	1.3 (1.22, 1.38)
TCE fat:blood partition coefficient	PFat	27 (16, 46)	31 (24, 42)	1.32 (1.22, 1.44)	1.32 (1.23, 1.43)
TCE gut:blood partition coefficient	PGut	1.3 (0.69, 3)	1.1 (0.79, 1.7)	1.36 (1.21, 1.79)	1.36 (1.2, 1.68)
TCE liver:blood partition coefficient	PLiv	1.5 (1.2, 1.9)	1.6 (1.3, 1.8)	1.15 (1.11, 1.2)	1.15 (1.11, 1.2)
TCE rapidly perfused:blood partition coefficient	PRap	1.3 (0.66, 2.7)	1.3 (0.82, 2.1)	1.35 (1.18, 1.82)	1.37 (1.2, 1.76)
TCE respiratory tissue:air partition coefficient	PResp	0.97 (0.48, 2.1)	1 (0.62, 1.6)	1.37 (1.19, 1.77)	1.36 (1.19, 1.78)
TCE kidney:blood partition coefficient	PKid	1.3 (0.77, 2.2)	1.2 (0.9, 1.7)	1.31 (1.19, 1.5)	1.3 (1.2, 1.45)
TCE slowly perfused:blood partition coefficient	PSlw	0.57 (0.35, 0.97)	0.73 (0.54, 0.97)	1.32 (1.23, 1.43)	1.33 (1.25, 1.46)
TCA blood:plasma concentration ratio	TCAPlas	0.78 (0.6, 0.96)	0.78 (0.71, 0.86)	1.12 (1.06, 1.22)	1.11 (1.07, 1.17)
Free TCA body:blood plasma partition coefficient	PBodTCA	0.7 (0.18, 2.2)	0.76 (0.46, 1.3)	1.72 (1.39, 2.81)	1.65 (1.4, 2.19)
Free TCA liver:blood plasma partition coefficient	PLivTCA	0.84 (0.25, 3.3)	1.1 (0.61, 2.1)	1.71 (1.39, 2.78)	1.66 (1.38, 2.37)

Table 3-38. Prior and posterior uncertainty and variability in rat PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Protein:TCA dissociation constant (μmole/L)	kDissoc	270 (95, 790)	280 (140, 530)	1.62 (1.31, 2.43)	1.6 (1.31, 2.31)
Maximum binding concentration (μmole/L)	B_{MAX}	320 (80, 1300)	320 (130, 750)	1.89 (1.5, 2.64)	1.84 (1.49, 2.57)
TCOH body:blood partition coefficient	PBodTCOH	1 (0.33, 4)	1.1 (0.51, 2.1)	1.71 (1.37, 2.69)	1.76 (1.38, 2.45)
TCOH liver:body partition coefficient	PLivTCOH	1.3 (0.39, 4.5)	1.2 (0.59, 2.8)	1.71 (1.37, 2.8)	1.78 (1.37, 2.75)
TCOG body:blood partition coefficient	PBodTCOG	0.48 (0.021, 14)	1.6 (0.091, 16)	1.39 (1.2, 1.97)	1.42 (1.21, 2.52)
TCOG liver:body partition coefficient	PLivTCOG	1.3 (0.078, 39)	10 (2.7, 41)	1.4 (1.2, 2.14)	1.42 (1.21, 2.3)
DCVG effective volume of distribution	VDCVG	0.27 (0.27, 0.27)	0.27 (0.27, 0.27)	1 (1, 1)	1 (1, 1)
TCE stomach absorption coefficient (/hr)	kAS	0.73 (0.0044, 400)	2.5 (0.32, 19)	4.16 (2.21, 20)	9.3 (4.07, 31.1)
TCE stomach-duodenum transfer coefficient (/hr)	kTSD	1.4 (0.04, 45)	3.2 (0.31, 19)	3.92 (2.13, 10.4)	5.54 (2.77, 10.7)
TCE duodenum absorption coefficient (/hr)	kAD	0.96 (0.0023, 260)	0.17 (0.038, 1)	4.17 (2.15, 20.8)	4.07 (2.51, 11.9)
TCA stomach absorption coefficient (/hr)	kASTCA	0.83 (0.0024, 240)	1.4 (0.13, 13)	4.15 (2.2, 18.7)	4.21 (2.4, 11.4)
V_{MAX} for hepatic TCE oxidation (mg/hr)	V_{MAX}	5.8 (2, 19)	5.3 (3.9, 7.7)	1.97 (1.54, 2.92)	1.69 (1.47, 2.15)
K_M for hepatic TCE oxidation (mg/L)	K_M	18 (1.9, 240)	0.74 (0.54, 1.4)	2.76 (1.89, 6.46)	1.84 (1.51, 2.7)
Fraction of hepatic TCE oxidation not to TCA+TCOH	FracOther	0.027 (0.0018, 0.59)	0.29 (0.047, 0.56)	1.42 (1.15, 2.33)	2.15 (1.32, 5.06)
Fraction of hepatic TCE oxidation to TCA	FracTCA	0.2 (0.027, 0.76)	0.046 (0.023, 0.087)	1.35 (1.11, 2.14)	1.84 (1.36, 2.8)
V_{MAX} for hepatic TCE GSH conjugation (mg/hr)	V_{MAX}DCVG	2 (0.015, 1,100)	5.8 (0.16, 340)	1.52 (1.3, 2.67)	1.57 (1.32, 2.93)
K_M for hepatic TCE GSH conjugation (mg/L)	K_MDCVG	1,500 (1.2, 1,800,000)	6300 (120, 720,000)	1.83 (1.45, 3.15)	1.88 (1.48, 3.49)

Table 3-38. Prior and posterior uncertainty and variability in rat PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
V_{MAX} for renal TCE GSH conjugation (mg/hr)	V_{MAX}KidDCVG	0.038 (0.00027, 13)	0.0024 (0.0005, 0.014)	1.52 (1.3, 2.81)	1.56 (1.29, 2.72)
K_M for renal TCE GSH conjugation (mg/L)	K_MKidDCVG	470 (0.47, 530,000)	0.25 (0.038, 2.2)	1.84 (1.47, 4.27)	1.93 (1.49, 3.57)
V_{MAX} for tracheo-bronchial TCE oxidation (mg/hr)	V_{MAX}Clara	0.2 (0.0077, 2.4)	0.17 (0.042, 0.69)	2.26 (1.71, 3.3)	4.35 (1.99, 6.7)
K_M for tracheo-bronchial TCE oxidation (mg/L)	K_MClara	0.016 (0.0014, 0.58)	0.025 (0.005, 0.15)	1.47 (1.26, 2.39)	1.65 (1.28, 10.5)
Fraction of respiratory metabolism to systemic circ.	FracLungSys	0.82 (0.027, 1)	0.73 (0.06, 0.98)	1.09 (1, 1.71)	1.13 (1.01, 1.86)
V_{MAX} for hepatic TCOH→TCA (mg/hr)	V_{MAX}TCOH	0.75 (0.037, 20)	0.71 (0.27, 2.2)	1.51 (1.25, 2.64)	1.68 (1.3, 3.23)
K_M for hepatic TCOH→TCA (mg/L)	K_MTCOH	1 (0.029, 23)	19 (3.6, 94)	1.52 (1.26, 2.7)	1.72 (1.26, 3.93)
V_{MAX} for hepatic TCOH→TCOG (mg/hr)	V_{MAX}Gluc	27 (0.83, 620)	11 (4.1, 32)	1.5 (1.25, 2.59)	2.3 (1.41, 5.19)
K_M for hepatic TCOH→TCOG (mg/L)	K_MGluc	31 (1, 570)	6.3 (1.2, 20)	1.5 (1.25, 2.74)	2.04 (1.3, 8.4)
Rate constant for hepatic TCOH→other (/hr)	kMetTCOH	4.2 (0.17, 150)	3 (0.57, 15)	1.49 (1.27, 2.67)	1.72 (1.3, 8.31)
Rate constant for TCA plasma→urine (/hr)	kUrnTCA	1.9 (0.21, 47)	0.92 (0.51, 1.7)	1.56 (1.33, 2.81)	1.58 (1.36, 2.25)
Rate constant for hepatic TCA→other (/hr)	kMetTCA	0.76 (0.037, 19)	0.47 (0.17, 1.2)	1.5 (1.26, 2.74)	1.52 (1.27, 2.45)
Rate constant for TCOG liver→bile (/hr)	kBile	1.4 (0.052, 31)	14 (2.7, 39)	1.5 (1.25, 2.8)	1.63 (1.29, 4.1)
Lumped rate constant for TCOG bile→TCOH liver (/hr)	kEHR	0.013 (0.00055, 0.64)	1.7 (0.34, 7.4)	1.5 (1.25, 2.49)	1.67 (1.26, 5.91)
Rate constant for TCOG→urine (/hr)	kUrnTCOG	11 (0.063, 1,000)	12 (0.45, 370)	1.74 (1.42, 2.99)	1.86 (1.43, 3.54)
Rate constant for hepatic DCVG→DCVC (/hr)	kDCVG	30,000 (30,000, 30,000)	30,000 (30,000, 30,000)	1 (1, 1)	1 (1, 1)

Table 3-38. Prior and posterior uncertainty and variability in rat PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Lumped rate constant for DCVC→urinary NAcDCVC (/hr)	kNAT	0.15 (0.00024, 84)	0.0029 (0.00066, 0.015)	1.49 (1.24, 2.8)	1.54 (1.26, 2.45)
Rate constant for DCVC bioactivation (/hr)	kKidBioact	0.12 (0.00023, 83)	0.0092 (0.0012, 0.043)	1.48 (1.24, 2.68)	1.52 (1.25, 2.5)

Table 3-39. Prior and posterior uncertainty and variability in human PBPK model parameters

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Cardiac output (L/hr)	QC	390 (280, 560)	330 (280, 390)	1.17 (1.1, 1.39)	1.39 (1.26, 1.54)
Alveolar ventilation (L/hr)	QP	380 (220, 640)	440 (360, 530)	1.27 (1.17, 1.52)	1.58 (1.44, 1.73)
Scaled fat blood flow	QFatC	0.051 (0.021, 0.078)	0.043 (0.033, 0.055)	1.64 (1.23, 2)	1.92 (1.72, 2.09)
Scaled gut blood flow	QGutC	0.19 (0.15, 0.23)	0.16 (0.14, 0.18)	1.16 (1.1, 1.21)	1.16 (1.12, 1.2)
Scaled liver blood flow	QLivC	0.063 (0.029, 0.099)	0.039 (0.026, 0.055)	1.62 (1.22, 1.92)	1.8 (1.62, 1.98)
Scaled slowly perfused blood flow	QSlwC	0.22 (0.13, 0.3)	0.17 (0.14, 0.21)	1.34 (1.18, 1.45)	1.39 (1.31, 1.46)
Scaled rapidly perfused blood flow	QRapC	0.29 (0.18, 0.4)	0.39 (0.34, 0.43)	1.31 (1.14, 1.57)	1.22 (1.16, 1.3)
Scaled kidney blood flow	QKidC	0.19 (0.16, 0.22)	0.19 (0.18, 0.21)	1.1 (1.07, 1.13)	1.1 (1.07, 1.12)
Respiratory lumen:tissue diffusive clearance rate (L/hr)	DResp	560 (44, 3300)	270 (130, 470)	1.37 (1.25, 1.61)	1.71 (1.52, 2.35)
Fat fractional compartment volume	VFatC	0.19 (0.088, 0.31)	0.16 (0.12, 0.21)	1.66 (1.23, 1.93)	1.65 (1.4, 1.9)
Gut fractional compartment volume	VGutC	0.02 (0.018, 0.022)	0.02 (0.019, 0.021)	1.07 (1.04, 1.08)	1.06 (1.05, 1.08)
Liver fractional compartment volume	VLivC	0.026 (0.018, 0.032)	0.026 (0.022, 0.03)	1.21 (1.12, 1.28)	1.2 (1.13, 1.26)
Rapidly perfused fractional compartment volume	VRapC	0.087 (0.079, 0.096)	0.088 (0.083, 0.093)	1.07 (1.05, 1.09)	1.06 (1.05, 1.08)
Fractional volume of respiratory lumen	VRespLumC	0.0024 (0.0018, 0.003)	0.0024 (0.0021, 0.0027)	1.18 (1.1, 1.23)	1.17 (1.12, 1.22)
Fractional volume of respiratory tissue	VRespEffC	0.00018 (0.00014, 0.00022)	0.00018 (0.00015, 0.00021)	1.18 (1.1, 1.24)	1.17 (1.13, 1.23)
Kidney fractional compartment volume	VKidC	0.0043 (0.0034, 0.0052)	0.0043 (0.0038, 0.0048)	1.15 (1.09, 1.19)	1.14 (1.1, 1.19)

Table 3-39. Prior and posterior uncertainty and variability in human PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Blood fractional compartment volume	VBldC	0.077 (0.066, 0.088)	0.078 (0.072, 0.084)	1.1 (1.06, 1.13)	1.1 (1.07, 1.13)
Slowly perfused fractional compartment volume	VSlwC	0.45 (0.33, 0.55)	0.48 (0.43, 0.52)	1.18 (1.1, 1.24)	1.16 (1.12, 1.22)
Plasma fractional compartment volume	VPlasC	0.044 (0.037, 0.051)	0.044 (0.04, 0.048)	1.11 (1.08, 1.14)	1.11 (1.08, 1.14)
TCA body fractional compartment volume [not incl. blood+liver]	VBodC	0.75 (0.74, 0.77)	0.75 (0.74, 0.76)	1.01 (1.01, 1.01)	1.01 (1.01, 1.01)
TCOH/G body fractional compartment volume [not incl. liver]	VBodTCOHC	0.83 (0.82, 0.84)	0.83 (0.83, 0.83)	1.01 (1, 1.01)	1.01 (1, 1.01)
TCE blood:air partition coefficient	PB	9.6 (6.5, 13)	9.2 (8.2, 10)	1.18 (1.13, 1.26)	1.21 (1.16, 1.28)
TCE fat:blood partition coefficient	PFat	68 (46, 98)	57 (49, 66)	1.18 (1.11, 1.33)	1.18 (1.11, 1.3)
TCE gut:blood partition coefficient	PGut	2.6 (1.3, 5.3)	2.9 (1.9, 4.1)	1.37 (1.2, 1.78)	1.41 (1.21, 1.77)
TCE liver:blood partition coefficient	PLiv	4 (1.9, 8.5)	4.1 (2.7, 5.9)	1.37 (1.22, 1.81)	1.33 (1.19, 1.6)
TCE rapidly perfused:blood partition coefficient	PRap	2.6 (1.2, 5.7)	2.4 (1.8, 3.2)	1.37 (1.21, 1.78)	1.5 (1.25, 1.87)
TCE respiratory tissue:air partition coefficient	PResp	1.3 (0.65, 2.7)	1.3 (0.9, 1.9)	1.36 (1.19, 1.81)	1.32 (1.2, 1.56)
TCE kidney:blood partition coefficient	PKid	1.6 (1.1, 2.3)	1.6 (1.3, 1.9)	1.17 (1.1, 1.33)	1.15 (1.09, 1.25)
TCE slowly perfused:blood partition coefficient	PSlw	2.1 (1.2, 3.5)	2.3 (1.9, 2.8)	1.28 (1.14, 1.53)	1.51 (1.36, 1.66)
TCA blood:plasma concentration ratio	TCAPlas	0.78 (0.55, 15)	0.65 (0.6, 0.77)	1.08 (1.03, 1.53)	1.52 (1.23, 2.03)
Free TCA body:blood plasma partition coefficient	PBodTCA	0.45 (0.19, 8.1)	0.44 (0.33, 0.55)	1.36 (1.19, 1.75)	1.67 (1.38, 2.2)
Free TCA liver:blood plasma partition coefficient	PLivTCA	0.59 (0.24, 10)	0.55 (0.39, 0.77)	1.36 (1.18, 1.76)	1.65 (1.37, 2.16)
Protein:TCA dissociation constant (μmole/L)	kDissoc	180 (160, 200)	180 (170, 190)	1.05 (1.03, 1.09)	1.04 (1.03, 1.07)

Table 3-39. Prior and posterior uncertainty and variability in human PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
Maximum binding concentration (μmole/L)	B_{MAX}	830 (600, 1100)	740 (630, 880)	1.17 (1.1, 1.3)	1.16 (1.1, 1.28)
TCOH body:blood partition coefficient	PBodTCOH	0.89 (0.51, 1.7)	1.5 (1.3, 1.7)	1.29 (1.16, 1.64)	1.34 (1.25, 1.47)
TCOH liver:body partition coefficient	PLivTCOH	0.58 (0.32, 1.1)	0.63 (0.45, 0.87)	1.29 (1.16, 1.65)	1.29 (1.17, 1.5)
TCOG body:blood partition coefficient	PBodTCOG	0.67 (0.036, 16)	0.72 (0.3, 1.8)	1.38 (1.2, 2.42)	7.83 (4.86, 12.6)
TCOG liver:body partition coefficient	PLivTCOG	1.8 (0.11, 28)	3.1 (0.87, 8.1)	1.38 (1.19, 2.04)	4.94 (2.73, 8.58)
DCVG effective volume of distribution	VDCVG	73 (5.2, 36000)	6.1 (5.4, 7.3)	1.27 (1.08, 1.95)	1.1 (1.07, 1.16)
TCE stomach absorption coefficient (/hr)	kAS	1.4 (1.4, 1.4)	1.4 (1.4, 1.4)	1 (1, 1)	1 (1, 1)
TCE stomach-duodenum transfer coefficient (/hr)	kTSD	1.4 (1.4, 1.4)	1.4 (1.4, 1.4)	1 (1, 1)	1 (1, 1)
TCE duodenum absorption coefficient (/hr)	kAD	0.75 (0.75, 0.75)	0.75 (0.75, 0.75)	1 (1, 1)	1 (1, 1)
TCA stomach absorption coefficient (/hr)	kASTCA	0.58 (0.0022, 210)	3 (0.061, 180)	4.26 (2.13, 17.6)	5.16 (2.57, 22.3)
TCOH stomach absorption coefficient (/hr)	kASTCOH	0.49 (0.0024, 210)	7.6 (0.11, 150)	4.19 (2.22, 21.5)	5.02 (2.44, 18.5)
V_{MAX} for hepatic TCE oxidation (mg/hr)	V_{MAX}	430 (130, 1500)	190 (130, 290)	1.98 (1.69, 2.31)	2.02 (1.77, 2.38)
K_M for hepatic TCE oxidation (mg/L)	K_M	3.7 (0.22, 63)	0.18 (0.078, 0.4)	2.74 (2.1, 5.62)	4.02 (2.9, 5.64)
Fraction of hepatic TCE oxidation not to TCA+TCOH	FracOther	0.12 (0.0066, 0.7)	0.11 (0.024, 0.23)	1.4 (1.11, 2.38)	2.71 (1.37, 5.33)
Fraction of hepatic TCE oxidation to TCA	FracTCA	0.19 (0.036, 0.56)	0.035 (0.024, 0.05)	2.55 (1.51, 3.96)	2.25 (1.89, 2.87)
V_{MAX} for hepatic TCE GSH conjugation (mg/hr)	V_{MAX}DCVG	100 (0.0057, 690,000)	340 (110, 1,100)	1.91 (1.55, 3.76)	6.18 (3.35, 11.3)
K_M for hepatic TCE GSH conjugation (mg/L)	K_MDCVG	3.1 (0.21, 42)	3.6 (1.2, 11)	1.52 (1.26, 2.91)	4.2 (2.48, 8.01)
V_{MAX} for renal TCE GSH conjugation (mg/hr)	V_{MAX}KidDCVG	220 (0.028, 6,700,000)	2.1 (0.17, 9.3)	1.86 (1.51, 3.33)	4.02 (1.57, 33.9)

Table 3-39. Prior and posterior uncertainty and variability in human PBPK model parameters (continued)

Parameter description	PBPK parameter	Prior population median: median (2.5%, 97.5%)	Posterior population median: median (2.5%, 97.5%)	Prior population GSD: median (2.5%, 97.5%)	Posterior population GSD: median (2.5%, 97.5%)
K_M for renal TCE GSH conjugation (mg/L)	K_MKidDCVG	2.7 (0.14, 41)	0.76 (0.29, 5.8)	1.5 (1.27, 2.56)	1.49 (1.27, 2.32)
V_{MAX} for tracheo-bronchial TCE oxidation (mg/hr)	V_{MAX}Clara	25 (1, 260)	18 (3.8, 41)	2.25 (1.85, 3.25)	2.9 (2.12, 6.49)
K_M for tracheo-bronchial TCE oxidation (mg/L)	K_MClara	0.019 (0.0017, 0.5)	0.31 (0.057, 1.4)	1.48 (1.25, 2.39)	10.8 (1.99, 37.6)
Fraction of respiratory metabolism to systemic circ.	FracLungSys	0.75 (0.051, 0.99)	0.96 (0.86, 0.99)	1.12 (1, 1.75)	1.02 (1, 1.1)
V_{MAX} for hepatic TCOH→TCA (mg/hr)	V_{MAX}TCOH	42 (0.77, 2,200)	9.2 (5.5, 20)	1.83 (1.46, 3.43)	3.15 (2.3, 5.44)
K_M for hepatic TCOH→TCA (mg/L)	K_MTCOH	5 (0.23, 81)	2.2 (1.3, 4.5)	1.49 (1.25, 2.57)	2.58 (1.75, 4.5)
V_{MAX} for hepatic TCOH→TCOG (mg/hr)	V_{MAX}Gluc	720 (12, 50,000)	900 (340, 2,000)	1.83 (1.48, 3.5)	2.29 (1.84, 4.57)
K_M for hepatic TCOH→TCOG (mg/L)	K_MGluc	10 (0.53, 190)	130 (47, 290)	1.5 (1.25, 2.6)	1.58 (1.26, 3.69)
Rate constant for hepatic TCOH→other (/hr)	kMetTCOH	0.83 (0.035, 10)	0.25 (0.042, 0.7)	1.5 (1.26, 3)	5.13 (2.72, 16.7)
Rate constant for TCA plasma→urine (/hr)	kUrnTCA	0.26 (0.038, 4)	0.11 (0.083, 0.15)	1.48 (1.29, 2.29)	1.86 (1.58, 2.28)
Rate constant for hepatic TCA→other (/hr)	kMetTCA	0.19 (0.01, 2.6)	0.096 (0.038, 0.19)	1.48 (1.26, 2.57)	2.52 (1.79, 4.34)
Rate constant for TCOG liver→bile (/hr)	kBile	1.2 (0.059, 16)	2.5 (1.1, 6.9)	1.47 (1.25, 2.75)	1.56 (1.27, 3.21)
Lumped rate constant for TCOG bile→TCOH liver (/hr)	kEHR	0.074 (0.004, 1.4)	0.053 (0.033, 0.087)	1.52 (1.26, 2.64)	1.72 (1.35, 2.51)
Rate constant for TCOG→urine (/hr)	kUrnTCOG	2.9 (0.061, 260)	2.4 (0.83, 7)	1.75 (1.4, 3.31)	18.7 (11.6, 31.8)
Rate constant for hepatic DCVG→DCVC (/hr)	kDCVG	0.044 (0.000063, 22)	2.5 (1.9, 3.4)	1.48 (1.25, 2.83)	1.51 (1.3, 1.86)
Lumped rate constant for DCVC→urinary NAcDCVC (/hr)	kNAT	0.00085 (0.000055, 0.041)	0.0001 (0.000047, 0.0007)	1.51 (1.25, 2.34)	1.47 (1.24, 2.48)
Rate constant for DCVC bioactivation (/hr)	kKidBioact	0.0022 (0.000095, 0.079)	0.023 (0.0062, 0.061)	1.51 (1.25, 2.57)	1.52 (1.25, 2.69)

Table 3-40. CI widths (ratio of 97.5–2.5% estimates) and fold-shift in median estimate for the PBPK model population median parameters, sorted in order of decreasing CI width[a]

Mouse				Rat				Human			
	Width of CI on population median		Fold-shift in population median		Width of CI on population median		Fold-shift in population median		Width of CI on population median		Fold-shift in population median
PBPK parameter	Prior	Posterior		PBPK parameter	Prior	Posterior		PBPK parameter	Prior	Posterior	
K_MDCVG	2,230,000,000	13,400,000	×**8.8**	K_MDCVG	1,500,000	5,800	×**4.29**	kASTCA	94,300	3,040	×**5.18**
K_MKidDCVG	1,170,000,000	3,540,000	×1.05	V_{MAX}DCVG	71,100	2,130	×2.86	kASTCOH	85,900	1,420	×**15.6**
V_{MAX}DCVG	400,000	46,200	÷**6.18**	kUrnTCOG	16,700	822	×1.04	V_{MAX}-KidDCVG	236,000,000	55.1	÷**105**
V_{MAX}KidDCVG	357,000	11,000	÷**12.8**	PBodTCOG	666	172	×**3.43**	K_MClara	289	23.9	×**16.2**
kASTCA	89,300	374	×**6.3**	kASTCA	98,200	95.7	×1.69	K_MKidDCVG	287	20	÷**3.48**
kTSD	1,190	51.1	×**3.26**	kTSD	1,130	61.8	×2.29	kMetTCOH	289	16.6	÷**3.28**
kEHR	412,000	42.1	÷**5.43**	kAS	91,000	60.2	×**3.41**	kNAT	756	15.1	÷**8.14**
FracOther	567	39.5	÷**18.5**	K_MKidDCVG	1,130,000	58.6	÷**1880**	V_{MAX}Clara	255	10.6	÷1.41
K_MClara	351,000	37.5	÷**134**	kKidBioact	366,000	35.6	÷**13.3**	kKidBioact	833	9.91	×**10.5**
kAS	91,900	35.9	×1	K_MClara	406	29.9	×1.53	V_{MAX}DCVG	122,000,000	9.78	×**3.29**
kUrnTCOG	4,0500,000	29.9	×**11.8**	V_{MAX}KidDCVG	48,500	27.5	÷**15.6**	FracOther	106	9.75	÷1.09
B_{MAX}	81.8	24.4	×1.66	kMetTCOH	891	26.4	÷1.41	PLivTCOG	253	9.32	×1.77
K_MGluc	344,000	24.3	×**16.3**	kAD	115,000	26.3	÷**5.53**	K_MDCVG	198	9.13	×1.18
kAD	84,900	23.8	÷**4.53**	K_MTCOH	781	26	×**18.7**	kUrnTCOG	4,290	8.5	÷1.19
kDissoc	60.3	21.8	×1.33	kNAT	351,000	22.7	÷**50.2**	kBile	274	6.54	×2.01
V_{MAX}Clara	131	15	×1.75	kEHR	1,160	21.9	×**134**	K_MGluc	365	6.07	×**13.4**
kMetTCOH	35,500,000	12.1	×**47.4**	K_MGluc	562	17.1	÷**4.98**	PBodTCOG	454	5.85	×1.08
kBile	390,000	11.3	×**8.23**	V_{MAX}Clara	305	16.5	÷1.21	V_{MAX}Gluc	4,330	5.71	×1.25
K_MTCOH	29,600,000	10.5	÷1.47	FracLungSys	36.7	16.3	÷1.12	K_M	288	5.1	÷**20.5**
V_{MAX}Gluc	23,600,000	8.28	×**41.1**	PLivTCOG	501	14.8	×**8.07**	kMetTCA	248	4.89	÷1.94

Table 3-40. CI widths (ratio of 97.5–2.5% estimates) and fold-shift in median estimate for the PBPK model population median parameters, sorted in order of decreasing CI width[a] (continued)

Mouse				Rat				Human			
	Width of CI on population median		Fold-shift in population median		Width of CI on population median		Fold-shift in population median		Width of CI on population median		Fold-shift in population median
PBPK parameter	Prior	Posterior		PBPK parameter	Prior	Posterior		PBPK parameter	Prior	Posterior	
PBodTCOG	4,770	6.27	÷1.95	kBile	588	14.8	×**9.67**	DResp	74.3	3.71	÷2.06
V_{MAX}TCOH	27,100,000	5.78	×1.8	FracOther	331	11.9	×**10.7**	V_{MAX}TCOH	2,900	3.62	÷**4.56**
K_M	386	5.76	÷**12.5**	V_{MAX}TCOH	550	8.25	÷1.06	K_MTCOH	359	3.48	÷2.33
kUrnTCA	4,540	5.76	÷**10.2**	V_{MAX}Gluc	740	7.79	÷2.4	kEHR	339	2.62	÷1.39
FracLungSys	608	5.55	×2.27	kMetTCA	507	6.93	÷1.61	V_{MAX}	11.5	2.27	÷2.33
kMetTCA	316,000	4.59	×**12**	B_{MAX}	16.2	5.79	×1	PResp	4.1	2.16	÷1.01
PLivTCOG	4,860	3.99	×1.04	DResp	180	4.81	×2.12	PLiv	4.44	2.14	×1.02
DResp	475,000	3.64	×**147**	PLivTCOH	11.5	4.7	÷1.09	QLivC	3.46	2.11	÷1.62
PLivTCA	58.3	2.88	×1	PBodTCOH	12.1	4.03	×1.03	PGut	4.21	2.1	×1.11
PResp	4	2.85	÷1.07	kDissoc	8.38	3.85	×1.04	FracTCA	15.5	2.06	÷**5.37**
PRap	3.78	2.79	÷1.03	FracTCA	28.1	3.85	÷**4.27**	PLivTCA	42.6	1.98	÷1.07
PGut	4.33	2.77	÷1.25	PLivTCA	13.3	3.49	×1.37	PLivTCOH	3.52	1.93	×1.08
V_{MAX}	10.7	2.67	÷1.58	kUrnTCA	219	3.28	÷2	kDCVG	344,000	1.8	×**55.7**
PBodTCA	62.6	2.55	×1.14	PBodTCA	12	2.8	×1.09	kUrnTCA	105	1.79	÷2.32
PSlw	4.04	2.54	÷1.06	PResp	4.32	2.6	×1.04	VFatC	3.49	1.76	÷1.21
PLiv	3.87	2.5	×1.26	K_M	123	2.56	÷**24**	PRap	4.66	1.74	÷1.09
FracTCA	3,060	2.49	×1.49	PRap	4.01	2.53	÷1.01	QFatC	3.7	1.7	÷1.19
TCAPlas	40.6	2.38	×1.46	PGut	4.35	2.16	÷1.17	PBodTCA	42.9	1.7	÷1.04
PKid	4.78	2.37	×1.2	V_{MAX}	9.5	1.98	÷1.11	PSlw	2.9	1.5	×1.11
QFatC	3.62	2.26	×1.02	QRapC	2.77	1.97	÷1	PKid	2.05	1.49	÷1.01
PLivTCOH	3.19	2.13	×1.48	VFatC	3.58	1.96	÷1	QP	2.97	1.48	×1.16
PBodTCOH	3.41	2.01	÷1.27	PKid	2.89	1.85	÷1.11	QSlwC	2.25	1.48	÷1.26
QKidC	2.39	1.91	÷1.01	QP	3.59	1.79	÷1.38	QC	2.04	1.39	÷1.19
PFat	3.01	1.89	÷1.01	PSlw	2.76	1.79	×1.28	B_{MAX}	1.92	1.38	÷1.12
QSlwC	2.04	1.88	÷1.02	PFat	2.91	1.77	×1.16	VLivC	1.79	1.36	×1.01
VPlasC	2.18	1.87	÷1.17	QSlwC	2.19	1.69	÷1.06	PFat	2.13	1.34	÷1.2
VFatC	3.49	1.83	×1.25	QFatC	3.47	1.66	×1.14	VDCVG	6,820	1.34	÷**12**
QP	2.75	1.82	÷1.02	VPlasC	2.17	1.55	×1.03	VRespEffC	1.66	1.33	÷1.02
VLivC	1.85	1.6	÷1.16	PB	2.37	1.51	÷1.15	PBodTCOH	3.32	1.32	×1.68

Table 3-40. CI widths (ratio of 97.5–2.5% estimates) and fold-shift in median estimate for the PBPK model population median parameters, sorted in order of decreasing CI width[a] (continued)

Mouse				Rat				Human			
	Width of CI on population median		Fold-shift in population median		Width of CI on population median		Fold-shift in population median		Width of CI on population median		Fold-shift in population median
PBPK parameter	Prior	Posterior		PBPK parameter	Prior	Posterior		PBPK parameter	Prior	Posterior	
QC	2.1	1.59	×1.2	QC	1.64	1.43	×1.15	VRespLumC	1.65	1.31	÷1
PB	2.3	1.54	÷1.07	VRespEffC	1.56	1.43	÷1	TCAPlas	26.9	1.29	÷1.21
QLivC	1.55	1.42	×1.02	VRespLumC	1.56	1.41	×1	VKidC	1.54	1.28	÷1.01
QRapC	1.51	1.41	÷1.03	VLivC	1.57	1.4	÷1.05	PB	2.04	1.28	÷1.04
VGutC	1.38	1.3	÷1.01	PLiv	1.67	1.37	×1.05	QRapC	2.22	1.25	×1.34
VBldC	1.34	1.27	÷1.02	QLivC	1.53	1.34	×1.04	QGutC	1.59	1.23	÷1.19
VRespLumC	1.32	1.26	÷1.01	VKidC	1.47	1.33	×1.01	VSlwC	1.66	1.21	×1.07
VRespEffC	1.31	1.26	÷1	QKidC	1.39	1.28	×1	VPlasC	1.39	1.2	×1.01
QGutC	1.52	1.24	×1.15	VGutC	1.38	1.28	÷1.01	QKidC	1.36	1.17	÷1
VKidC	1.29	1.24	÷1	VBldC	1.34	1.25	×1.01	VBldC	1.34	1.17	×1.02
VRapC	1.3	1.23	÷1.01	VRapC	1.34	1.23	×1	FracLungSys	19.4	1.14	×1.29
VSlwC	1.19	1.11	÷1.01	QGutC	1.53	1.22	×1.14	VRapC	1.22	1.12	×1
VBodC	1.05	1.03	×1.01	TCAPlas	1.6	1.21	÷1.01	kDissoc	1.23	1.12	÷1.01
VBodTCOHC	1.04	1.03	×1.01	VSlwC	1.15	1.09	×1	VGutC	1.22	1.11	×1.01
				VBodC	1.04	1.03	×1	VBodC	1.04	1.02	÷1
				VBodTCOHC	1.02	1.01	×1	VBodTCOHC	1.02	1.01	÷1

[a]Shifts in the median estimate greater than threefold are in bold to denote larger shifts between the prior and posterior distributions

However, for some parameters, the posterior distributions in the population medians had CIs >100-fold. In mice, the absorption parameter for TCA still had posterior CI of 400-fold, reflecting the fact that the absorption rate is poorly estimated from the few available studies with TCA dosing. In addition, mouse metabolism parameters for GSH conjugation have posterior CIs >10,000-fold, reflecting the lack of any direct data on GSH conjugation in mice. In rats, two parameters related to TCOH and TCOG had CIs between 100- and 1,000-fold, reflecting the poor identifiability of these parameters given the available data. In humans, only the oral absorption parameters for TCA and TCOH had CIs >100-fold, reflecting the fact that the absorption rate is poorly estimated from the few available studies with TCOH and TCA dosing.

In terms of general consistency between prior and posterior distributions, in most cases, the central estimate of the population median shifted by less than threefold. In almost all of the cases that the shift was greater (see bold entries in Table 3-40), the prior distribution had a wide distribution, with CI greater (sometimes substantially greater) than 100-fold. The only exception was the fraction of TCE oxidation directly producing TCA, which shifted by fourfold in rats and fivefold in mice, with prior CIs of 28- and 16-fold, respectively. These shifts are still relatively modest in comparison to the prior CI, and moreover, the posterior CI is quite narrow (fourfold in rats, twofold in humans), suggesting that the parameter is well identified by the in vivo data.

In addition, there were only a few cases in which the interquartile regions of the prior and posterior distributions did not overlap. In most of these cases, including the diffusion rate from respiratory lumen to tissue, the K_M values for renal TCE GSH conjugation and respiratory TCE oxidation, and several metabolite kinetic parameters, the prior distributions themselves were noninformative. For a noninformative prior, the lack of overlap would only be an issue if the posterior distributions were affected by the truncation limit, which was not the case. The only other parameter for which there was a lack of interquartile overlap between the prior and posterior distribution was the K_M for hepatic TCE oxidation in mice and in rats, though the prior and posterior 95% CIs did overlap within each species. As discussed Section 3.3, there is some uncertainty in the extrapolation of in vitro K_M values to in vivo values (within the same species). In addition, in mice, it has been known for some time that K_M values appear to be discordant among different studies (Greenberg et al., 1999; Abbas and Fisher, 1997; Fisher et al., 1991).

In terms of estimates of population variability, for the vast majority of parameters, the posterior estimate of the population GSD was either twofold or less, indicating modest variability. In some cases, while the posterior population GSD was greater than twofold, it was similar to the prior estimate of the population GSD, indicating limited additional informative data on variability. This was the case for oral absorption parameters, which are expected to be highly variable because the current model lumps parameters for different oral dosing vehicles together, and a relatively wide prior distribution was given. In addition, in some cases, this was due to in vitro data showing a higher degree of variability. Examples of this include TCA plasma binding parameters in the mouse, and the V_{MAX} for hepatic oxidation and the fraction of

oxidation to TCA in humans. In a few other cases, the in vivo data appeared to indicate greater than twofold variability between subjects, and these are discussed in more detail below.

In the mouse, the two parameters for which this is the case are the V_{MAX} for respiratory tract oxidation and the urinary excretion rate for TCOG. In the first case, the variability is driven by the need for a higher respiratory tract V_{MAX} for males in the Fisher et al. (1991) study as compared to other studies. In the second case, it is driven by the relatively low estimate of urinary excretion of TCOG in the Abbas and Fisher (1997), Abbas et al. (1997), and Greenberg et al. (1999) studies as compared with the relatively high estimate in Green and Prout (1985) and Prout et al. (1985).

In the rat, the two parameters for which the in vivo data suggest greater than twofold variability are the fraction of oxidation not producing TCA or TCOH, and the V_{MAX} for respiratory tract oxidation. In the first case, this is driven by three studies that appeared to require greater (Bernauer et al., 1996; Kimmerle and Eben, 1973b) or lower (Hissink et al., 2002) estimates for this parameter as compared with the other studies. Nonetheless, the degree of variability is not much greater than twofold, with a central estimate population GSD of 2.15-fold. In the case of the V_{MAX} for respiratory tract oxidation, two studies appeared to require higher (Fisher et al., 1989) or lower (Simmons et al., 2002) values for this parameter as compared with the other studies.

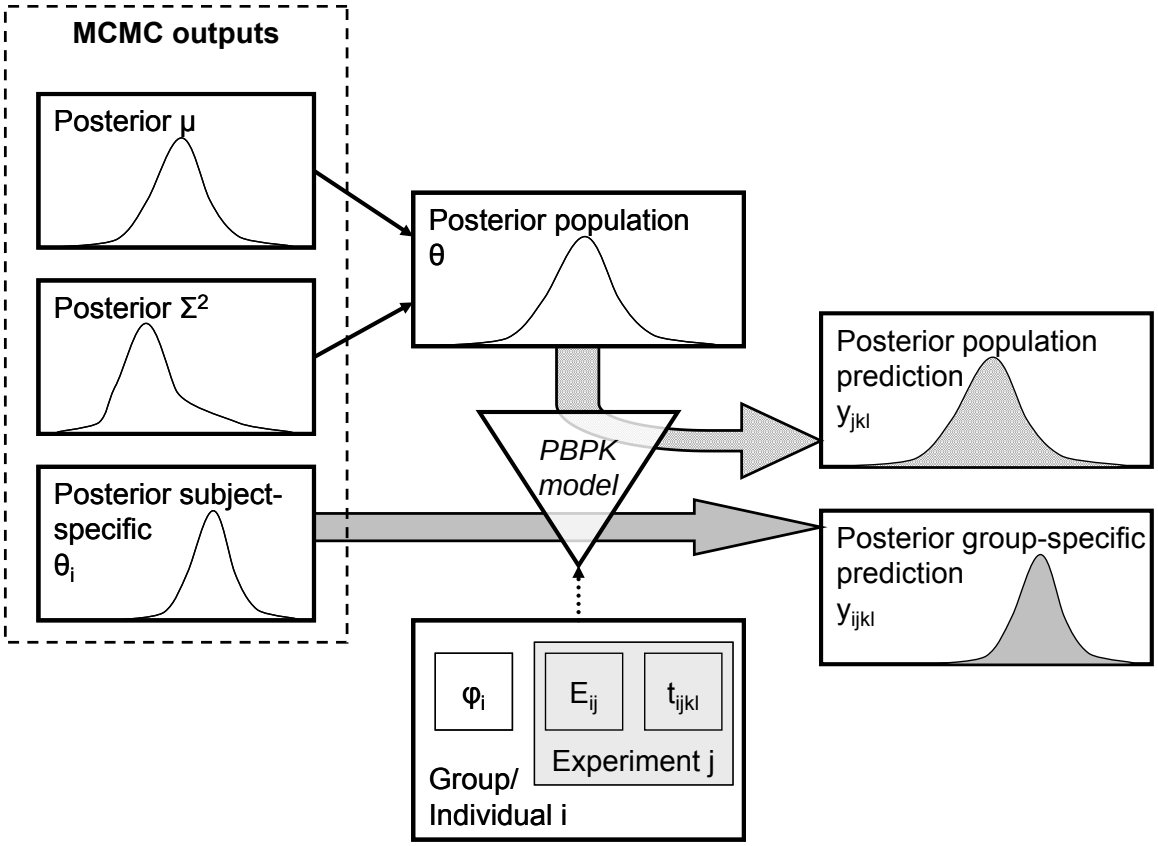
In humans, as would be expected, more parameters appeared to exhibit greater than twofold variability. In terms of distribution, the partition coefficients for TCOG had rather large posterior estimates for the population GSD of eightfold for the body and fivefold for the liver. In terms of the body, a few of the subjects in Fisher et al. (1998) and all of the subjects in Monster et al. (1976) appeared to require much higher partition coefficients for TCOG. For the liver, the variability did not have a discernable trend across studies. In addition, almost all of the metabolism and clearance parameters had posterior estimates for population variability of greater than a twofold GSD. The largest of these was the urinary excretion rate for TCOG, with a GSD of 19-fold. In this case, the variability was driven by individuals in the Chiu et al. (2007) 1 ppm study, who were predicted to have much lower rate of urinary excretion as compared to that estimated in the other, higher exposure studies.

In sum, the Bayesian analysis of the updated PBPK model and data exhibited no major inconsistencies in prior and posterior parameter distributions. The most significant issue in terms of population central estimates was the K_M for hepatic oxidative metabolism, for which the posterior estimates were low compared to, albeit somewhat uncertain, in vitro estimates, and it could be argued that a wider prior distribution would have been better. However, the central estimates were not at or near the truncation boundary, so it is unlikely that wider priors would change the results substantially. In terms of population variability, in rodents, the estimates of variability were generally modest, which is consistent with more homogeneous and controlled experimental subjects and conditions, whereas the estimates of human population variability

were greater—particularly for metabolism and clearance. Overall, there were no indications based on this evaluation of prior and posterior distributions either that prior distributions were overly restrictive or that model specification errors led to pathological parameter estimates.

3.5.6.3. Comparison of Model Predictions With Data

Comparisons of model predictions and data for each species are discussed in the sub-sections below. First, as an overall summary, for each species and each output measurement, the data and predictions generated from a random sample of the MCMC chain are scatter-plotted to show the general degree of consistency between data and predictions. Next, as with the Hack et al. (2006) model, the sampled subject-specific parameters were used to generate predictions for comparison to the calibration data (see Figure 3-8). Thus, the predictions for a particular data set are conditioned on the posterior parameter distributions for same data set. Because these parameters were "optimized" for each experiment, these subject-specific predictions should be accurate by design—and, on the whole, were so. In addition, the "residual error" estimate for each measurement (see Table 3-41) provides some quantitative measure of the degree to which there were deviations due to intrastudy variability and model misspecification, including any difficulties fitting multiple dose levels in the same study using the same model parameters.



Two sets of posterior predictions were generated: population predictions (diagonal hashing) and subject-specific predictions (vertical hashing). (Same as Figure A-2 in Appendix A)

Figure 3-8. Schematic of how posterior predictions were generated for comparison with experimental data.

Table 3-41. Estimates of the residual-error

Measurement abbreviation	Measurement description	GSD for "residual" error (median estimate)		
		Mouse	Rat	Human
RetDose	Retained TCE dose (mg)	-	-	1.13
CAlvPPM	TCE concentration in alveolar air (ppm)	-	-	1.44~1.83
CInhPPM	TCE concentration in closed-chamber (ppm)	1.18	1.11~1.12	-
CMixExh	TCE concentration in mixed exhaled air (mg/L)	-	1.5	-
CArt	TCE concentration in arterial blood (mg/L)	-	1.17~1.52	-
CVen	TCE concentration in venous blood (mg/L)	**2.68**	1.22~**4.46**	1.62~**2.95**
CBldMix	TCE concentration in mixed arterial and venous blood (mg/L)	1.61	1.5	-
CFat	TCE concentration in fat (mg/L)	**2.49**	1.85~**2.66**	-
CGut	TCE concentration in gut (mg/L)	-	1.86	-
CKid	TCE concentration in kidney (mg/L)	**2.23**	1.47	-
CLiv	TCE concentration in liver (mg/L)	1.71	1.67~1.78	-
CMus	TCE concentration in muscle (mg/L)	-	1.65	-
AExhpost	Amount of TCE exhaled postexposure (mg)	1.23	1.12~1.17	-

Table 3-41. Estimates of the residual-error (continued)

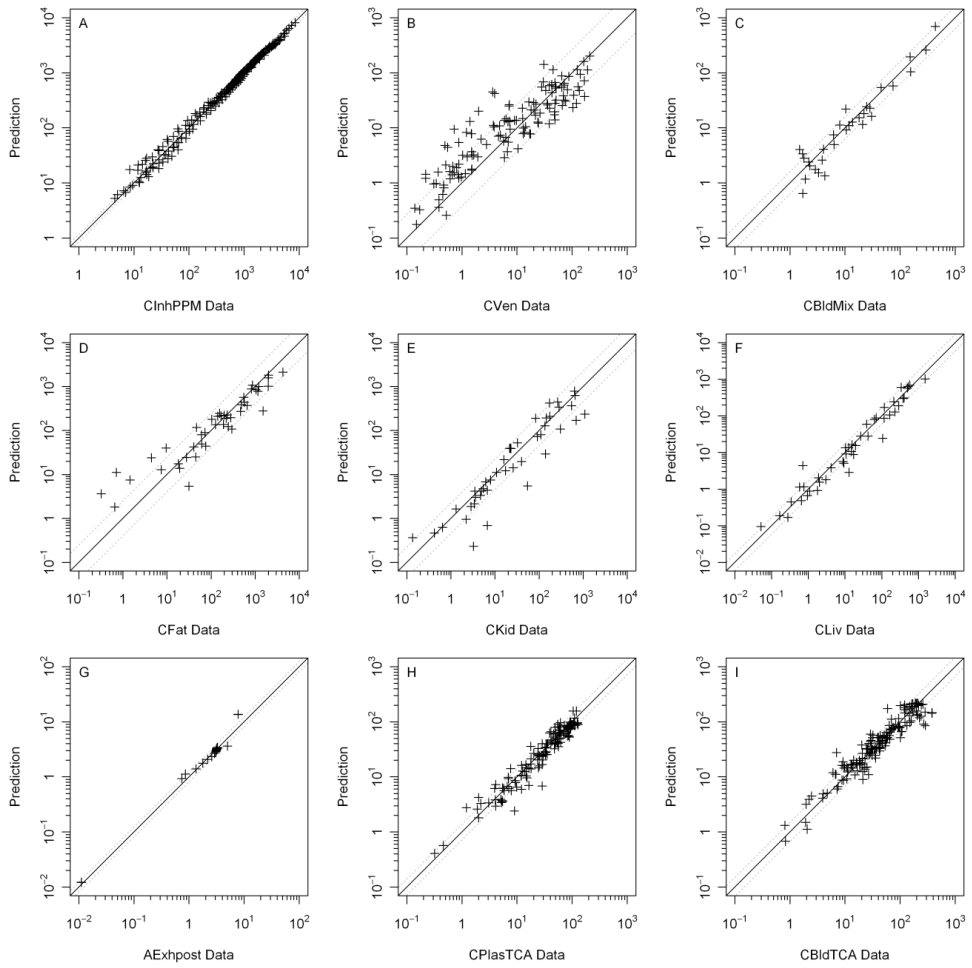
Measurement abbreviation	Measurement description	GSD for "residual" error (median estimate)[a]		
		Mouse	Rat	Human
CPlasTCA	TCA concentration in plasma (mg/L)	1.40	1.13~1.21	1.12~1.17
CBldTCA	TCA concentration in blood (mg/L)	1.49	1.13~1.59	1.12~1.49
CLivTCA	TCA concentration in liver (mg/L)	1.34	1.67	-
AUrnTCA	Cumulative amount of TCA excreted in urine (mg)	1.34	1.18~1.95	1.11~1.54
AUrnTCA_collect	Cumulative amount of TCA collected in urine (noncontinuous sampling) (mg)	-	-	**2~2.79**
CTCOH	Free TCOH concentration in blood (mg/L)	1.54	1.14~1.64	1.14~**2.1**
CLivTCOH	Free TCOH concentration in liver (mg/L)	1.59	-	-
TotCTCOH	Total TCOH concentration in blood (mg/L)	1.85	1.49	1.2~1.69
ABileTCOG	Cumulative amount of bound TCOH excreted in bile (mg)	-	**2.13**	-
CTCOG	Bound TCOH concentration in blood	-	**2.76**	-
CTCOGTCOH	Bound TCOH concentration in blood in free TCOH equivalents	1.49	-	-
CLivTCOGTCOH	Bound TCOH concentration in liver in free TCOH equivalents (mg/L)	1.63	-	-
AUrnTCOGTCOH	Cumulative amount of total TCOH excreted in urine (mg)	1.26	1.12~**2.27**	1.11~1.13
AUrnTCOGTCOH_ collect	Cumulative amount of total TCOH collected in urine (noncontinuous sampling) (mg)	-	-	1.3~1.63
AUrnTCTotMole	Cumulative amount of TCA+total TCOH excreted in urine (mmol)	-	1.12~1.54	-
CDCVGmol	DCVG concentration in blood (mmol/L)	-	-	1.53
AUrnNDCVC	Cumulative amount of NAcDCVC excreted in urine (mg)	-	1.17	1.17

[a]Values higher than twofold are in bold.

Next, only samples of the population parameters (means and variances) were used, and new subjects were sampled from appropriate distribution using these population means and variances (see Figure 3-8). That is, the predictions were only conditioned on the population-level parameters distributions, representing an "average" over all of the data sets, and not on the specific predictions for that data set. These "new" subjects then represent the predicted population distribution, incorporating variability in the population as well as uncertainty in the population means and variances. Because of the limited amount of mouse data, all available data for that species were utilized for calibration, and there were no data available for "out-of-sample" evaluation (often referred to as "validation data," but this term is not used here due to ambiguities as to its definition). In rats, several studies that contained primarily blood TCE data, which were abundant, were used for out-of-sample evaluation. In humans, there were substantial individual and aggregated (mean of individuals in a study) data that were available for out-of-sample evaluation, as computational intensity limited the number of individuals who could be used in the MCMC-based calibration.

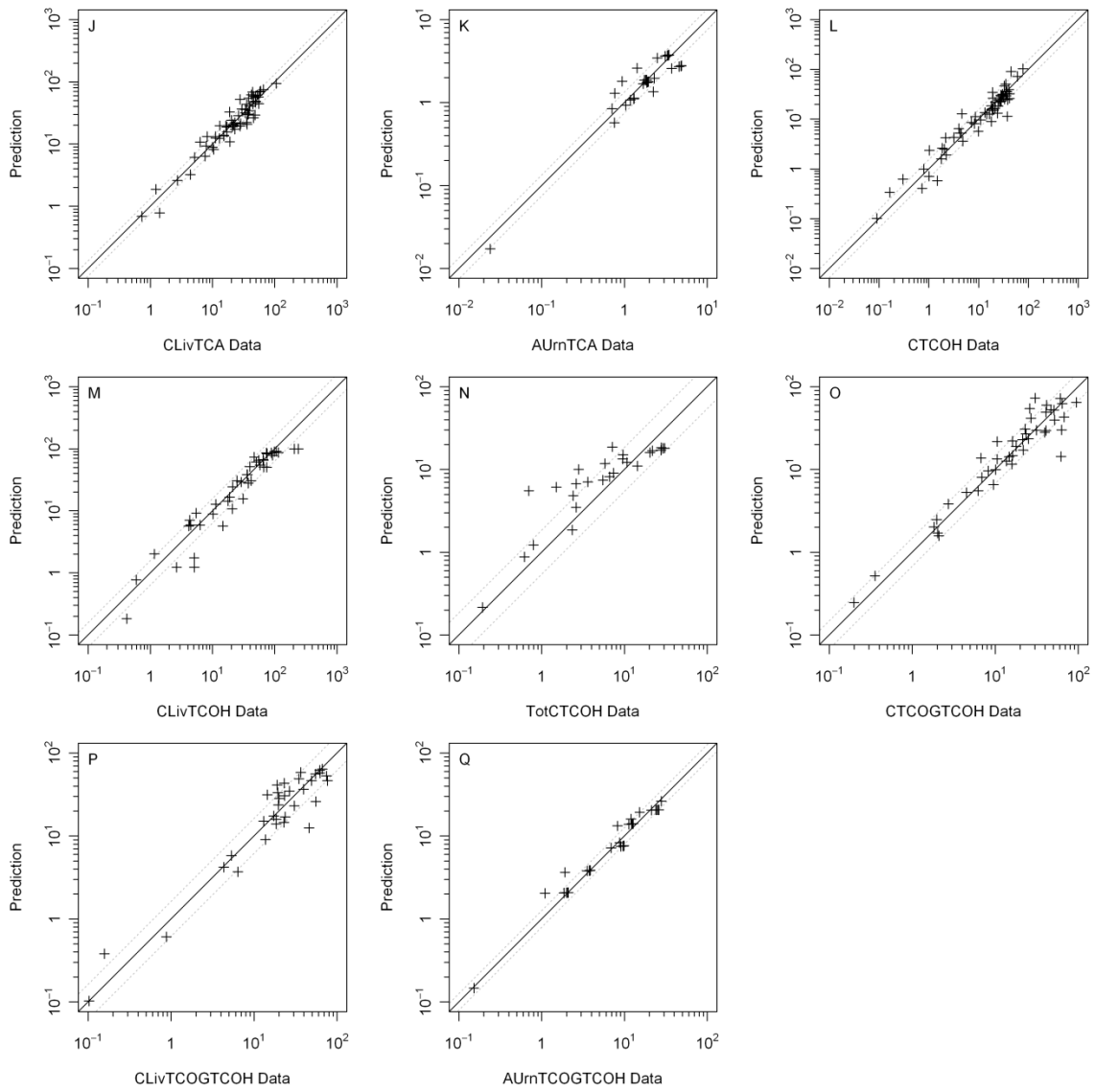
3.5.6.3.1. Mouse model and data

Each panel of Figure 3-9 shows a scatter plot of the calibration data and a random posterior prediction for each of the measured endpoint. The endpoint abbreviations are listed in Table 3-41, as are the implied GSDs for the "residual" errors, which include intrastudy variability, interindividual variability, and measurement and model errors. The residual-error GSDs are also shown as grey dotted lines in Figure 3-9. Table 3-42 provides an evaluation of the predictions of the mouse model for each data set, with figures showing individual time-course data and predictions in Appendix A.



Each panel shows results for a different measurement. The solid line represents prediction = data, and the grey dotted lines show prediction = data × GSD_{err} and data ÷ GSD_{err}, where GSD_{err} is the median estimate of the residual-error GSD shown in Table 3-41.

Figure 3-9. Comparison of mouse data and PBPK model predictions from a random posterior sample.



Each panel shows results for a different measurement. The solid line represents prediction = data, and the grey dotted lines show prediction = data × GSD_{err} and data ÷ GSD_{err}, where GSD_{err} is the median estimate of the residual-error GSD shown in Table 3-41.

Figure 3-9 (continued). Comparison of mouse data and PBPK model predictions from a random posterior sample.

Table 3-42. Summary comparison of updated PBPK model predictions and in vivo data in mice

Study	Exposure(s)	Discussion
Abbas and Fisher (1997)	TCE gavage (corn oil)	Generally, model predictions were quite good, especially with respect to tissue concentrations of TCE, TCA, and TCOH. There were some discrepancies in TCA and TCOG urinary excretion and TCA and TCOG concentrations in blood due to the requirement (unlike in Hack et al., 2006) that all experiments in the same study utilize the same parameters. Thus, for instance, TCOG urinary excretion was accurately predicted at 300 mg/kg, underpredicted at 600 mg/kg, overpredicted at 1,200 mg/kg, and underpredicted again at 2,000 mg/kg, suggesting significant intraexperimental variability (not addressed in the model). Population predictions were quite good, with the almost all of the data within the 95% CI of the predictions, and most within the interquartile region.
Abbas et al. (1997)	TCOH, TCA i.v.	Both subject-specific and population predictions were quite good. Urinary excretion, which was overpredicted by the Hack et al. (2006) model, was accurately predicted due to the allowance of additional "untracked" clearance. In the case of population predictions, almost all of the data were within the 95% CI of the predictions, and most within the interquartile region.
Fisher and Allen (1993)	TCE gavage (corn oil)	Both subject-specific and population predictions were quite good. Some discrepancies in the time-course of TCE blood concentrations were evidence across doses in the subject-specific predictions, but not in the population predictions, suggesting significant intrasubject variability (not addressed in the model).
Fisher et al. (1991)	TCE inhalation	Blood TCE levels during and following inhalation exposures were still overpredicted at the higher doses. However, there was the stringent requirement (absent in Hack et al., 2006) that the model utilize the same parameters for all doses and in both the closed and open-chamber experiments. Moreover, the Hack et al. (2006) model required significant differences in the parameters for the different closed-chamber experiments, while predictions here were accurate utilizing the same parameters across different initial concentrations. These conclusions were the same for subject-specific and population predictions (e.g., TCE blood levels remained overpredicted in the later case).
Green and Prout (1985)	TCE gavage (corn oil)	Both subject-specific and population predictions were adequate, though the data collection was sparse. In the case of population predictions, almost all of the data were within the 95% CI of the predictions, and about half within the interquartile region.
Greenberg et al. (1999)	TCE inhalation	Model predictions were quite good across a wide variety of measures that included tissue concentrations of TCE, TCA, and TCOH. However, as with the Hack et al. (2006) predictions, TCE blood levels were overpredicted by up to twofold. Population predictions were quite good, with the exception of TCE blood levels. Almost all of the other data was within the 95% CI of the predictions, and most within the interquartile region.
Larson and Bull (1992a)	TCE gavage (aqueous)	Both subject-specific and population predictions were quite good, though the data collection was somewhat sparse. In the case of population predictions, all of the data were within the 95% CI of the predictions.
Larson and Bull (1992b)	TCA gavage (aqueous)	Both subject-specific and population predictions were quite good. In the case of population predictions, most of the data were within the interquartile region.
Merdink et al. (1998)	TCE i.v.	Both subject-specific and population predictions were quite good, though the data collection was somewhat sparse. In the case of population predictions, all of the data were within the 95% CI of the predictions.

Table 3-42. Summary comparison of updated PBPK model predictions and in vivo data in mice (continued)

Study	Exposure(s)	Discussion
Prout et al. (1985)	TCE gavage (corn oil)	Both subject-specific and population predictions were adequate, though there was substantial scatter in the data due to the use of single animals at each data point.
Templin et al. (1993)	TCE gavage (aqueous)	Both subject-specific and population predictions were quite good. With respect to population predictions, almost all of the other data was within the 95% CI of the predictions, and most within the interquartile region.

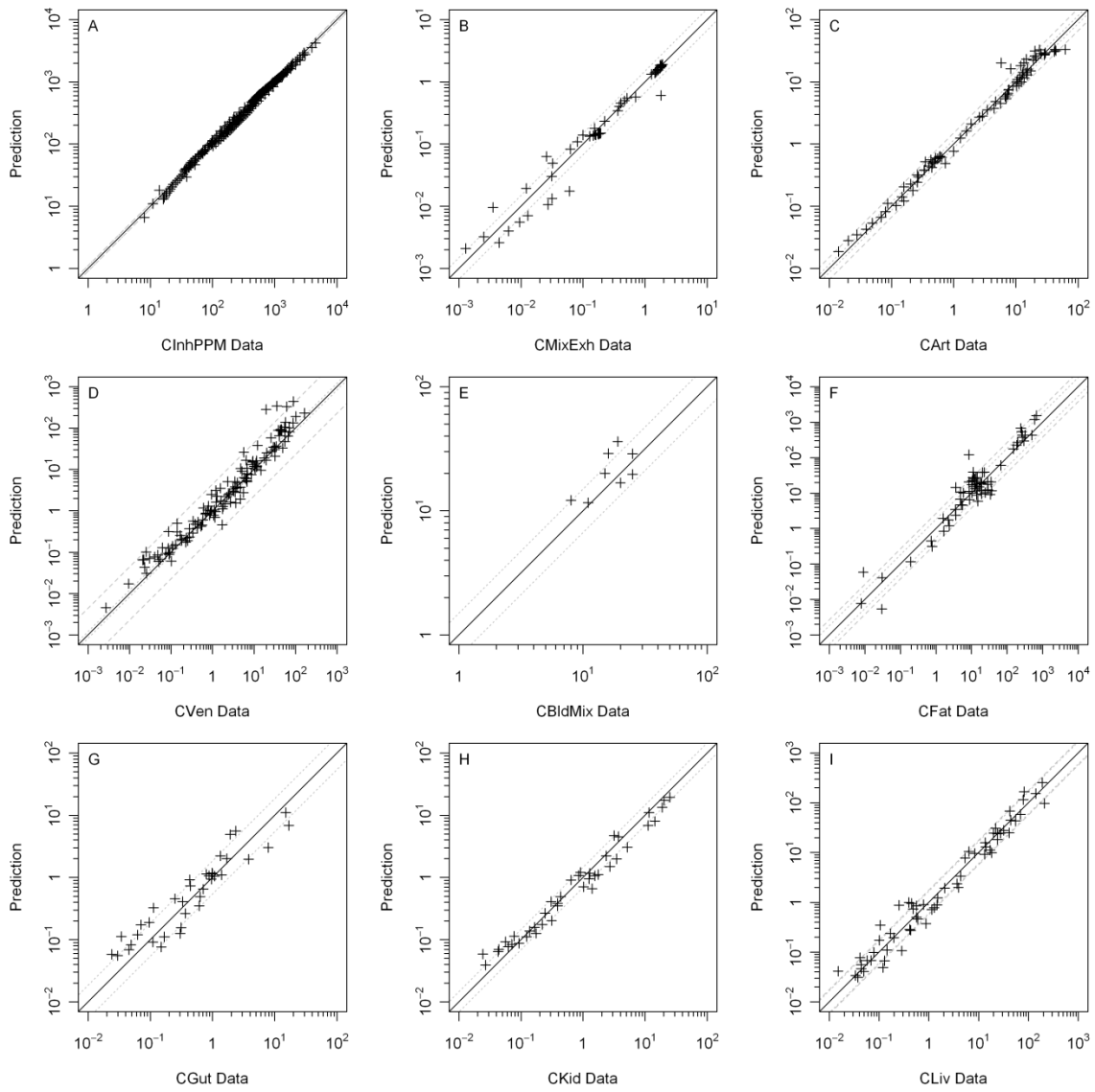
In terms of total metabolism, closed-chamber data (see Figure 3-9, panel A) were fit accurately with the updated model, with a small residual-error GSD of 1.18. While the previous analyses of Hack et al. (2006) allowed for each chamber experiment to be fit with different parameters, the current analysis made the more restrictive assumption that all experiments in a single study utilize the same parameters. Furthermore, the accuracy of closed-chamber predictions did not require the very high values for cardiac output that were used by Fisher et al. (1991), confirming the suggestion (discussed in Appendix A) that additional respiratory metabolism would resolve this discrepancy. The accurate model means that uncertainty with respect to possible wash-in/wash-out, respiratory metabolism, and extrahepatic metabolism could be well characterized. For instance, the absence of in vivo data on GSH metabolism in mice means that this pathway remains relatively uncertain; however, the current model should be reliable for estimating lower and upper bounds on the GSH pathway flux.

In terms of the parent compound TCE (see Figure 3-9, panels B-G), the parent PBPK model (for TCE) appears to now be robust, with the exception of the remaining overprediction of TCE in blood following inhalation exposure. As expected, the venous-blood TCE concentration had the largest residual-error, with a GSD of 2.7, reflecting largely the difficulty in fitting TCE blood levels following inhalation exposure. In addition, the fat and kidney TCE concentrations also are somewhat uncertain, with a GSD for the residual-error of 2.5 and 2.2, respectively. These tissues were only measured in two studies, Abbas and Fisher (1997) and Greenberg et al. (1999), and the residual-error reflects the difficulties in simultaneously fitting the model to the different dose levels with the same parameters. Residual-error GSDs for other TCE measurements were less than twofold. Thus, most of the problems previously encountered with the Abbas and Fisher (1997) gavage data were solved by allowing absorption from both the stomach and duodenal compartments. Notably, the addition of possible wash-in/wash-out, respiratory metabolism, and extrahepatic metabolism (i.e., kidney GSH conjugation) was insufficient to remove the long-standing discrepancy of PBPK models overpredicting TCE blood levels from mouse inhalation exposures, suggesting another source of model or experimental error is the cause. However, the availability of tissue concentration levels of TCE somewhat ameliorates this limitation.

In terms of TCA and TCOH, the overall mass balance and metabolic disposition to these metabolites also appeared to be robust, as urinary excretion following dosing with TCE, TCOH, and TCA could be modeled accurately (see Figure 3-9, panels K and Q). The residual GSDs for the urinary excretions are small: 1.34 for TCA and 1.26 for total TCOH. In addition, the blood and tissue concentrations were also accurately predicted (see Figure 3-9, panels H-J, L-P). All of the residual GSDs were less than twofold, with those for TCA measurements <1.5-fold. This improvement over the Hack et al. (2006) model was likely due in part to the addition of nonurinary clearance ("untracked" metabolism) of TCA and TCOH. Also, the addition of a liver compartment for TCOH and TCOG, so that first-pass metabolism could be properly accounted for, was essential for accurate simulation of the metabolite pharmacokinetics both from intravenous (i.v.) dosing of TCOH and from exposure to TCE.

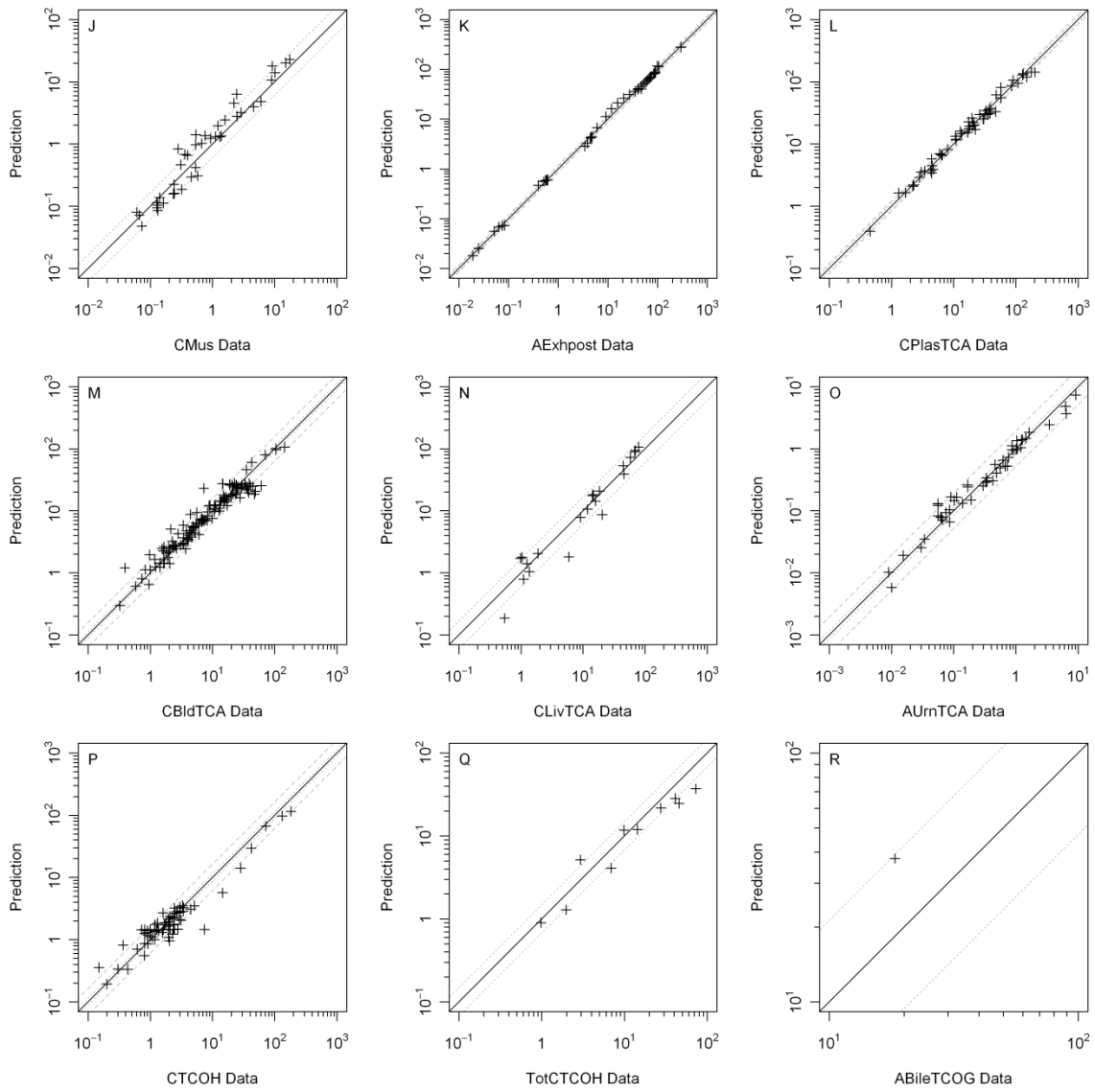
3.5.6.3.2. Rat model and data

Each panel of Figure 3-10 shows a scatter plot of the calibration data and a random posterior prediction for each of the measured endpoint. The endpoint abbreviations are listed in Table 3-41, as are the implied GSDs for the "residual" errors, which include intrastudy variability, interindividual variability, and measurement and model errors. The residual-error GSDs are also shown as grey dashed or dotted lines in Figure 3-10. A summary evaluation of the predictions of the rat model as compared to the data are provided in Tables 3-43 and 3-44, with figures showing individual time-course data and predictions in Appendix A.



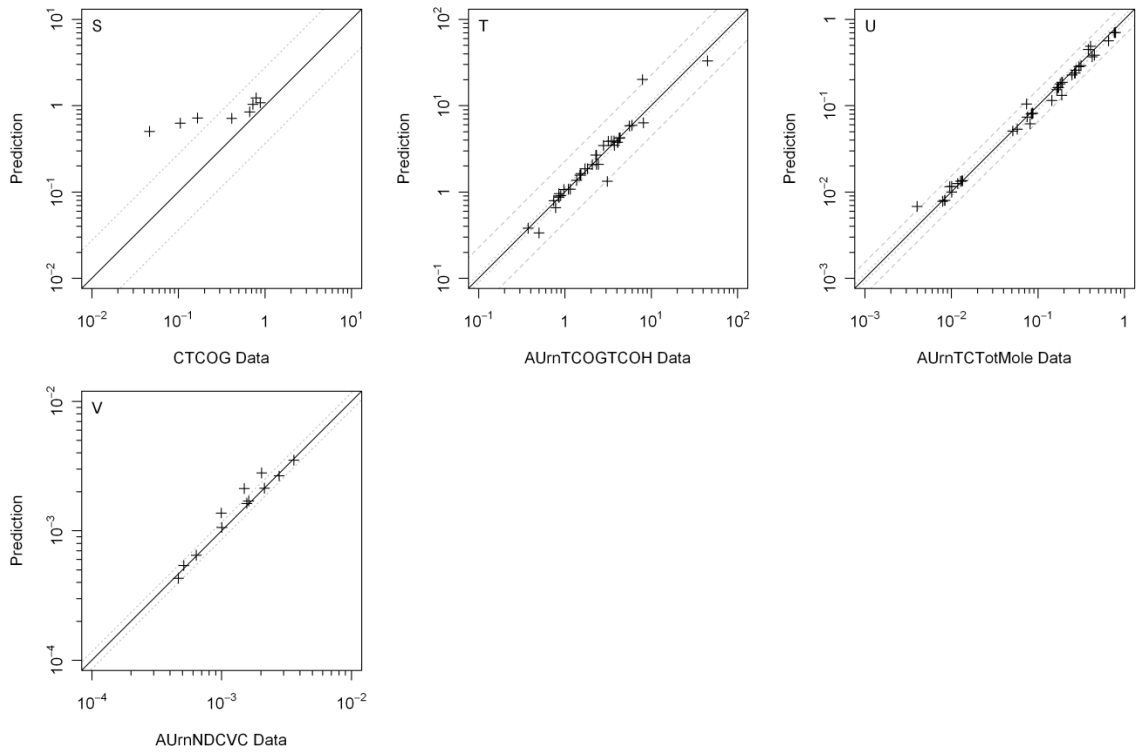
Each panel shows results for a different measurement. The solid line represents prediction = data, and the grey lines show prediction = data × GSD_{err} and data ÷ GSD_{err}, where GSD_{err} is the lowest (dotted) and highest (dashed) median estimate of the residual-error GSD shown in Table 3-41.

Figure 3-10. Comparison of rat data and PBPK model predictions from a random posterior sample.



Each panel shows results for a different measurement. The solid line represents prediction=data, and the grey lines show prediction = data × GSD_{err} and data ÷ GSD_{err}, where GSD_{err} is the lowest (dotted) and highest (dashed) median estimate of the residual-error GSD shown in Table 3-41.

Figure 3-10 (continued). Comparison of rat data and PBPK model predictions from a random posterior sample.



Each panel shows results for a different measurement. The solid line represents prediction=data, and the grey lines show prediction = data × GSD_{err} and data ÷ GSD_{err}, where GSD_{err} is the lowest (dotted) and highest (dashed) median estimate of the residual-error GSD shown in Table 3-41.

Figure 3-10 (continued). Comparison of rat data and PBPK model predictions from a random posterior sample.

Table 3-43. Summary comparison of updated PBPK model predictions and in vivo data used for —calibration" in rats

Study	Exposure(s)	Discussion
Bernauer et al. (1996)	TCE inhalation	Posterior fits to these data were adequate, especially with the requirement that all predictions for dose levels utilize the same PBPK model parameters. Predictions of TCOG and TCA urinary excretion was relatively accurate, though the time-course of TCA excretion seemed to proceed more slowly with increasing dose, an aspect not captured in the model. Importantly, unlike the Hack et al. (2006) results, the time-course of NAcDCVC excretion was quite well simulated, with the excretion rate remaining nonnegligible at the last time point (48 hrs). It is likely that the addition of the DCVG submodel between TCE and DCVC, along with prior distributions that accurately reflected the lack of reliable, independent (e.g., in vitro) data on bioactivation, allowed for the better fit.
Dallas et al. (1991)	TCE inhalation	These data, consisting of arterial blood and exhaled breath concentrations of TCE, were accurately predicted by the model using both subject-specific and population-sampled parameters. In the case of population predictions, most of the data were within the 95% CI of the predictions.
Fisher et al. (1989)	TCE inhalation	These data, consisting of closed-chamber TCE concentrations, were accurately simulated by the model using both subject-specific and population-sampled parameters. In the case of population predictions, most of the data were within the 95% CI of the predictions.
Fisher et al. (1991)	TCE inhalation	These data, consisting of TCE blood, and TCA blood and urine time-courses, were accurately simulated by the model using both subject-specific and population-sampled parameters. In the case of population predictions, most of the data were within the 95% CI of the predictions.

Table 3-43. Summary comparison of updated PBPK model predictions and in vivo data used for —calibration" in rats (continued)

Study	Exposure(s)	Discussion
Green and Prout (1985)	TCE gavage (corn oil) TCA i.v. TCA gavage (aqueous)	For TCE treatment, these data, consisting of one time point each in urine for TCA, TCA +TCOG, and TCOG, were accurately simulated by both subject-specific and population predictions. For TCA i.v. treatment, the single datum of urinary TCA+TCOG at 24 hrs was at the lower 95% CI in the subject-specific simulations, but accurately predicted with the population-sampled parameters, suggesting intrastudy variability is adequately accounted for by population variability. For TCA gavage treatment, the single datum of urinary TCA+TCOG at 24 hrs was accurately simulated by both subject-specific and population predictions.
Hissink et al. (2002)	TCE gavage (corn oil) TCE i.v.	These data, consisting of TCE blood, and TCA+TCOG urinary excretion time-courses, were accurately simulated by the model using subject-specific parameters. In the case of population predictions, TCA+TCOH urinary excretion appeared to be somewhat underpredicted.
Kaneko et al. (1994)	TCE inhalation	These data, consisting of TCE blood and TCA and TCOG urinary excretion time-courses, were accurately predicted by the model using both subject-specific and population-sampled parameters. In the case of population predictions, TCA+TCOH urinary excretion appeared to be somewhat underpredicted, However, all of the data were within the 95% CI of the predictions.

Table 3-43. Summary comparison of updated PBPK model predictions and in vivo data used for "calibration" in rats (continued)

Study	Exposure(s)	Discussion
Keys et al. (2003)	TCE inhalation, gavage (aqueous), i.a.	These data, consisting of TCE blood, gut, kidney, liver, muscle, and fat concentration time-courses, were accurately predicted by the model using both subject-specific and population-sampled parameters. In the case of population predictions, most of the data were within the 95% CI of the predictions.
Kimmerle and Eben (1973b)	TCE inhalation	Some inaccuracies were noted in subject-specific predictions, particularly with TCA and TCOG urinary excretion, TCE exhalation postexposure, and TCE venous blood concentrations. In the case of TCA excretion, the rate was underpredicted at the lowest dose (49 mg/kg) and overpredicted at 330 ppm. In terms of TCOG urinary excretion, the rate was overpredicted at 175 ppm and underpredicted at 330 ppm. Similarly for TCE exhaled postexposure, there was some overprediction at 175 ppm and some underprediction at 300 ppm. Finally, venous blood concentrations were overpredicted at 3,000 ppm. However, for population predictions, most of the data were within the 95% confidence region.
Larson and Bull (1992b)	TCA gavage (aqueous)	These data, consisting of TCA plasma time-courses, were accurately predicted by the model using both subject-specific and population-sampled parameters. In the case of population predictions, all of the data were within the 95% CI of the predictions.
Larson and Bull (1992a)	TCE gavage (aqueous)	These data, consisting of TCE, TCA, and TCOH in blood, were accurately predicted by the model using both subject-specific and population-sampled parameters. In the case of population predictions, all of the data were within the 95% CI of the predictions.
Lee et al. (2000a; Lee et al., 2000b)	TCE i.v., p.v.	These data, consisting of TCE concentration time course in mixed arterial and venous blood and liver, were predicted using both the subject specific and population predictions. In both cases, most of the data were within the 95% CI of the predictions.
Merdink et al. (1999)	TCOH i.v.	TCOH blood concentrations were accurately predicted using subject-specific parameters. However, population-based parameters seemed to lead to some underprediction, though most of the data were within the 95% CI of the predictions.
Prout et al. (1985)	TCE gavage (corn oil)	Most of these data were accurately predicted using both subject-specific and population-sampled parameters. However, at the highest two doses (1,000 and 2,000 mg/kg), there were some discrepancies in the (very sparsely collected) urinary excretion measurements. In particular, using subject-specific parameters, TCA+TCOH urinary excretion was underpredicted at 1,000 mg/kg and overpredicted at 2,000 mg/kg. Using population-sampled parameters, this excretion was underpredicted in both cases, though not entirely outside of the 95% CI.
Simmons et al. (2002)	TCE inhalation	Most of these data were accurately predicted using both subject-specific and population-sampled parameters. In the open-chamber experiments, there was some scatter in the data that did not seem to be accounted for in the model. The closed-chamber data were accurately fit.

Table 3-43. Summary comparison of updated PBPK model predictions and in vivo data used for "calibration" in rats (continued)

Study	Exposure(s)	Discussion
Stenner et al. (1997)	TCE intraduodenal TCOH i.v. TCOH i.v., bile-cannulated	These data, consisting of TCA and TCOH in blood and TCA and TCOG in urine, were generally accurately predicted by the model using both subject-specific and population-sampled parameters. However, using subject-specific parameters, the amount of TCOG in urine was overpredicted for 100 TCOH mg/kg i.v. dosing, though total TCOH in blood was accurately simulated. In addition, in bile-cannulated rats, the TCOG excretions at 5 and 20 mg/kg i.v. were underpredicted, while the amount at 100 mg/kg was accurately predicted. On the other hand, in the case of population predictions, all of the data were within the 95% CI of the predictions, and mostly within the interquartile region, even for TCOG urinary excretion. This suggests that intrastudy variability may be a source of the poor fit in using the subject-specific parameters.
Templin et al. (1995b)	TCE oral (aqueous)	These data, consisting of TCE, TCA, and TCOH in blood, were accurately predicted by the model using both subject-specific and population-sampled parameters. In the case of population predictions, all of the data were within the 95% CI of the predictions.
Yu et al. (2000)	TCA i.v.	These data, consisting of TCA in blood, liver, plasma, and urine, were generally accurately predicted by the model using both subject-specific and population-sampled parameters. The only notable discrepancy was at the highest dose of 50 mg/kg, in which the rate of urinary excretion from 0 to 6 hrs appeared to more rapid than the model predicted. However, all of the data were within the 95% CI of the predictions based on population-sampled parameters.

Table 3-44. Summary comparison of updated PBPK model predictions and in vivo data used for "out-of-sample" evaluation in rats

Study	Exposure(s)	Discussion
Andersen et al. (1987a)	TCE inhalation	These closed-chamber data were well within the 95% CI of the predictions based on population-sampled parameters.
Bruckner et al. unpublished	TCE inhalation	These data on TCE in blood, liver, kidney, fat, muscle, gut, and venous blood were generally accurately predicted based on population-sampled parameters. The only notable exception was TCE in the kidney during the exposure period at the 500 ppm level, which was somewhat underpredicted (though levels postexposure were accurately predicted).
Fisher et al. (1991)	TCE inhalation	These data on TCE in blood were well within the 95% CI of the predictions based on population-sampled parameters.
Jakobson et al. (1986)	TCE inhalation	These data on TCE in arterial blood were well within the 95% CI of the predictions based on population-sampled parameters.
Lee et al. (1996)	TCE i.a., i.v., p.v., gavage	Except at some very early time-points (<0.5 hr), these data on TCE in blood were well within the 95% CI of the predictions based on population-sampled parameters.
Lee et al. (2000a; 2000b)	TCE gavage	These data on TCE in blood were well within the 95% CI of the predictions based on population-sampled parameters.

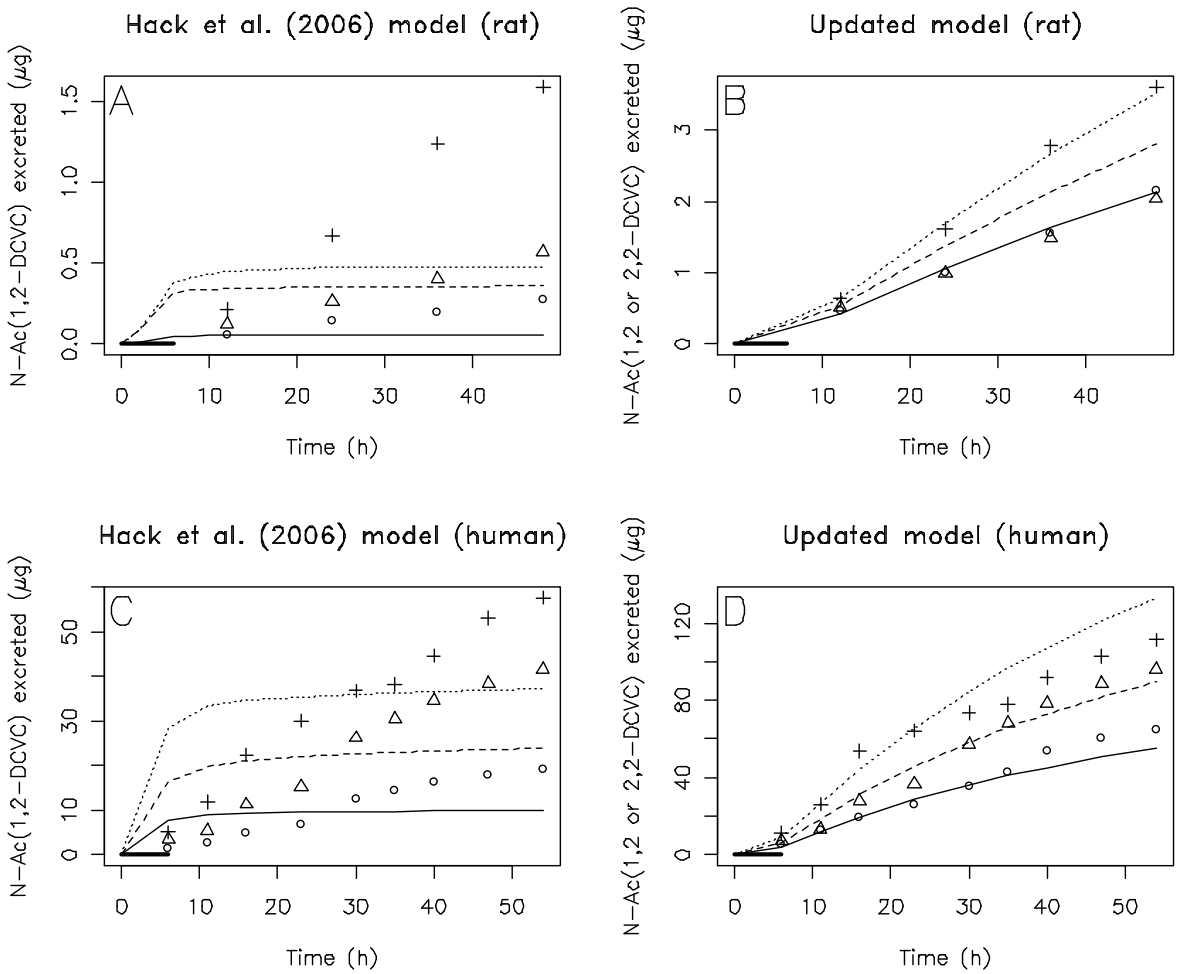
Similar to previous analyses (Hack et al., 2006), the TCE submodel for the rat appears to be robust, accurately predicting blood and tissue concentrations (see Figure 3-10, panels A-K), with residual-error GSDs generally less than twofold. The only exceptions are the predictions of

venous blood from Kimmerle and Eben (1973b), which have residual-error GSDs greater than fourfold, and the predictions of fat concentrations from Simmons et al. (2002); with residual-error GSD of 2.7-fold. For Kimmerle and Eben (1973b), the inaccuracy was primarily at the 3,000-ppm exposure, which might reflect other factors related to the high exposure. For Simmons et al. (2002), the high residual-error appears to reflect scatter due to intrastudy variability. Unlike in the mouse, some data consisting of TCE blood and tissue concentrations were used for "out-of-sample evaluation" (sometimes loosely termed "validation"). These data were generally well simulated (see Table 3-44); most of the data were within the 95% CI of posterior predictions. This provides additional confidence in the predictions for the parent compound.

In terms of TCA and TCOH, as with the mouse, the overall mass balance and metabolic disposition to these metabolites also appeared to be robust: urinary excretion following dosing with TCE, TCOH, and TCA could be modeled accurately (see Figure 3-10 panels O, T, and U), with the residual-errors also indicating good predictions in most cases. Residual-error for these measurements was larger for Green and Prout (1985), Prout et al. (1985), and Stenner et al. (1997), ranging from a GSD of 1.8 to 2.3, reflecting largely intrastudy variability. Residual-errors for the other studies had GSDs of 1.1–1.5. This improvement over the Hack et al. (2006) model was likely due in part to the addition of nonurinary clearance ("untracked" metabolism) of TCA and TCOH. In addition, adding a liver compartment for TCOH and TCOG, so that first-pass metabolism could be properly accounted for, was essential for accurate simulation of the metabolite pharmacokinetics both from i.v. dosing of TCOH and from TCE exposure. Blood and plasma concentrations of TCA and free or total TCOH were also fairly well simulated (see Figure 3-10, panels L, M, P, Q, and S), with GSDs for the residual-error of 1.1–1.6. A bit more discrepancy (residual-error GSD of 1.7) was evident with TCA liver concentrations (see Figure 3-10, panel N). However, TCA liver concentrations were only available in one study (Yu et al., 2000), and the data show a change in the ratio of liver to blood concentrations at the last time point, which may be the source of the added residual-error. Predictions of biliary excretion of TCOG in bile-cannulated rats (see Figure 3-10, panel R), from Green and Prout (1985), and TCOG in blood (see Figure 3-10, panel S), from Stenner et al. (1997), were less accurate, with residual-error GSDs >2. However, the biliary excretion data consisted of a single measurement, and the amount of free TCOH in the same experiment from Stenner et al. (1997) was accurately predicted.

In terms of total metabolism, as with the mouse, closed-chamber data (see Figure 3-10, panel A) were fit accurately with the updated model (residual-error GSD of about 1.1). In addition, the data on NAcDCVC urinary excretion was well predicted (see Figure 3-10, panel V), with residual-error GSD of 1.18. In particular, the fact that excretion was still ongoing at the end of the experiment was accurately predicted (see Figure 3-11, panels A and B). Thus, there is greater confidence in the estimate of the flux through the GSH pathway than there was from the

Hack et al. (2006) model. However, the overall flux is still estimated indirectly, and there remains some ambiguity as to the relative contributions of respiratory wash-in/wash-out, respiratory metabolism, extrahepatic metabolism, DCVC bioactivation vs. *N*-acetylation, and oxidation in the liver producing something other than TCOH or TCA. Therefore, there remains a large range of possible values for the flux through the GSH conjugation and other indirectly estimated pathways that are nonetheless consistent with all of the available in vivo data. The use of noninformative priors for the metabolism parameters for which there were no in vitro data means that a fuller characterization of the uncertainty in these various metabolic pathways could be achieved. Thus, the model should be reliable for estimating lower and upper bounds on several of these pathways.



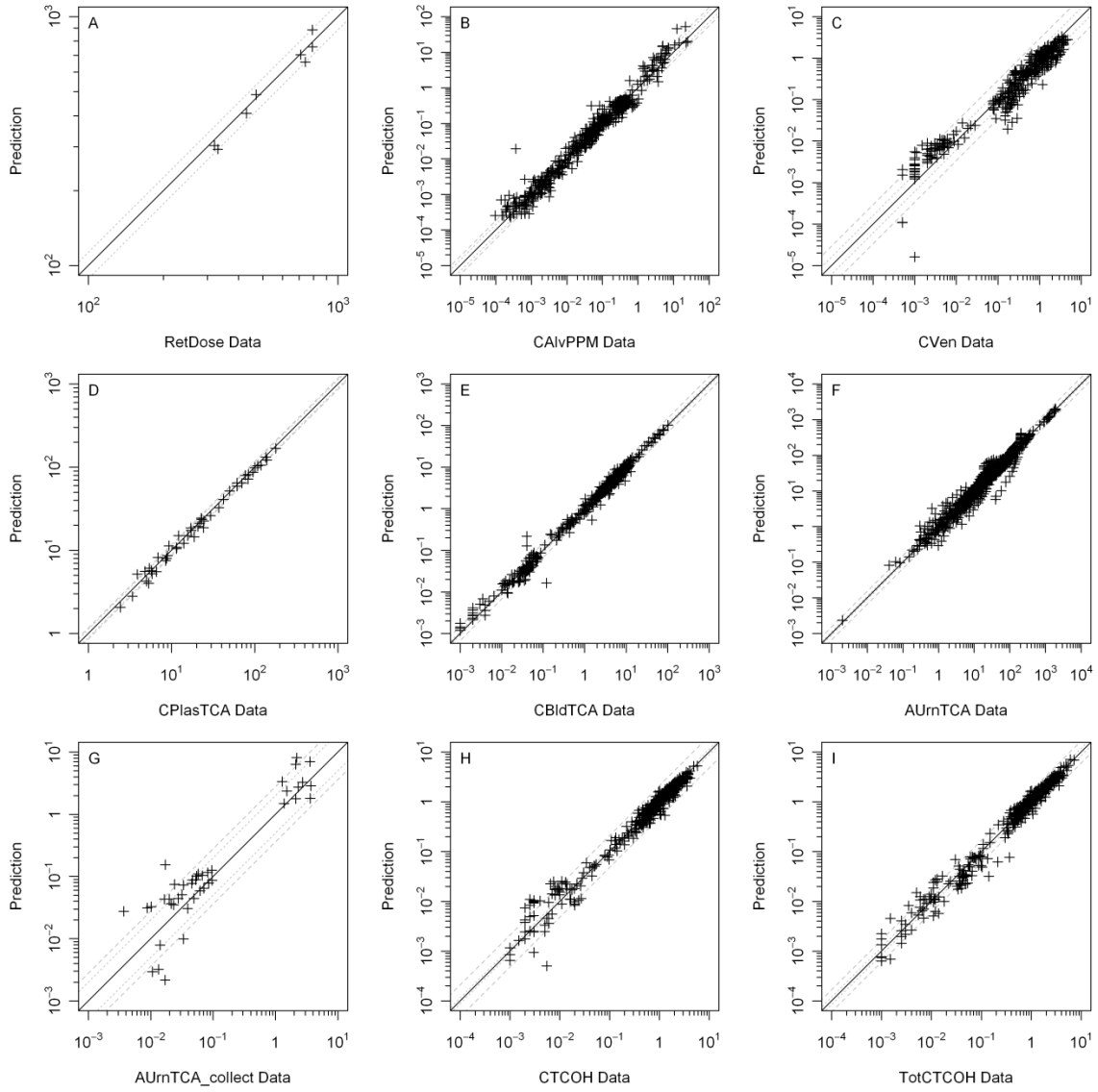
Data are from Bernauer et al. (1996) for (A and B) rats or (C and D) humans exposed for 6 hour to 40 (○), 80 (Δ), or 160 (+) ppm in air (thick horizontal line denotes the exposure period). Predictions from Hack et al. (2006) and the corresponding data (A and C) are only for the 1,2 isomer, whereas those from the updated model (B and D) are for both isomers combined. Parameter values used for each prediction are a random sample from the subject-specific parameters from the rat and human MCMC chains (the last iteration of the first chain was used in each case). Note that in the Hack et al. (2006) model, each dose group had different model parameters, whereas in the updated model, all dose groups are required to have the same model parameters. See files linked to Appendix A for comparisons with the full distribution of predictions.

Figure 3-11. Comparison of urinary excretion data for NAcDCVC and predictions from the Hack et al. (2006) and the updated PBPK models.

3.5.6.3.3. Human model and data

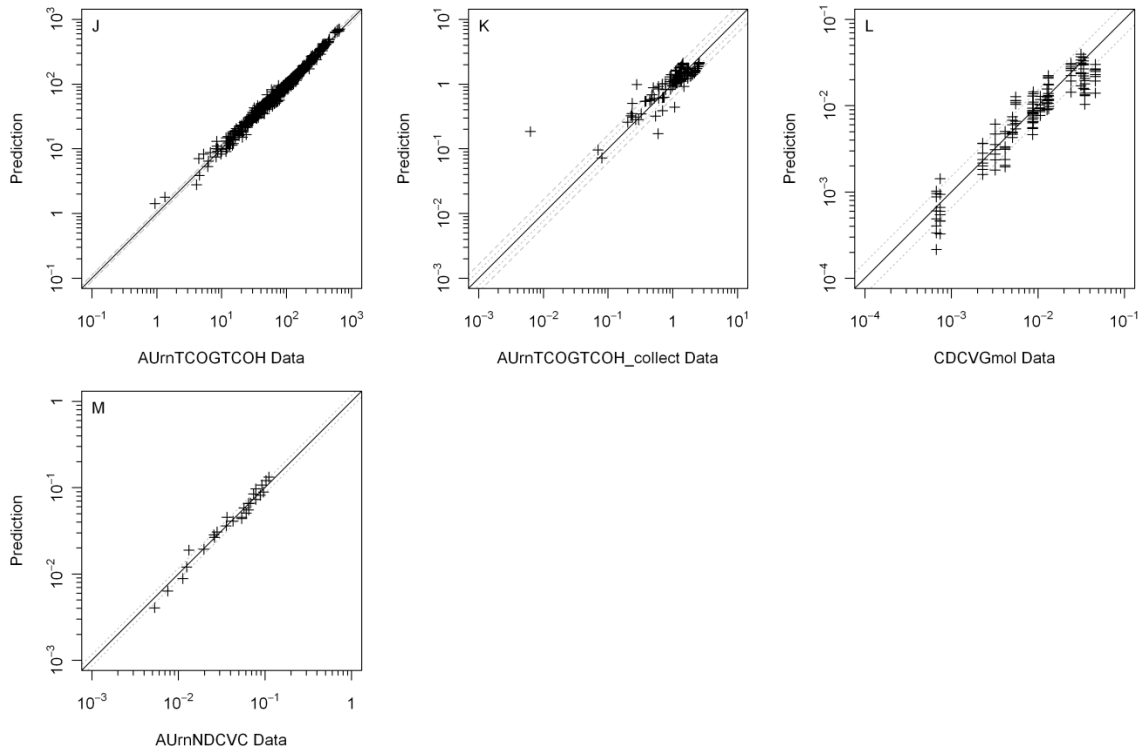
Each panel of Figure 3-12 shows a scatter plot of the calibration data and a random posterior prediction for each of the measured endpoint. The endpoint abbreviations are listed in Table 3-41, as are the implied GSDs for the ~~"r~~esidual" errors, which include intrastudy

variability, interindividual variability, and measurement and model errors. The residual-error GSDs are also shown as grey dashed or dotted lines in Figure 3-12. Table 3-45–3-46 provide a summary evaluation of the predictions of the model as compared to the human data, with figures showing individual time-course data and predictions in Appendix A.



Each panel shows results for a different measurement. The solid line represents prediction = data, and the grey lines show prediction = data × GSD_{err} and data ÷ GSD_{err}, where GSD_{err} is the lowest (dotted) and highest (dashed) median estimate of the residual-error GSD shown in Table 3-41.

Figure 3-12. Comparison of human data and PBPK model predictions from a random posterior sample.



Each panel shows results for a different measurement. The solid line represents prediction = data, and the grey lines show prediction = data × GSD_{err} and data ÷ GSD_{err}, where GSD_{err} is the lowest (dotted) and highest (dashed) median estimate of the residual-error GSD shown in Table 3-41.

Figure 3-12 (continued). Comparison of rat data and PBPK model predictions from a random posterior sample.

Table 3-45. Summary comparison of updated PBPK model predictions and in vivo data used for —calibration" in humans

Reference	Exposure(s)	Discussion
Bernauer et al. (1996)	TCE inhalation	These data, consisting of TCA, TCOG and NAcDCVC excreted in urine, were accurately predicted by the model using both individual-specific and population-sampled parameters. The posterior NAcDCVC predictions were an important improvement over the predictions of Hack et al. (2006), which predicted much more rapid excretion than observed. The fit improvement is probably a result of the addition of the DCVG submodel between TCE and DCVC, along with the broader priors on DCVC excretion and bioactivation. Interestingly, in terms of population predictions, the NAcDCVC excretion data from this study were on the low end, though still within the 95% CI.

Table 3-45. Summary comparison of updated PBPK model predictions and in vivo data used for "calibration" in humans (continued)

Reference	Exposure(s)	Discussion
Chiu et al. (2007)	TCE inhalation	Overall, posterior predictions were quite accurate across most of the individuals and exposure occasions. TCE alveolar breath concentrations were well simulated for both individual-specific and population-generated simulations, though there was substantial scatter (intraoccasion variability). However, TCE blood concentrations were consistently overpredicted in most of the experiments, both using individual-specific and population-generated parameters. This was not unexpected, as Chiu et al. (2007) noted the TCE blood measurements to be lower by about twofold relative to previously published studies. As discussed in Chiu et al. (2007) wash-in/wash-out and extrahepatic (including respiratory) metabolism were not expected to be able to account for the difference, and indeed all of these processes were added to the current model without substantially improving the discrepancy. With respect to metabolite data, TCA and total TCOH in blood were relatively accurately predicted. There was individual experimental variability observed for both TCA and TCOH in blood at 6 hrs (end of exposure). The population-generated simulations overpredicted TCA in blood, while they were accurate in predicting blood TCOH. Predictions of free TCOH in blood also showed overprediction for individual experiments, with variability at the end of exposure timepoint. However, TCOH fits were improved for the population-generated simulations. TCA and TCOG urinary excretion was generally well simulated, with simulations slightly under- or overpredicting the individual experimental data in some cases.
Fisher et al. (1998)	TCE inhalation	The majority of the predictions for these data were quite accurate. Interestingly, in contrast to the predictions for Chiu et al. (2007), TCE blood levels were somewhat underpredicted in a few cases, both from using individual-specific and population-generated predictions. These two results together suggest some unaccounted-for study-to-study variance, though interindividual variability cannot be discounted as the data from Chiu et al. (2007) were from individuals in the Netherlands and that from Fisher et al. (1998) were from individuals in the United States. As reported by Fisher et al. (1998), TCE in alveolar air was somewhat overpredicted in several cases; however, the discrepancies seemed smaller than originally reported for the Fisher et al. model.
Fisher et al. (1998) (continued)	TCE inhalation (continued)	With respect to metabolite data, TCOH and TCA in blood and TCOG and TCA in urine were generally well predicted, though data for some individuals appeared to exhibit inter- and/or intraoccasion variability. For example, in one case in which the same individual (female) was exposed to both 50 and 100 ppm, the TCOH blood data was overpredicted at the higher one exposure. In addition, in one individual, initial individual-specific simulations for TCA in urine were underpredicted but shifted to overpredictions towards the end of the simulations. The population-generated results overpredicted TCA in urine for the same individual. Given the results from Chiu et al. (2007), interoccasion variability is likely to be the cause, though some dose-related effect cannot be ruled out. Finally, DCVG data was well predicted in light of the high variability in the data and availability of only grouped data or data from multiple individuals who cannot be matched to the appropriate TCE and oxidative metabolite data set. In all cases, the basic shape (plateau and then sharp decline) and order of magnitude of the time-course were well predicted, Furthermore, the range of the data was well-captured by the 95% CI of the population-generated predictions.
Kimmerle and Eben (1973a)	TCE inhalation	These data were well fit by the model, using either individual-specific or population-generated parameters.

Table 3-45. Summary comparison of updated PBPK model predictions and in vivo data used for —calibration" in humans (continued)

Reference	Exposure(s)	Discussion
Monster et al. (1976)	TCE inhalation	The data simulated in this case were exhaled alveolar TCE, TCE in venous blood, TCA in blood, TCA in urine, and TCOG in urine. Both using individual-specific and population-generated simulations, all fits are within the 95% CI. The one exception was the retained dose for a male exposed to 65 ppm, which was outside the 95% CI for the population-generated results.
Muller et al. (1974)	TCA, TCOH oral	The data measured after oral TCA was timecourse TCA measured in plasma and urine. Individual-specific predictions were accurate, but both data sets were overpredicted in the population-generated simulations. The data measured after oral TCOH were timecourse TCOH in blood, TCOG in urine, TCA in plasma, and TCA in urine. Individual-specific predictions were accurate, but the population-generated simulations overpredicted TCOH in blood and TCOG in urine. The population-based TCA predictions were accurate. These results indicate that —unusual" parameter values were necessary in the individual-specific simulations to give accurate predictions.
Paykoc et al. (1945)	TCA i.v.	These data were well fit by the model, using either individual-specific or population-generated parameters.

Table 3-46. Summary comparison of updated PBPK model predictions and in vivo data used for —out-of-sample" evaluation in humans

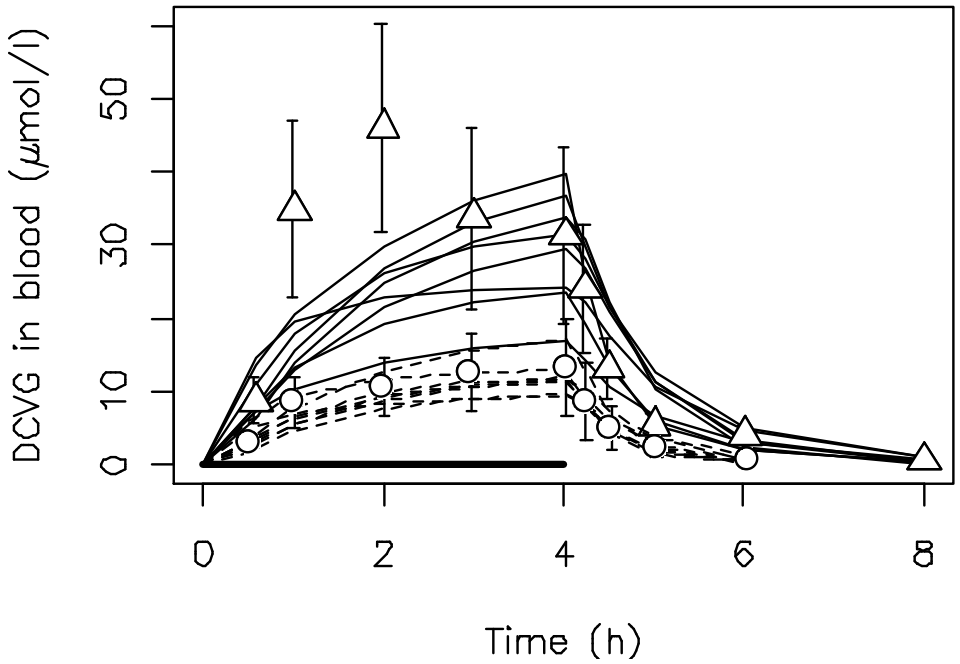
Reference	Exposure(s)	Discussion
Bartonicek (1962)	TCE inhalation	While these data were mostly within the 95% CI of the predictions, they tended to be at the high end for all of the individuals in the study.
Bloemen et al. (2001)	TCE inhalation	These data were all well within the 95% CI of the predictions.
Fernandez et al. (1977)	TCE inhalation	These data were all well within the 95% CI of the predictions.
Lapare et al. (1995)	TCE inhalation	These data were all well within the 95% CI of the predictions.
Monster et al. (1979a)	TCE inhalation	These data were all well within the 95% CI of the predictions.
Muller et al. (1975; 1974)	TCE inhalation	Except for TCE in alveolar air, which was overpredicted during exposure, these data were all well within the 95% CI of the predictions.
Sato et al. (1977)	TCE inhalation	These data were all well within the 95% CI of the predictions.
Stewart et al. (1970)	TCE inhalation	These data were all well within the 95% CI of the predictions.
Triebig et al. (1976)	TCE inhalation	Except for TCE in alveolar air, these data were all well within the 95% CI of the predictions.

With respect to the TCE submodel, retained dose, blood, and exhaled air measurements (see Figure 3-12, panels A-C) appeared more robust than previously found from the Hack et al. (2006) model. TCE blood concentrations from most studies were well predicted, with residual-error GSD in most studies of less than twofold. However, those from Chiu et al. (2007) were consistently overpredicted (i.e., data <0.1 mg/L in Figure 3-12, panel C), with residual-error GSD of almost threefold, and a few of those from Fisher et al. (1989) were consistently underpredicted. Alveolar breath concentrations and retained dose of TCE were well predicted (residual-error GSD <1.5-fold) from all studies except Fisher et al. (1998), which had a residual-error GSD of 1.8-fold. However, the discrepancy in alveolar breath appeared smaller than that originally reported by Fisher et al. (1998) for their PBPK model. In addition, the majority of the "out-of-sample" evaluation data consisted of TCE in blood or breath, and were generally well predicted (see Table 3-46), lending confidence to the model predictions for the parent compound.

In terms of TCA and TCOH, as with the mouse and rat, the overall mass balance and metabolic disposition to these metabolites also appeared to be robust, as urinary excretion following TCE exposure could be modeled accurately (see Figure 3-12, panels F, G, J, and K). In most cases, the residual-error GSD was less than twofold. However, TCA urinary data from Chiu et al. (2007) (panel G in Figure 3-12) indicated greater interoccasion variability, reflected in the residual-error GSD of 2.8. In this study, the same individual exposed to the same concentration on different occasions sometimes had substantial differences in urinary excretion. In addition, many TCA urine measurements in this study were saturated, and had to be omitted, and the fact that the remaining data were sparse and possibly censored may have contributed to the greater intrastudy variability. Blood and plasma concentrations of TCA and free TCOH (see Figure 3-12, panels D, E, and H) were fairly well simulated, with GSD for the residual-error of 1.1–1.4, though total TCOH in blood (see Figure 3-12, panel I) had slightly greater residual-error with GSD of about 1.6. This partially reflects the "sharper" peak concentrations of total TCOH in the Chiu et al. (2007) data relative to the model predictions. In addition, TCA and TCOH blood and urine data were available from several studies for "out-of-sample" evaluation and were generally well predicted by the model (see Table 3-46), lending further confidence to the model predictions for these metabolites.

In terms of total metabolism, no closed-chamber data exist in humans, but, as discussed above, alveolar breath concentrations and retained dose (see Figure 3-12, panels A and B) were generally well simulated, suggesting that total metabolism may be fairly robust. In addition, as with the rat, the data on NAcDCVC urinary excretion was well predicted (see Figure 3-11, Figure 3-12 panel M), with residual-error GSD of 1.12). In particular, the model accurately predicted the fact that excretion was still ongoing at the end of the experiment (48 hours after the end of exposure). Thus, there is greater confidence in the estimate of the flux through this part of the GSH pathway than there was from the Hack et al. (2006) model, in which excretion was completed within the first few hours after exposure (see Figure 3-11, panels C and D).

If only urinary NAcDCVC data were available, as is the case for the rat, the overall GSH conjugation flux would still be estimated indirectly, and there would remain some ambiguity as to the relative contributions of respiratory wash-in/wash-out, respiratory metabolism, extrahepatic metabolism, DCVC bioactivation vs. *N*-acetylation, and oxidation in the liver producing something other than TCOH or TCA. However, unlike in the rat, the blood DCVG data, while highly variable, nonetheless provide substantial constraints (at least a strong lower bound) on the flux of GSH conjugation, and is well fit by the model (see Figure 3-12, panel L, and Figure 3-13). Importantly, the high residual-error GSD for blood DCVG reflects the fact that only grouped or unmatched individual data were available, so in this case, the residual-error includes interindividual variability, which is not included in the other residual-error estimates. However, as discussed above in Section 3.3.3.2.1, there are uncertainties as to the accuracy of analytical method used by Lash et al. (1999b) in the measurement of DCVG in blood. Because these data are so determinative of the overall GSH conjugation flux, these analytical uncertainties are important to consider in the overall evaluation of the PBPK model predictions (see below, Section 3.5.7).



Data are mean concentrations for males (Δ) and females (○) reported in Lash et al. (1999b) for humans exposed for 4 hours to 100 ppm TCE in air (thick horizontal line denotes the exposure period). Data for oxidative metabolites from the same individuals were reported in Fisher et al. (1998) but could not be matched with the individual DCVG data (Lash 2007, personal communication). The vertical error bars are SEs of the mean as reported in Lash et al. (1999b) (n = 8, so SD is 80.5-fold larger). Lines are PBPK model predictions for individual male (solid) and female (dashed) subjects. Parameter values used for each prediction are a random sample from the individual-specific parameters from the human MCMC chains (the last iteration of the 1st chain was used). See files linked to Appendix A for comparisons with the full distribution of predictions.

Figure 3-13. Comparison of DCVG concentrations in human blood and predictions from the updated model.

For the other indirectly estimated pathways, there remain a large range of possible values that are nonetheless consistent with all of the available in vivo data. The use of noninformative priors for the metabolism parameters for which there were no in vitro data means that a fuller characterization of the uncertainty in these various metabolic pathways could be achieved. Thus, as with the rat, the model should be reliable for estimating lower and upper bounds on several of these pathways.

3.5.6.4. Sensitivity Analysis With Respect to Calibration Data

To assess the informativeness of the calibration data to the parameters, local sensitivity analysis is performed with respect to the calibration data points. For each scaling parameter, the

central difference is used to estimate the partial derivatives by centering on the sample mean of its estimated population mean, and then increasing and decreasing by 5%. The relative change in the model output f(θ) is used to estimate a local sensitivity coefficient (SC) as follows:

$$SC = 10 \times \{f(\theta_+) - f(\theta_-)\}/[½ \times \{f(\theta_+) + f(\theta_-)\}]$$

Here, $f(\theta)$ is one of the model predictions of the calibration data, $\theta_\pm$ is the maximum likelihood estimate (MLE) or baseline value of ± 5%. For log-transformed parameters, 0.05 was added or subtracted from the baseline value, whereas for untransformed parameters, the baseline value was multiplied by 1.05 or 0.95. The resulting values of SC are binned into five categories according to their sensitivity coefficient: negligible ($|SC| \leq 0.01$) very low ($0.01 < |SC| \leq 0.1$), low ($0.1 < |SC| \leq 0.5$), medium ($0.5 < |SC| \leq 1.0$), and high ($|SC| > 1.0$).

Note that local sensitivity analyses as typically performed in deterministic PBPK modeling can only inform the "primary" effects of parameter uncertainties (i.e., the direct change on the quantity of interest due to change in a parameter). They cannot address the *propagation* of uncertainties, such as those that can arise due to parameter correlations in the parameter fitting process. Those can only be addressed in a global sensitivity analysis, which is left for future research.

The results of local sensitivity analyses are shown in Figures 3-14–3-16. For each parameter, the number of data points (out of the entire calibration set) that have sensitivity coefficients in the various categories are shown graphically. As summarized in Table 3-47, most of the parameters have at least some calibration data to which they are at least moderately sensitive ($|SC| > 0.5$). Across species, the cardiac output (lnQCC), ventilation-perfusion ratio (lnVPRC), blood-air partition coefficient (lnPBC), V_{MAX} for oxidation ($lnV_{MAX}C$), and VLivC are consistently among the most sensitive parameters, with >10% of the calibration data exhibiting $|SC| > 0.5$ to these parameters. Note that the reason the liver volume is sensitive is that it is used to scale the capacity or clearance rate for oxidation.

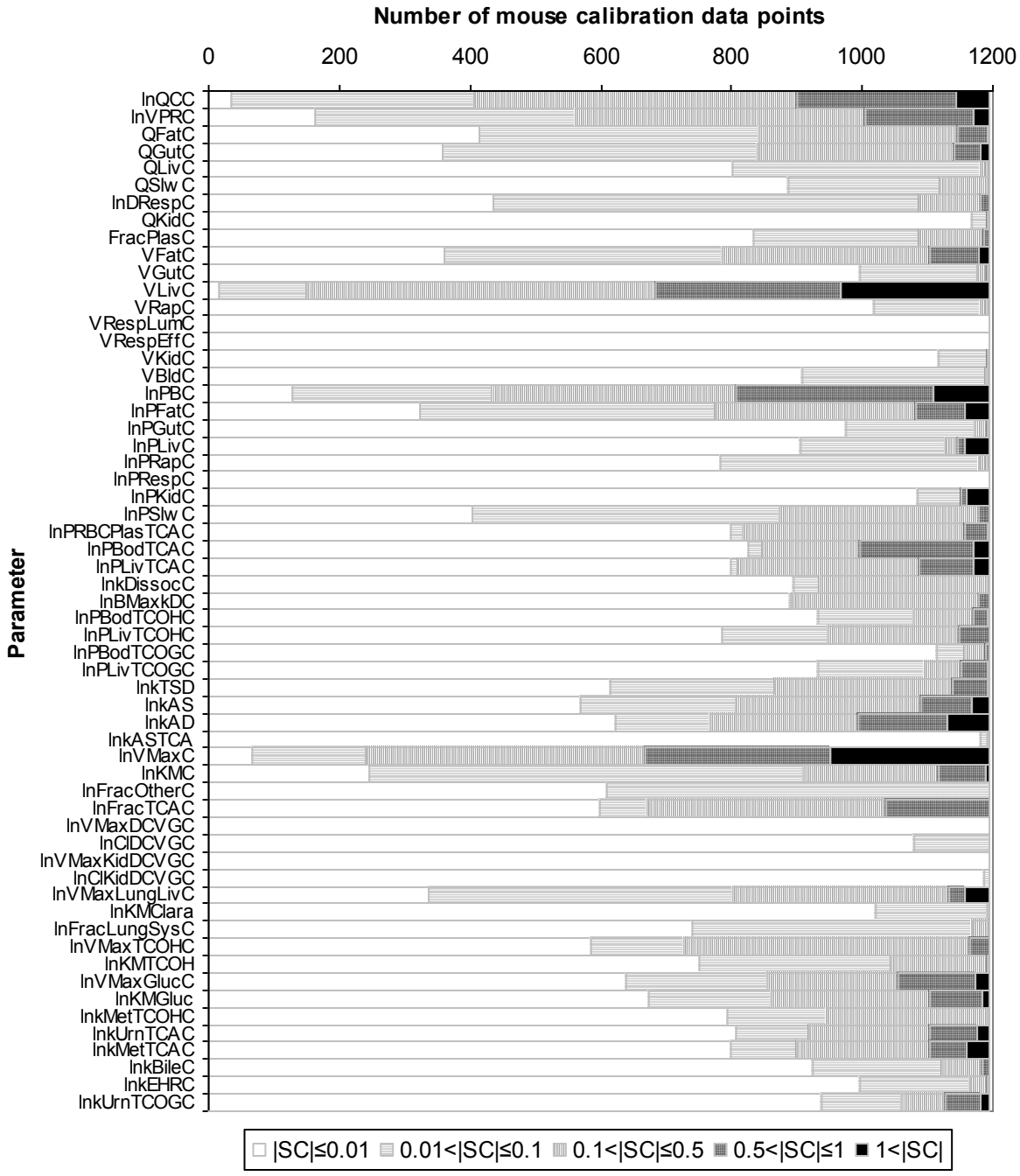


Figure 3-14. Sensitivity analysis results: Number of mouse calibration data points with SC in various categories for each scaling parameter.

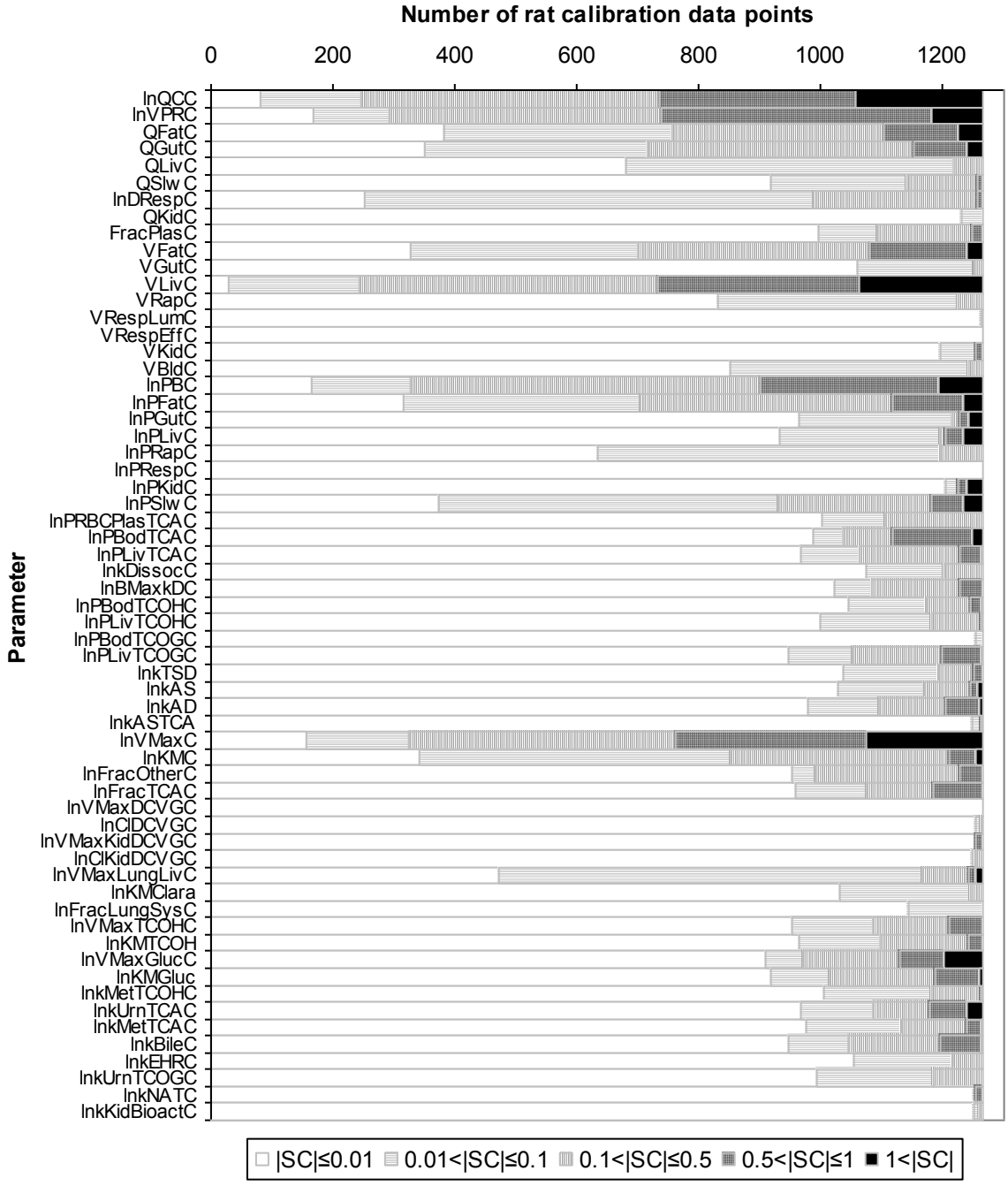


Figure 3-15. Sensitivity analysis results: Number of rat calibration data points with SC in various categories for each scaling parameter.

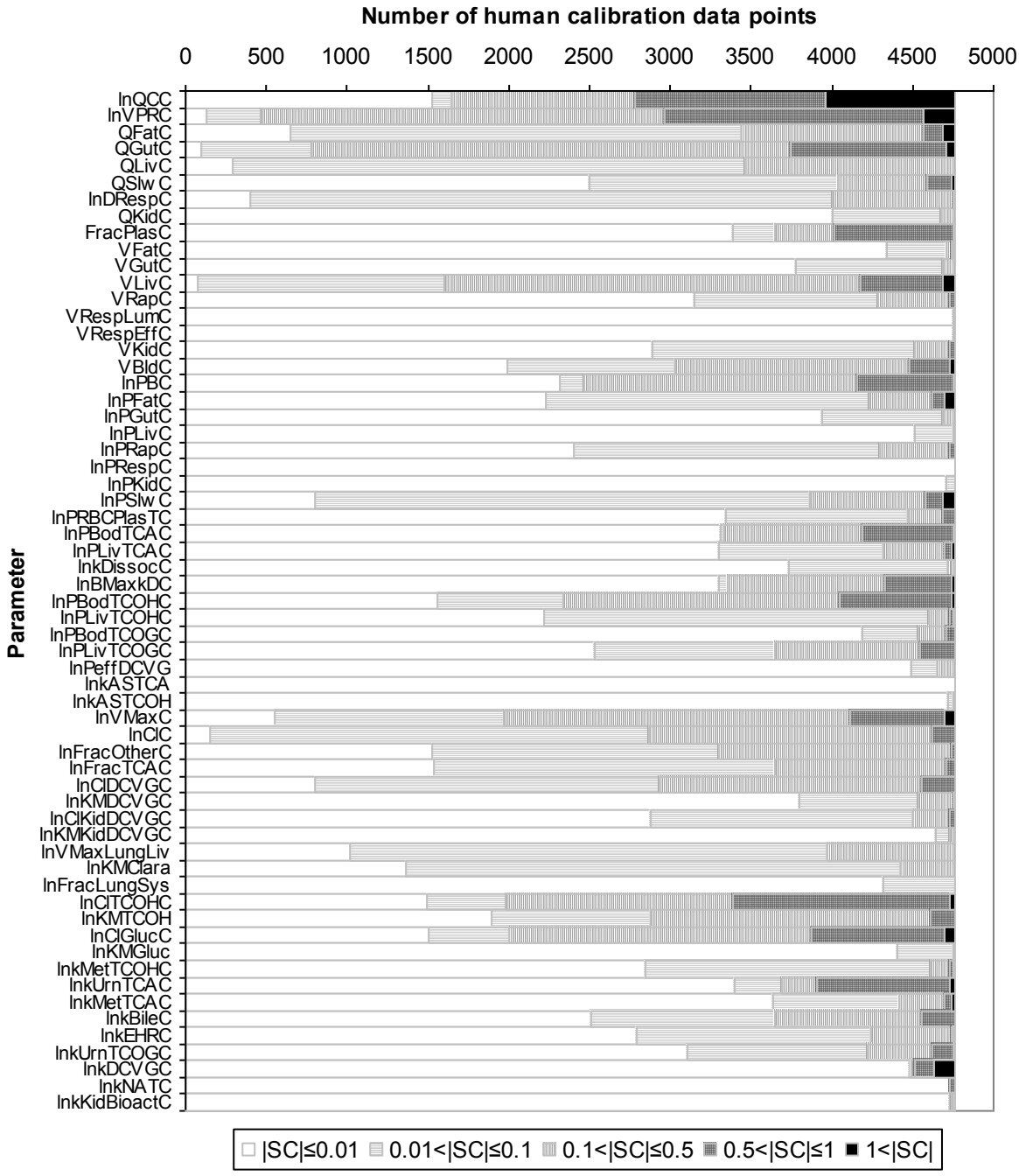


Figure 3-16. Sensitivity analysis results: Number of human calibration data points with SC in various categories for each scaling parameter.

Table 3-47. Summary of scaling parameters ordered by fraction of calibration data of moderate or high sensitivity

Mouse		Rat		Human	
Parameter[a]	Fraction with |SC|>0.5	Parameter[a]	Fraction with |SC|>0.5	Parameter[a]	Fraction with |SC|>0.5
lnV_{MAX}C	0.4405	VLivC	0.4213	lnQCC	0.4159
VLivC	0.428	lnQCC	0.4182	lnVPRC	0.3777
lnPBC	0.3233	lnVPRC	0.4158	lnClTCOHC	0.2871
lnQCC	0.2454	lnV_{MAX}C	0.3984	QGutC	0.2137
lnkAD	0.1675	lnPBC	0.2893	lnClGlucC	0.186
lnPBodTCAC	0.1642	VFatC	0.1455	lnkUrnTCAC	0.1789
lnVPRC	0.1575	QFatC	0.1273	FracPlasC	0.1553
lnFracTCAC	0.1323	lnPBodTCAC	0.1162	lnPBodTCOHC	0.1486
lnV_{MAX}GlucC	0.1147	lnPFatC	0.1154	lnV_{MAX}C	0.1358
lnPFatC	0.093	lnV_{MAX}GlucC	0.1083	lnPBC	0.1269
lnPLivTCAC	0.0896	QGutC	0.0885	VLivC	0.1225
lnkAS	0.0863	lnkUrnTCAC	0.0696	lnPBodTCAC	0.12
VFatC	0.0762	lnPSlwC	0.0664	lnBMaxkDC	0.0897
lnKMGluc	0.0762	lnFracTCAC	0.064	VBldC	0.0586
lnkMetTCAC	0.0762	lnKMGluc	0.0625	lnkDCVGC	0.0515
lnkUrnTCAC	0.0754	lnkBileC	0.0538	lnPLivTCOGC	0.0446
lnKMC	0.0653	lnPLivTCOGC	0.0514	lnClDCVGC	0.0435
lnkUrnTCOGC	0.0544	lnPLivC	0.0482	lnkBileC	0.0422
lnV_{MAX}LungLivC	0.0511	lnkAD	0.0474	QFatC	0.0401
lnkTSD	0.0469	lnKMC	0.0427	lnPSlwC	0.0372
QGutC	0.0452	lnV_{MAX}TCOHC	0.0427	QSlwC	0.0345
QFatC	0.0402	lnPKidC	0.0324	lnKMTCOH	0.0305
lnPLivC	0.0402	lnPGutC	0.03	lnPFatC	0.0292
lnPLivTCOHC	0.0377	lnFracOtherC	0.03	lnClC	0.0288
lnPKidC	0.0352	lnPLivTCAC	0.0292	lnkUrnTCOGC	0.0282
lnPLivTCOGC	0.0352	lnBMaxkDC	0.0285	lnPRBCPlasTCAC	0.0147
lnPRBCPlasTCAC	0.031	lnkMetTCAC	0.0213	lnPLivTCAC	0.0135
lnV_{MAX}TCOHC	0.0235	lnV_{MAX}LungLivC	0.0182	lnkMetTCAC	0.013
lnPBodTCOHC	0.0201	lnKMTCOH	0.0182	lnFracTCAC	0.0103
lnPSlwC	0.0134	lnkAS	0.0158	lnPBodTCOGC	0.0095
lnBMaxkDC	0.0134	lnPBodTCOHC	0.015	VRapC	0.0063
lnDRespC	0.0109	FracPlasC	0.0126	VKidC	0.0057
lnkBileC	0.0084	lnkTSD	0.0103	lnClKidDCVGC	0.0057
FracPlasC	0.0059	VKidC	0.0095	lnkNATC	0.0057
lnPBodTCOGC	0.005	lnV_{MAX}KidDCVGC	0.0095	lnPRapC	0.005
VGutC	0.0025	lnkNATC	0.0095	lnPLivTCOHC	0.005
lnPGutC	0.0025	lnDRespC	0.0063	lnkMetTCOHC	0.005
lnKMTCOH	0.0017	QSlwC	0.0055	lnFracOtherC	0.0046
lnkMetTCOHC	0.0017	lnPLivTCOHC	0.0016	VFatC	0.0036
lnkEHRC	0.0017	lnkASTCA	0.0016	lnkEHRC	0.0036
QKidC	0.0008	lnkMetTCOHC	0.0016	lnDRespC	0.0011
VKidC	0.0008	VGutC	0.0008	lnKMDCVGC	0.0011
		lnPRBCPlasTCAC	0.0008	lnkKidBioactC	0.0002
		lnkUrnTCOGC	0.0008		

[a]Parameters not shown have no data with |SC| > 0.5.

For scaling parameters for which all of the calibration data are negligibly sensitive (|SC| < 0.01), it is important that they either have informative prior data or are unimportant for dose-metric predictions. For mice, these parameters are the volumes of the respiratory lumen and tissue (VRespLumC, VRespEffC), the partition coefficient for the respiratory tissue (lnPRespC), and the V_{MAX} values for GSH conjugation in the liver and kidney. For the respiratory tract parameters, there are prior data to identify the parameters. Moreover, none of the dose-metric predictions are sensitive to these parameters (see Section 3.5.7.2, below). For GSH conjugation, it should be noted that for the clearance in the liver and lung (V_{MAX}/K_M), some data are available with sensitivity 0.01 < |SC| < 0.1. The data are not at all informative as to the maximum capacity for GSH conjugation.

For rats, all of the scaling parameters have at least one calibration data point with |SC| > 0.01. However, for the volumes of the respiratory lumen and tissue (VRespLumC, VRespEffC), the partition coefficient for the respiratory tissue (lnPRespC), and the V_{MAX} values for GSH conjugation in the liver, these consist of only one or two data points. As with mice, there are prior data to help identify the respiratory tract parameters. Moreover, none of the dose-metric predictions are sensitive to the respiratory tract parameters (see Section 3.5.7.2, below). The data are not very informative as to maximum capacity for GSH conjugation in the liver. However, there are some data that have low or moderate informativeness (0.1 < |SC| < 1) as to the maximum capacity for GSH conjugation in the kidney, and clearance via GSH conjugation (V_{MAX}/K_M) in the liver and kidney, which have much greater impact on the dose-metric predictions than the maximum capacity in the liver (see Section 3.5.7.2, below).

For humans, all of the scaling parameters have at least one calibration data point with |SC| > 0.01. However, for the volumes of the respiratory lumen and tissue (VRespLumC, VRespEffC), the partition coefficient for the respiratory tissue (lnPRespC), and the oral absorption rate for TCA, these consist of only one or two data points. As with mice and rats, there are prior data to help identify the respiratory tract parameters. Moreover, none of the dose-metric predictions are sensitive to the respiratory or TCA oral absorption parameters (see Section 3.5.7.2, below).

Therefore, the local sensitivity analysis with respect to calibration data confirms that most of the scaling parameters are informed by at least some of the calibration data. In addition, the parameters for which the calibration data have very little or negligible sensitivity are either informed by prior data or have little impact on dose-metric predictions.

3.5.6.5. Summary Evaluation of Updated PBPK Model

Overall, the updated PBPK model, utilizing parameters consistent with the available physiological and in vitro data from published literature, provides reasonable fits to an extremely large database of in vivo pharmacokinetic data in mice, rats, and humans. Posterior parameter distributions were obtained by MCMC sampling using a hierarchical Bayesian population

statistical model and a large fraction of this in vivo database. Convergence of the MCMC samples for model parameters was good for mice, and adequate for rats and humans. Evaluation of posterior parameter distributions suggests reasonable results in light of prior expectations and the nature of the available calibration data. In addition, in rats and humans, the model produced predictions that are consistent with in vivo data from many studies not used for calibration (insufficient studies were available in mice for such "out of sample" evaluation). Finally, the local sensitivity analysis with respect to calibration data confirms that most of the scaling parameters are informed by at least some of the calibration data, and those that were not either were informed by prior data or would not have great impact on dose-metric predictions.

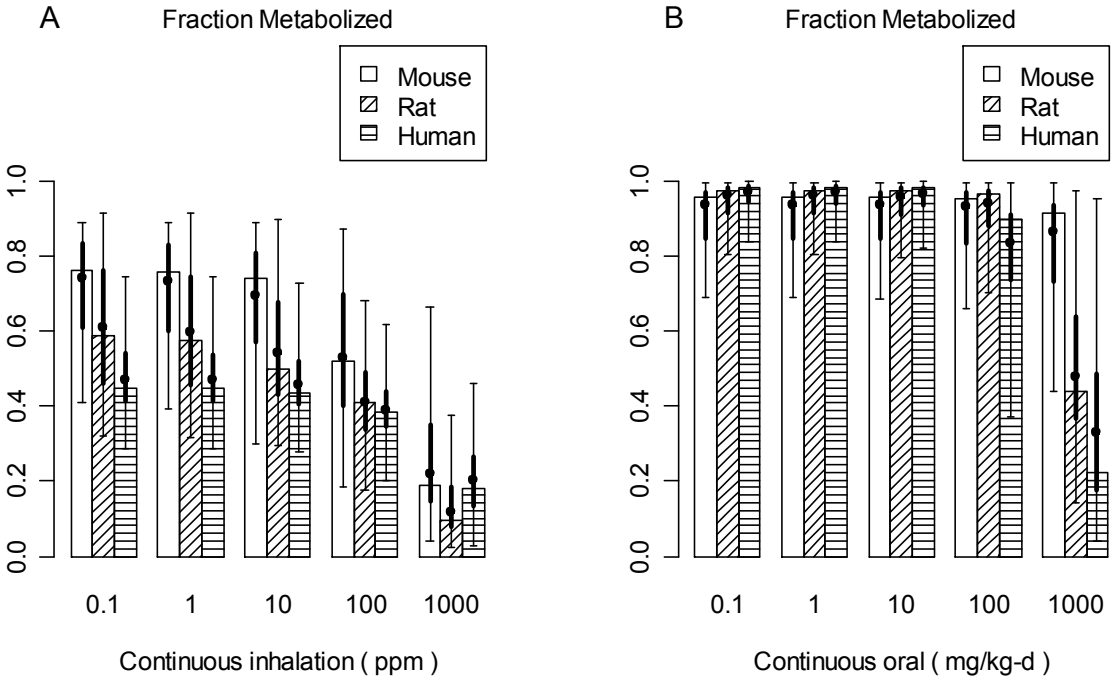
3.5.7. PBPK Model Dose-Metric Predictions

3.5.7.1. Characterization of Uncertainty and Variability

Since it is desirable to characterize the contributions from both uncertainty in population parameters and variability within the population, the following procedure is adopted. First, 500 sets of population parameters (i.e., population mean and variance for each parameter) are extracted from the posterior MCMC samples—these represent the uncertainty in the population parameters. To minimize autocorrelation, they were obtained by "thinning" the chains to the appropriate degree. From each of these sets of population parameters, 100 subject-specific parameters were generated by Monte Carlo—each of these represents the population variability, given a *particular* set of population parameters. Thus, a total of 50,000 subjects, representing 100 (variability) each for 500 different populations (uncertainty), were generated.

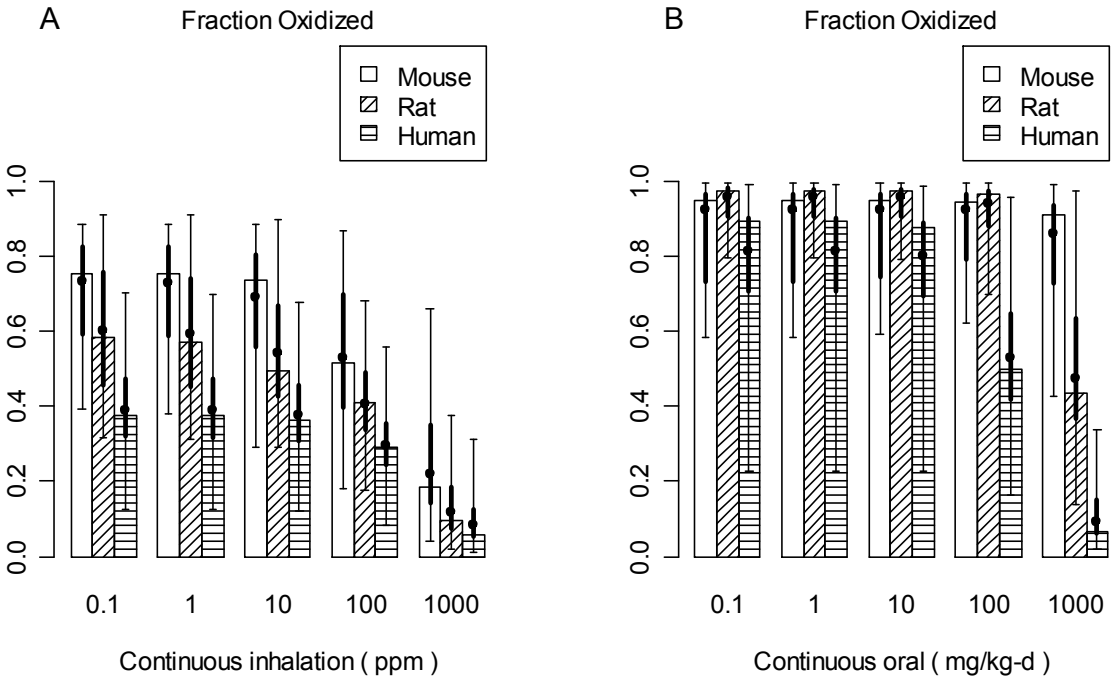
Each set was run for a variety of generic exposure scenarios. The combined distribution of all 50,000 individuals reflects both uncertainty and variability (i.e., the case in which one is trying to predict the dosimetry for a single random subject). In addition, for each dose-metric, the mean predicted internal dose was calculated from each of the 500 sets of 100 individuals, resulting in a distribution for the uncertainty in the population mean. Comparing the combined uncertainty and variability distribution with the uncertainty distribution in the population mean gives a sense of how much of the overall variation is due to uncertainty vs. variability.

Figures 3-17–3-25 show the results of these simulations for a number of representative dose-metrics across species continuously exposed via inhalation or orally. For display purposes, dose-metrics have been scaled by total intake (resulting in a predicted "fraction" metabolized) or exposure level (resulting in an internal dose per ppm for inhalation or per mg/kg-day for oral exposures). In these figures, the thin error bars represent the 95% CI for overall uncertainty and variability, and the thick error bars represent the 95% CI for the uncertainty in the population mean. The interpretation of these figures is that if the thick error bars are much smaller (or greater) than the thin error bars, then variability (or uncertainty) contributes the most to overall uncertainty and variability.



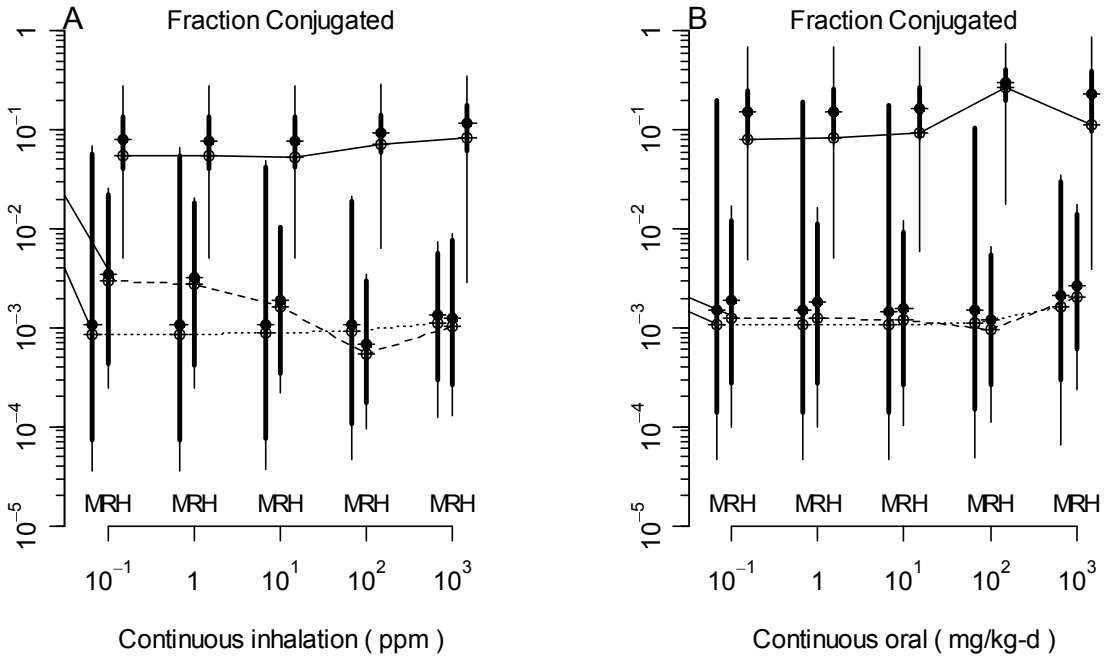
Bars and thin error bars represent the median estimate and 95% CI for a random subject, and reflect combined uncertainty and variability. Circles and thick error bars represent the median estimate and 95% CI for the population mean, and reflect uncertainty only.

Figure 3-17. PBPK model predictions for the fraction of intake that is metabolized under continuous inhalation (A) and oral (B) exposure conditions in mice (white), rats (diagonal hashing), and humans (horizontal hashing).



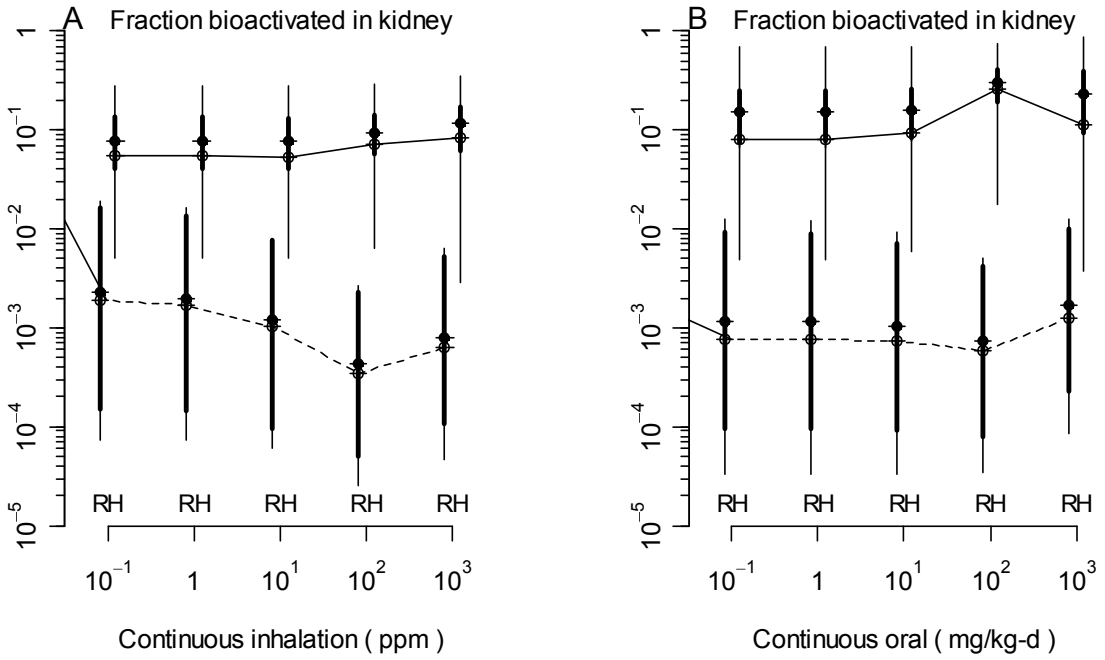
Bars and thin error bars represent the median estimate and 95% CI for a random subject, and reflect combined uncertainty and variability. Circles and thick error bars represent the median estimate and 95% CI for the population mean, and reflect uncertainty only.

Figure 3-18. **PBPK model predictions for the fraction of intake that is metabolized by oxidation (in the liver and lung) under continuous inhalation (A) and oral (B) exposure conditions in mice (white), rats (diagonal hashing), and humans (horizontal hashing).**



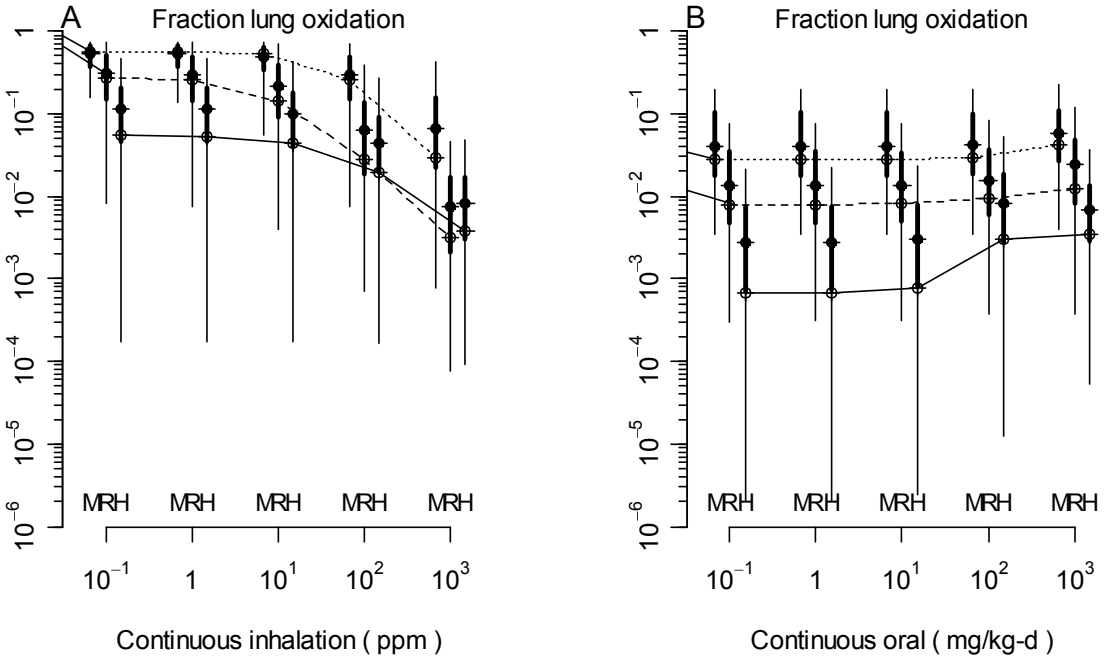
X-values are slightly offset for clarity. Open circles (connected by lines) and thin error bars represent the median estimate and 95% CI for a random subject, and reflect combined uncertainty and variability. Filled circles and thick error bars represent the median estimate and 95% CI for the population mean, and reflect uncertainty only.

Figure 3-19. PBPK model predictions for the fraction of intake that is metabolized by GSH conjugation (in the liver and kidney) under continuous inhalation (A) and oral (B) exposure conditions in mice (dotted line), rats (dashed line), and humans (solid line).



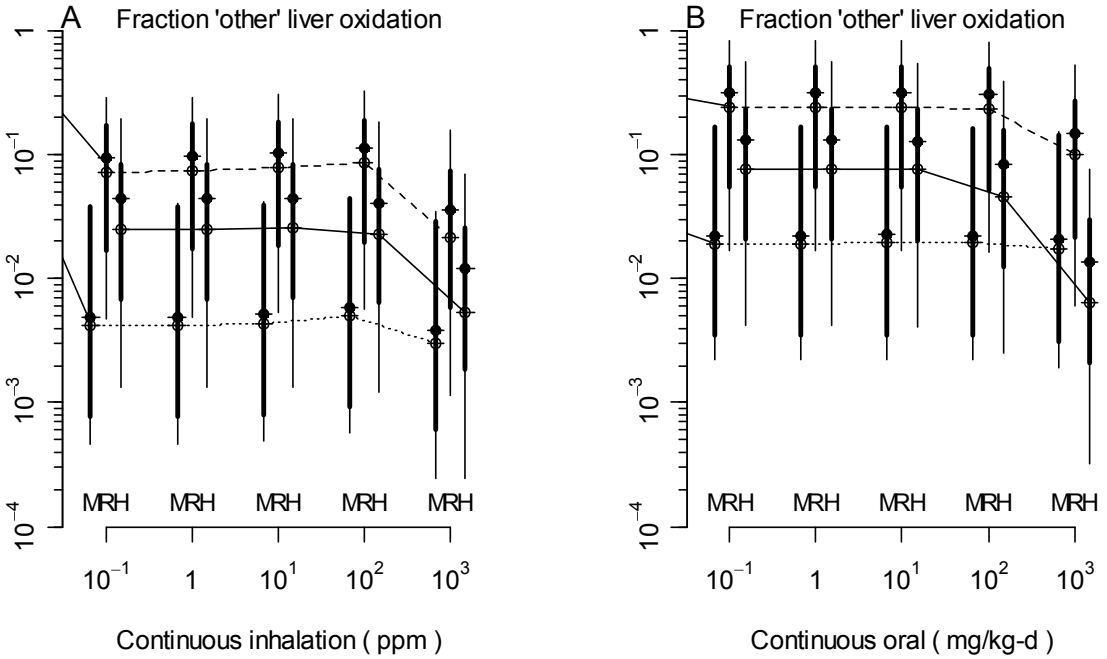
X-values are slightly offset for clarity. Open circles (connected by lines) and thin error bars represent the median estimate and 95% CI for a random subject, and reflect combined uncertainty and variability. Filled circles and thick error bars represent the median estimate and 95% CI for the population mean, and reflect uncertainty only.

Figure 3-20. **PBPK model predictions for the fraction of intake that is bioactivated DCVC in the kidney under continuous inhalation (A) and oral (B) exposure conditions in rats (dashed line) and humans (solid line).**



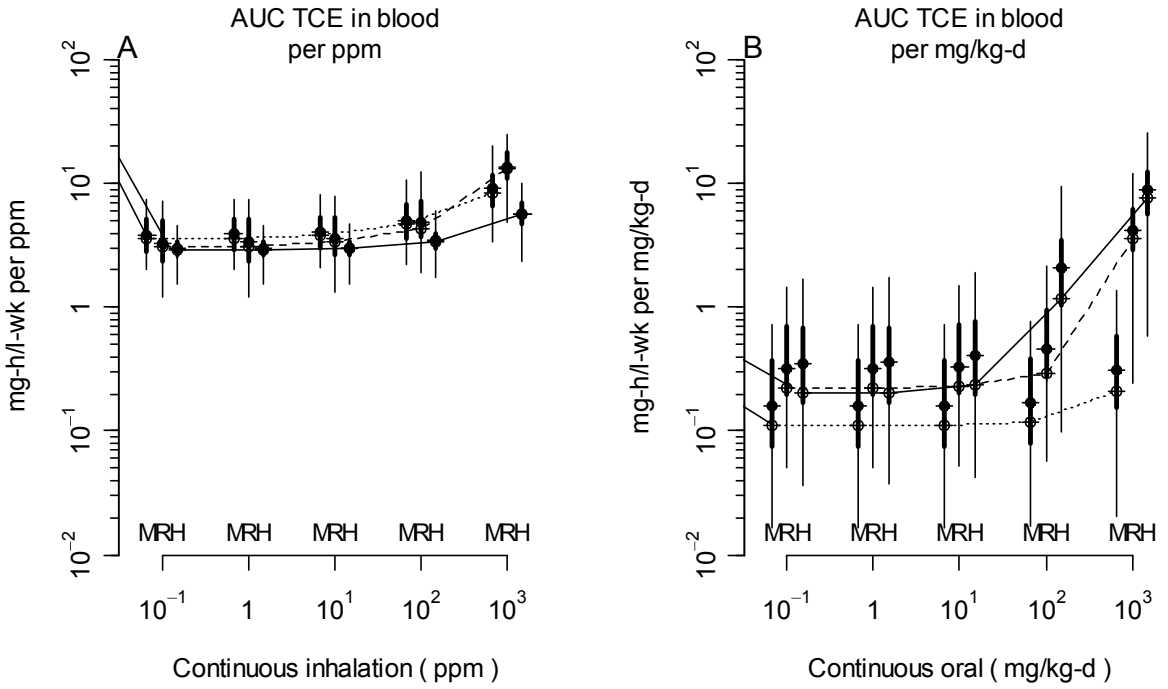
X-values are slightly offset for clarity. Open circles (connected by lines) and thin error bars represent the median estimate and 95% CI for a random subject, and reflect combined uncertainty and variability. Filled circles and thick error bars represent the median estimate and 95% CI for the population mean, and reflect uncertainty only.

Figure 3-21. **PBPK model predictions for fraction of intake that is oxidized in the respiratory tract under continuous inhalation (A) and oral (B) exposure conditions in mice (dotted line), rats (dashed line), and humans (solid line).**



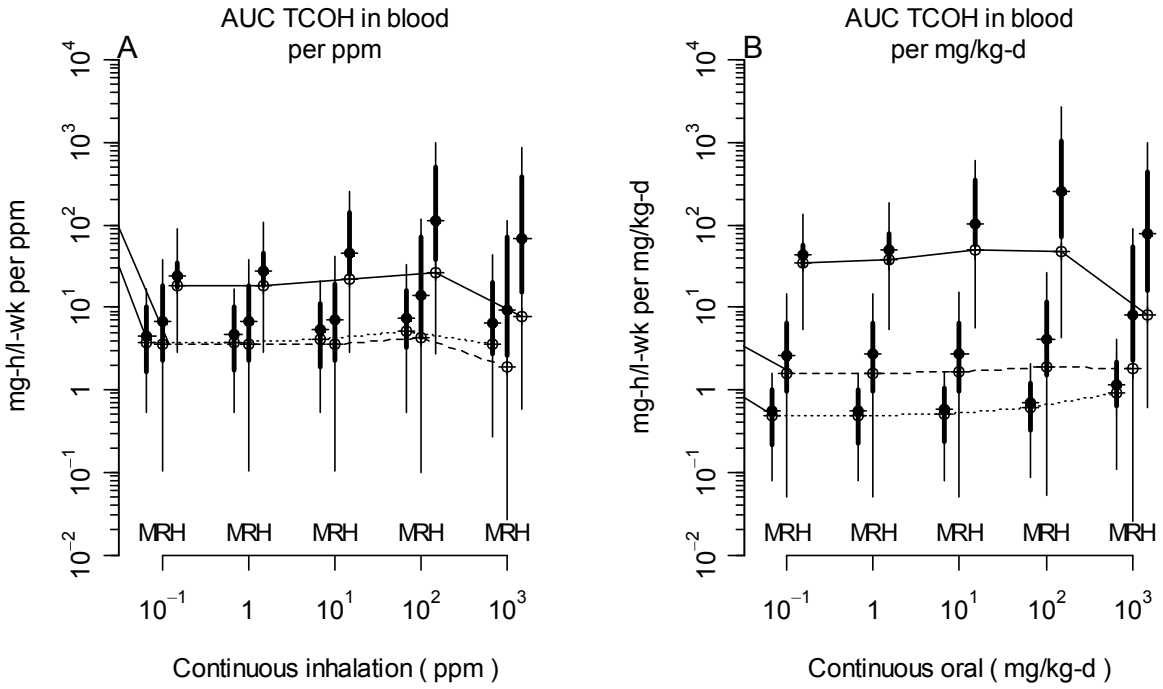
X-values are slightly offset for clarity. Open circles (connected by lines) and thin error bars represent the median estimate and 95% CI for a random subject, and reflect combined uncertainty and variability. Filled circles and thick error bars represent the median estimate and 95% CI for the population mean, and reflect uncertainty only.

Figure 3-22. PBPK model predictions for the fraction of intake that is "untracked" oxidation of TCE in the liver under continuous inhalation (A) and oral (B) exposure conditions in mice (dotted line), rats (dashed line), and humans (solid line).



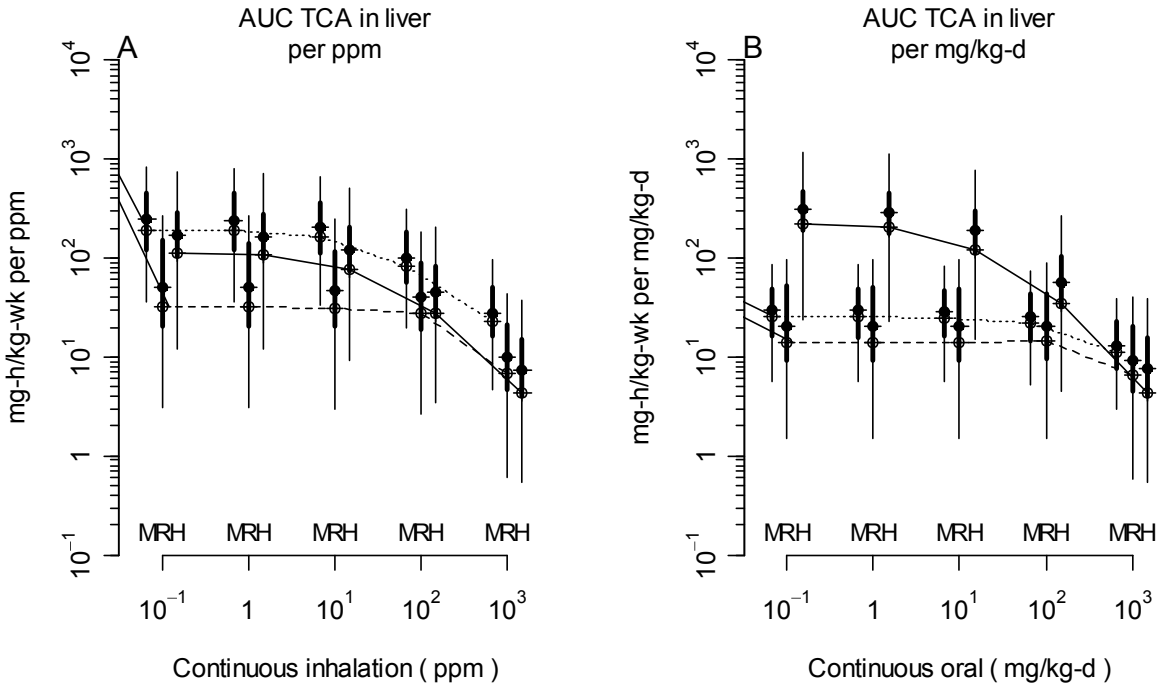
X-values are slightly offset for clarity. Open circles (connected by lines) and thin error bars represent the median estimate and 95% CI for a random subject, and reflect combined uncertainty and variability. Filled circles and thick error bars represent the median estimate and 95% CI for the population mean, and reflect uncertainty only.

Figure 3-23. PBPK model predictions for the weekly AUC of TCE in venous blood (mg-hour/L-week) per unit exposure (ppm or mg/kg-day) under continuous inhalation (A) and oral (B) exposure conditions in mice (dotted line), rats (dashed line), and humans (solid line).



X-values are slightly offset for clarity. Open circles (connected by lines) and thin error bars represent the median estimate and 95% CI for a random subject, and reflect combined uncertainty and variability. Filled circles and thick error bars represent the median estimate and 95% CI for the population mean, and reflect uncertainty only.

Figure 3-24. **PBPK model predictions for the weekly AUC of TCOH in blood (mg-hour/L-week) per unit exposure (ppm or mg/kg-day) under continuous inhalation (A) and oral (B) exposure conditions in mice (dotted line), rats (dashed line), and humans (solid line).**



X-values are slightly offset for clarity. Open circles (connected by lines) and thin error bars represent the median estimate and 95% CI for a random subject, and reflect combined uncertainty and variability. Filled circles and thick error bars represent the median estimate and 95% CI for the population mean, and reflect uncertainty only.

Figure 3-25. PBPK model predictions for the weekly AUC of TCA in the liver (mg-hour/L-week) per unit exposure (ppm or mg/kg-day) under continuous inhalation (A) and oral (B) exposure conditions in mice (dotted line), rats (dashed line), and humans (solid line).

For application to human health risk assessment, the uncertainty in and variability among rodent internal dose estimates both contribute to uncertainty in human risk estimates. Therefore, it is appropriate to combine uncertainty and variability when applying rodent dose-metric predictions to quantitative risk assessment. The median and 95% CI for each dose-metric at some representative exposures in rodents are given in Tables 3-48 and 3-49, and the CI in these tables includes both uncertainty in the population mean and variance as well as variability in the population. On the other hand, for use in predicting human risk, it is often necessary to separate, to the extent possible, interindividual variability from uncertainty, and this disaggregation is summarized in Table 3-50.

3.5.7.2. Local Sensitivity Analysis With Respect to Dose-Metric Predictions

To assess the parameter sensitivity of dose-metric predictions, a local sensitivity analysis is performed. The representative exposure scenarios in Tables 3-48–3-50 are used, but with

metabolic flux dose-metrics converted to "fraction of intake" (i.e., amount metabolized through a pathway divided by total dose). Each parameter is centered on the sample mean of its estimated population mean, and then increased and decreased by 5%. The relative change in the model output $f(\theta)$ is used to estimate a local SC as follows:

$$SC = 10 \times \{f(\theta_+) - f(\theta_-)\} / [½ \times \{f(\theta_+) + f(\theta_-)\}]$$

Here, $f(\theta)$ is one of dose-metric predictions, $\theta_\pm$ is the MLE or baseline value of ± 5%. For log-transformed parameters, 0.05 was added or subtracted from the baseline value, whereas for untransformed parameters, the baseline value was multiplied by 1.05 or 0.95.

Note that local sensitivity analyses as typically performed in deterministic PBPK modeling can only inform the "primary" effects of parameter uncertainties (i.e., the direct change on the quantity of interest due to change in a parameter). They cannot address the *propagation* of uncertainties through an analysis, such as those that can arise due to parameter correlations in the parameter fitting process. Those can only be addressed in a global sensitivity analysis, which is left for future research.

The results of local sensitivity analyses are shown in Figures 3-26–3-31. As expected, each dose-metric is sensitive to a only a small fraction of the scaling parameters. Many of these are well-specified a priori, either due to their being physiological parameters or partition coefficients that can be measured in vitro. The remaining sensitive parameters are generally related to metabolism or clearance.

Table 3-48. Posterior predictions for representative internal doses: mouse[a]

Dose-metric	Posterior predictions for mouse dose-metrics: median (2.5%, 97.5%)				Units
	100 ppm, 7 hr/d, 5 d/wk	600 ppm, 7 hr/d, 5 d/wk	300 mg/kg-d, 5 d/wk	1,000 mg/kg-d, 5 d/wk	
ABioactDCVCBW34	0.304 (0.000534, 12.4)	2.35 (0.00603, 37)	0.676 (0.00193, 18.4)	2.81 (0.0086, 42.4)	mg/wk-kg$^{3/4}$
ABioactDCVCKid	43.7 (0.0774, 1780)	336 (0.801, 5,240)	96.8 (0.281, 2,550)	393 (1.23, 6,170)	mg/wk-kg tissue
AMetGSHBW34	0.684 (0.0307, 17.6)	5.15 (0.285, 44.9)	1.66 (0.0718, 24.5)	6.37 (0.567, 49.4)	mg/wk-kg$^{3/4}$
AMetLiv1BW34	170 (61.2, 403)	878 (342, 2,030)	400 (125, 610)	874 (233, 1,960)	mg/wk-kg$^{3/4}$
AMetLivOtherBW34	3.81 (0.372, 38.4)	20 (1.86, 192)	8.38 (0.773, 80.1)	20 (1.55, 202)	mg/wk-kg$^{3/4}$
AMetLivOtherLiv	196 (19, 2,070)	1,030 (96.5, 10,100)	437 (39.5, 4,180)	1,020 (82.1, 10,400)	mg/wk-kg tissue
AMetLngBW34	187 (7.75, 692)	263 (10.9, 2,240)	38.5 (3.49, 147)	127 (8.59, 484)	mg/wk-kg$^{3/4}$
AMetLngResp	638,000 (26,500, 2,510,000)	918,000 (36,800, 7,980,000)	134,000 (12,500, 514,000)	433,000 (30,200, 1,690,000)	mg/wk-kg tissue
AUCCBld	96.9 (45, 211)	822 (356, 2,040)	110 (6.95, 411)	592 (56, 1,910)	mg-hr/L-wk
AUCCTCOH	87.9 (9.9, 590)	480 (42.1, 4,140)	132 (14.4, 670)	389 (34, 2,600)	mg-hr/L-wk
AUCLivTCA	1,880 (444, 7,190)	5,070 (1,310, 18,600)	2,260 (520, 8,750)	4,660 (939, 18,900)	mg-hr/L-wk
TotMetabBW34	377 (140, 917)	1,260 (475, 3,480)	472 (165, 617)	1,110 (303, 2,010)	mg/wk-kg$^{3/4}$
TotOxMetabBW34	375 (139, 916)	1,250 (451, 3,450)	465 (161, 616)	1,100 (294, 2,010)	mg/wk-kg$^{3/4}$
TotTCAInBW	272 (88.9, 734)	729 (267, 1,950)	334 (106, 875)	694 (185, 1,910)	mg/wk-kg

[a]Mouse body weight is assumed to be 0.03 kg. Predictions are weekly averages over 10 weeks of the specified exposure protocol. CI reflects both uncertainties in population parameters (mean, variance) as well as population variability.

Table 3-49. Posterior predictions for representative internal doses: rat[a]

Dose-metric	Posterior predictions for rat dose-metrics: median (2.5%,97.5%)				Units
	100 ppm, 7 hr/d, 5 d/wk	600 ppm, 7 hr/d, 5 d/wk	300 mg/kg-d, 5 d/wk	1,000 mg/kg-d, 5 d/wk	
ABioactDCVCBW34	0.341 (0.0306, 2.71)	2.3 (0.175, 22.6)	2.15 (0.17, 20.2)	8.89 (0.711, 84.1)	mg/wk-kg$^{3/4}$
ABioactDCVCKid	67.8 (6.03, 513)	450 (35.4, 4,350)	420 (31.6, 3,890)	1,720 (134, 15,800)	mg/wk-kg tissue
AMetGSHBW34	0.331 (0.0626, 2.16)	2.27 (0.315, 19.3)	2.13 (0.293, 16)	8.84 (1.35, 69.3)	mg/wk-kg$^{3/4}$
AMetLiv1BW34	176 (81.1, 344)	623 (271, 1,270)	539 (176, 1,060)	951 (273, 2,780)	mg/wk-kg$^{3/4}$
AMetLivOtherBW34	45.5 (2.52, 203)	160 (7.84, 749)	134 (6.83, 659)	238 (11.3, 1390)	mg/wk-kg$^{3/4}$
AMetLivOtherLiv	1,870 (92.1, 8,670)	6,660 (313, 31,200)	5,490 (280, 27,400)	9,900 (492, 59,600)	mg/wk-kg tissue
AMetLngBW34	15 (0.529, 173)	24.5 (0.819, 227)	15.1 (0.527, 115)	32.1 (1.01, 311)	mg/wk-kg$^{3/4}$
AMetLngResp	41,900 (1,460, 496,000)	67,900 (2,350, 677,000)	40,800 (1,500, 325,000)	85,700 (2,660, 877,000)	mg/wk-kg tissue
AUCCBld	86.7 (39.2, 242)	1,160 (349, 2,450)	670 (47.8, 1,850)	3,340 (828, 8,430)	mg-hr/L-wk
AUCCTCOH	83.6 (1.94, 1,560)	446 (6, 10,900)	304 (4.71, 7,590)	685 (8.14, 32,500)	mg-hr/L-wk
AUCLivTCA	587 (53.7, 4,740)	2,030 (186, 13,400)	1,730 (124, 11,800)	3,130 (200, 21,000)	mg-hr/L-wk
TotMetabBW34	206 (103, 414)	682 (288, 1,430)	572 (199, 1,080)	1,030 (302, 2,920)	mg/wk-kg$^{3/4}$
TotOxMetabBW34	206 (103, 414)	677 (285, 1,430)	568 (191, 1,080)	1,010 (286, 2,910)	mg/wk-kg$^{3/4}$
TotTCAInBW	31.7 (3.92, 174)	110 (13.8, 490)	90.1 (10.4, 417)	164 (17.3, 800)	mg/wk-kg

[a]Rat body weight is assumed to be 0.3 kg. Predictions are weekly averages over 10 weeks of the specified exposure protocol. CI reflects both uncertainties in population parameters (mean, variance) as well as population variability.

Table 3-50. Posterior predictions for representative internal doses: human[a]

Dose-metric	Posterior predictions for human dose-metrics: 2.5% population: median (2.5%, 97.5%) 50% population: median (2.5%, 97.5%) 97.5% population: median (2.5%, 97.5%)			
	Female 0.001 ppm continuous	**Male 0.001 ppm continuous**	**Female 0.001 mg/kg-d continuous**	**Male 0.001 mg/kg-d continuous**
ABioactDCVCBW34	0.000256 (6.97×10^{-5}, 0.000872)	0.000254 (6.94×10^{-5}, 0.000879)	0.000197 (6.13×10^{-5}, 0.000502)	0.0002 (6.24×10^{-5}, 0.000505)
	0.00203 (0.00087, 0.00408)	0.00202 (0.000859, 0.00413)	0.00262 (0.0012, 0.00539)	0.00271 (0.00125, 0.00559)
	0.0119 (0.00713, 0.0177)	0.012 (0.00699, 0.0182)	0.021 (0.0118, 0.0266)	0.022 (0.0124, 0.0277)
ABioactDCVCKid	0.02 (0.00549, 0.0709)	0.0207 (0.00558, 0.0743)	0.0152 (0.0048, 0.0384)	0.016 (0.00493, 0.0407)
	0.16 (0.0671, 0.324)	0.163 (0.0679, 0.342)	0.207 (0.0957, 0.43)	0.22 (0.102, 0.459)
	0.95 (0.56, 1.45)	0.979 (0.563, 1.51)	1.68 (0.956, 2.26)	1.81 (1.03, 2.43)
AMetGSHBW34	0.000159 (4.38×10^{-5}, 0.000539)	0.000157 (4.37×10^{-5}, 0.00054)	0.000121 (3.82×10^{-5}, 0.000316)	0.000123 (3.82×10^{-5}, 0.000323)
	0.00126 (0.000536, 0.00253)	0.00125 (0.000528, 0.00254)	0.00161 (0.000748, 0.00331)	0.00167 (0.000777, 0.00343)
	0.00736 (0.00442, 0.011)	0.00736 (0.00434, 0.0112)	0.013 (0.00725, 0.0164)	0.0136 (0.00759, 0.0171)
AMetLiv1BW34	0.00161 (0.000619, 0.00303)	0.00157 (0.000608, 0.00292)	0.00465 (0.00169, 0.0107)	0.00498 (0.00184, 0.0112)
	0.00637 (0.00501, 0.00799)	0.00619 (0.00484, 0.00779)	0.0172 (0.0153, 0.0183)	0.018 (0.0161, 0.0191)
	0.0157 (0.0118, 0.0206)	0.0152 (0.0115, 0.02)	0.0192 (0.019, 0.0193)	0.02 (0.0198, 0.0201)
AMetLivOtherBW34	4.98×10^{-5} (8.59×10^{-6}, 0.000222)	4.87×10^{-5} (8.33×10^{-6}, 0.000214)	0.000143 (2.35×10^{-5}, 0.000681)	0.00015 (2.49×10^{-5}, 0.000713)
	0.000671 (0.000134, 0.00159)	0.000652 (0.000129, 0.00153)	0.00166 (0.00035, 0.00365)	0.00173 (0.000365, 0.00382)
	0.00507 (0.00055, 0.00905)	0.00491 (0.000531, 0.00885)	0.00993 (0.00109, 0.0153)	0.0103 (0.00113, 0.0159)
AMetLivOtherLiv	0.000748 (0.000138, 0.00335)	0.00065 (0.000119, 0.00288)	0.00214 (0.000354, 0.00979)	0.00197 (0.00033, 0.00907)
	0.0104 (0.00225, 0.0237)	0.00898 (0.00193, 0.0203)	0.0253 (0.00564, 0.0543)	0.0234 (0.00526, 0.0503)
	0.0805 (0.00871, 0.147)	0.0691 (0.00751, 0.127)	0.157 (0.0188, 0.251)	0.146 (0.0173, 0.232)
AMetLngBW34	6.9×10^{-6} (6.13×10^{-7}, 7.99×10^{-5})	7.25×10^{-6} (6.44×10^{-7}, 8.39×10^{-5})	7.54×10^{-8} (6.59×10^{-9}, 7.85×10^{-7})	7.05×10^{-8} (6.1×10^{-9}, 7.25×10^{-7})
	0.00122 (0.000309, 0.0032)	0.00127 (0.000325, 0.00329)	1.51×10^{-5} (3.44×10^{-6}, 4.6×10^{-5})	1.39×10^{-5} (3.21×10^{-6}, 4.24×10^{-5})
	0.0123 (0.00563, 0.0197)	0.0124 (0.00582, 0.0199)	0.000396 (0.000104, 0.00097)	0.000366 (9.54×10^{-5}, 0.000906)

Table 3-50. Posterior predictions for representative internal doses: human[a] (continued)

Dose-metric	Posterior predictions for human dose-metrics: 2.5% population: median (2.5%, 97.5%) 50% population: median (2.5%, 97.5%) 97.5% population: median (2.5%, 97.5%) Female 0.001 ppm continuous	Male 0.001 ppm continuous	Female 0.001 mg/kg-d continuous	Male 0.001 mg/kg-d continuous
AMetLngResp	0.0144 (0.00116, 0.155)	0.0146 (0.00118, 0.157)	0.00015 (1.27×10^{-5}, 0.00153)	0.000134 (1.15×10^{-5}, 0.00137)
	2.44 (0.613, 6.71)	2.44 (0.621, 6.65)	0.0313 (0.00725, 0.0963)	0.0279 (0.00644, 0.086)
	25.8 (12.4, 42.3)	25.3 (12.2, 41.2)	0.813 (0.216, 2.13)	0.716 (0.189, 1.9)
AUCCBld	0.00151 (0.00122, 0.00186)	0.00158 (0.00127, 0.00191)	4.33×10^{-5} (3.3×10^{-5}, 6.23×10^{-5})	3.84×10^{-5} (2.89×10^{-5}, 5.61×10^{-5})
	0.00285 (0.00252, 0.00315)	0.00295 (0.00262, 0.00326)	0.000229 (0.000122, 0.000436)	0.000204 (0.000109, 0.000391)
	0.00444 (0.00404, 0.00496)	0.00456 (0.00416, 0.00507)	0.00167 (0.000766, 0.00324)	0.00153 (0.000693, 0.00303)
AUCCTCOH	0.00313 (0.00135, 0.00547)	0.00305 (0.00134, 0.00532)	0.00584 (0.00205, 0.0122)	0.00615 (0.00213, 0.0127)
	0.0181 (0.0135, 0.0241)	0.0179 (0.0133, 0.0238)	0.0333 (0.025, 0.0423)	0.035 (0.0264, 0.0445)
	0.082 (0.0586, 0.118)	0.0812 (0.0585, 0.117)	0.115 (0.0872, 0.163)	0.122 (0.0919, 0.172)
AUCLivTCA	0.0152 (0.00668, 0.0284)	0.0137 (0.00598, 0.0258)	0.029 (0.0116, 0.0524)	0.0279 (0.0114, 0.0501)
	0.126 (0.0784, 0.194)	0.114 (0.0704, 0.177)	0.227 (0.138, 0.343)	0.219 (0.133, 0.33)
	0.754 (0.441, 1.38)	0.699 (0.408, 1.3)	1.11 (0.661, 1.87)	1.09 (0.64, 1.88)
TotMetabBW34	0.0049 (0.00383, 0.00595)	0.00482 (0.0038, 0.00585)	0.0163 (0.0136, 0.0181)	0.0173 (0.0147, 0.019)
	0.0107 (0.00893, 0.0129)	0.0105 (0.00877, 0.0127)	0.0191 (0.0188, 0.0193)	0.0199 (0.0196, 0.0201)
	0.0246 (0.0185, 0.0326)	0.0244 (0.0183, 0.0324)	0.0194 (0.0194, 0.0194)	0.0202 (0.0202, 0.0202)
TotOxMetabBW34	0.00273 (0.00143, 0.00422)	0.00269 (0.00143, 0.00415)	0.0049 (0.00183, 0.0108)	0.00516 (0.00194, 0.0114)
	0.00871 (0.0069, 0.0111)	0.00857 (0.00675, 0.011)	0.0173 (0.0154, 0.0183)	0.018 (0.0161, 0.0191)
	0.0224 (0.0158, 0.0309)	0.0222 (0.0155, 0.0308)	0.0192 (0.019, 0.0193)	0.02 (0.0198, 0.0201)

Table 3-50. Posterior predictions for representative internal doses: human[a] (continued)

Dose-metric	Posterior predictions for human dose-metrics: 2.5% population: median (2.5%, 97.5%) 50% population: median (2.5%, 97.5%) 97.5% population: median (2.5%, 97.5%)			
	Female 0.001 ppm continuous	**Male 0.001 ppm continuous**	**Female 0.001 mg/kg-d continuous**	**Male 0.001 mg/kg-d continuous**
TotTCAInBW	0.000259 (0.000121, 0.000422)	0.000246 (0.000114, 0.000397)	0.000501 (0.000189, 0.000882)	0.000506 (0.000192, 0.00089)
	0.00154 (0.00114, 0.00202)	0.00146 (0.00109, 0.00193)	0.00286 (0.00222, 0.00357)	0.00289 (0.00222, 0.0036)
	0.00525 (0.00399, 0.00745)	0.00499 (0.0038, 0.0071)	0.00659 (0.00579, 0.00724)	0.00662 (0.00581, 0.00726)

[a]Human body weight is assumed to be 70 kg for males, 60 kg for females. Predictions are weekly averages over 100 weeks of continuous exposure (dose-metric units same as previous tables). Each row represents a different population percentile (2.5, 50, and 97.5%), and the CI in each entry reflects uncertainty in population parameters (mean, variance).

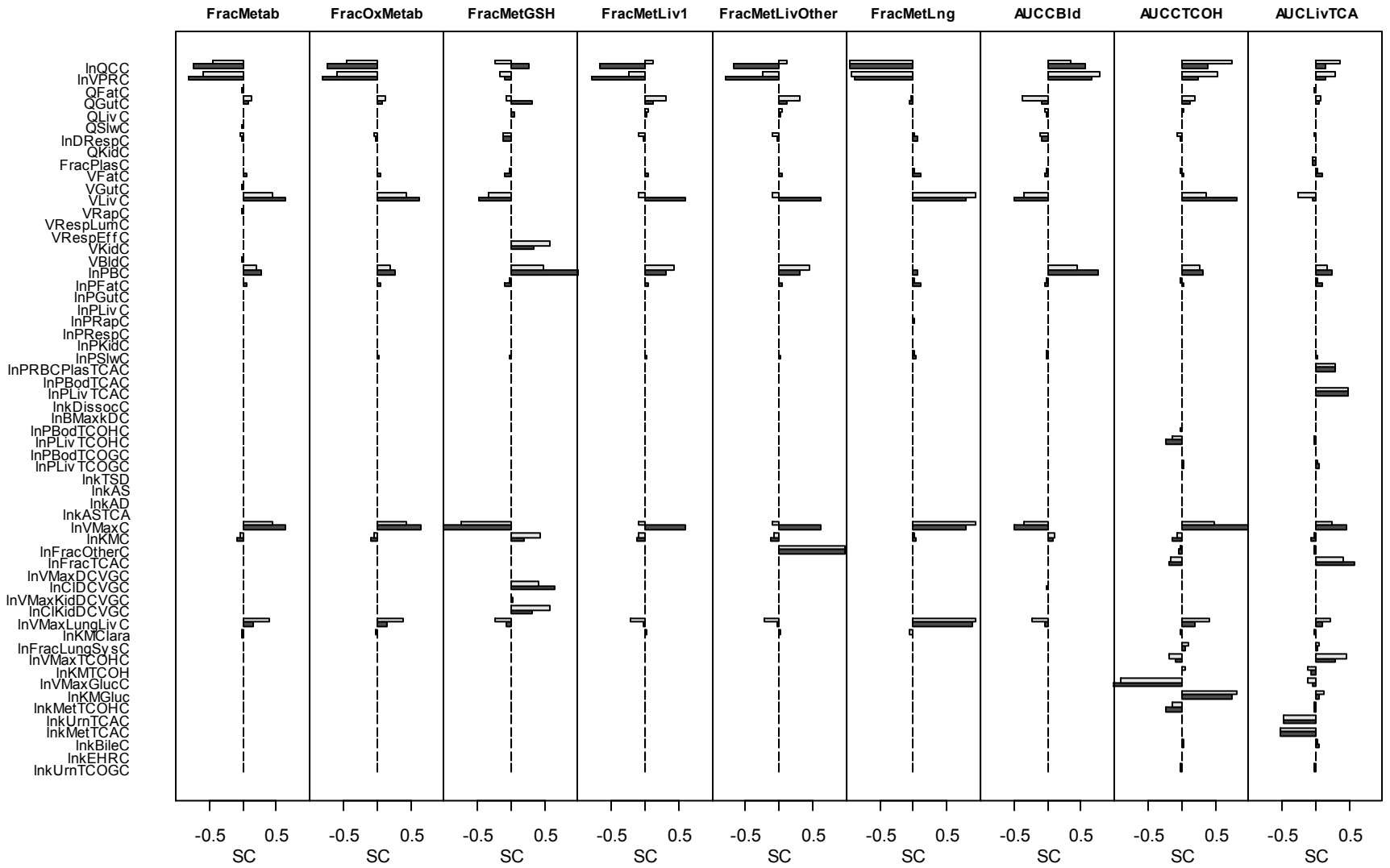


Figure 3-26. Sensitivity analysis results: SC for mouse scaling parameters with respect to dose-metrics following 100 ppm (light bars) and 600 ppm (dark bars), 7 hours/day, 5 days/week inhalation exposures.

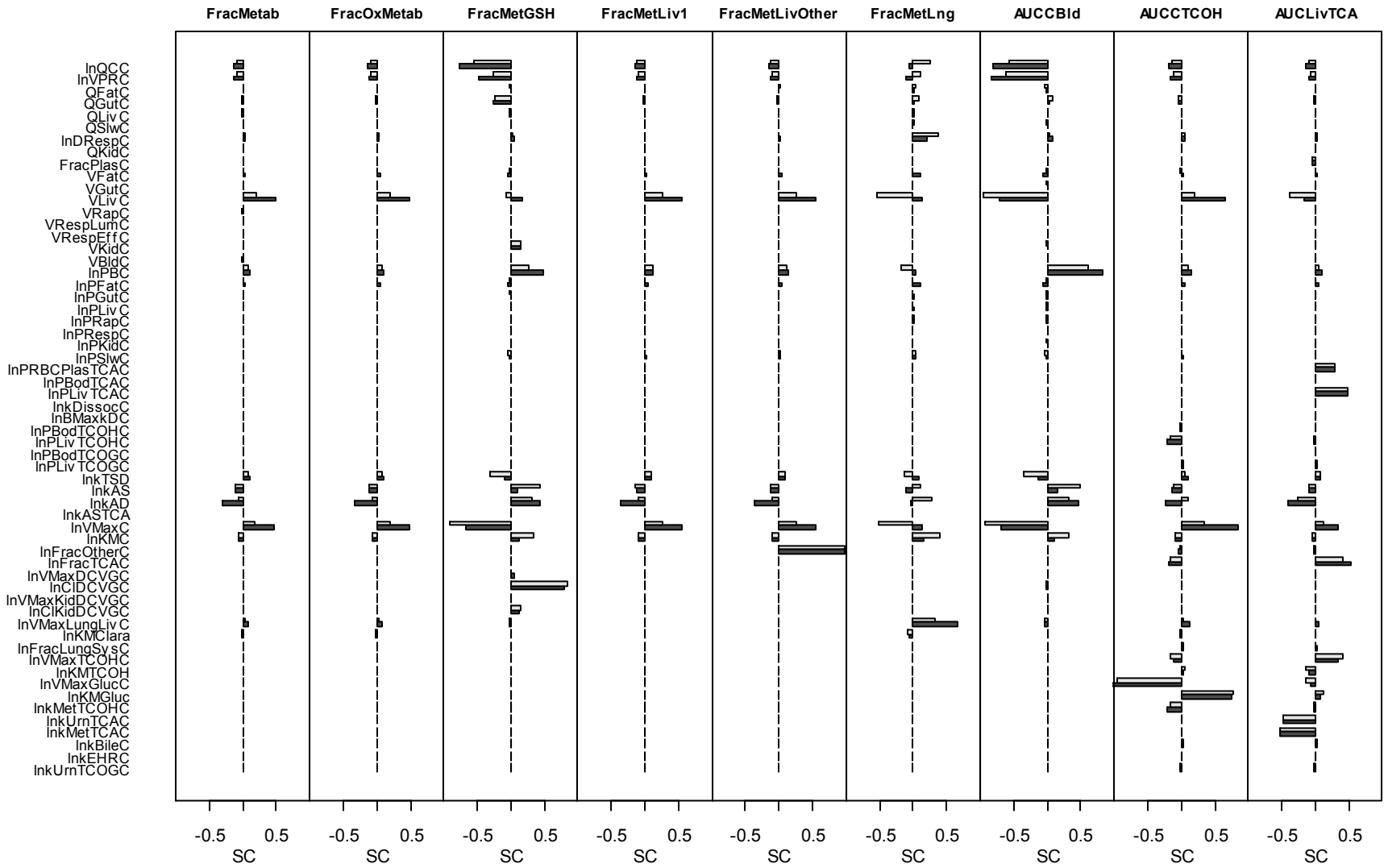


Figure 3-27. Sensitivity analysis results: SC for mouse scaling parameters with respect to dose-metrics following 300 mg/kg-day (light bars) and 1,000 mg/kg-day (dark bars), 5 days/week gavage exposures.

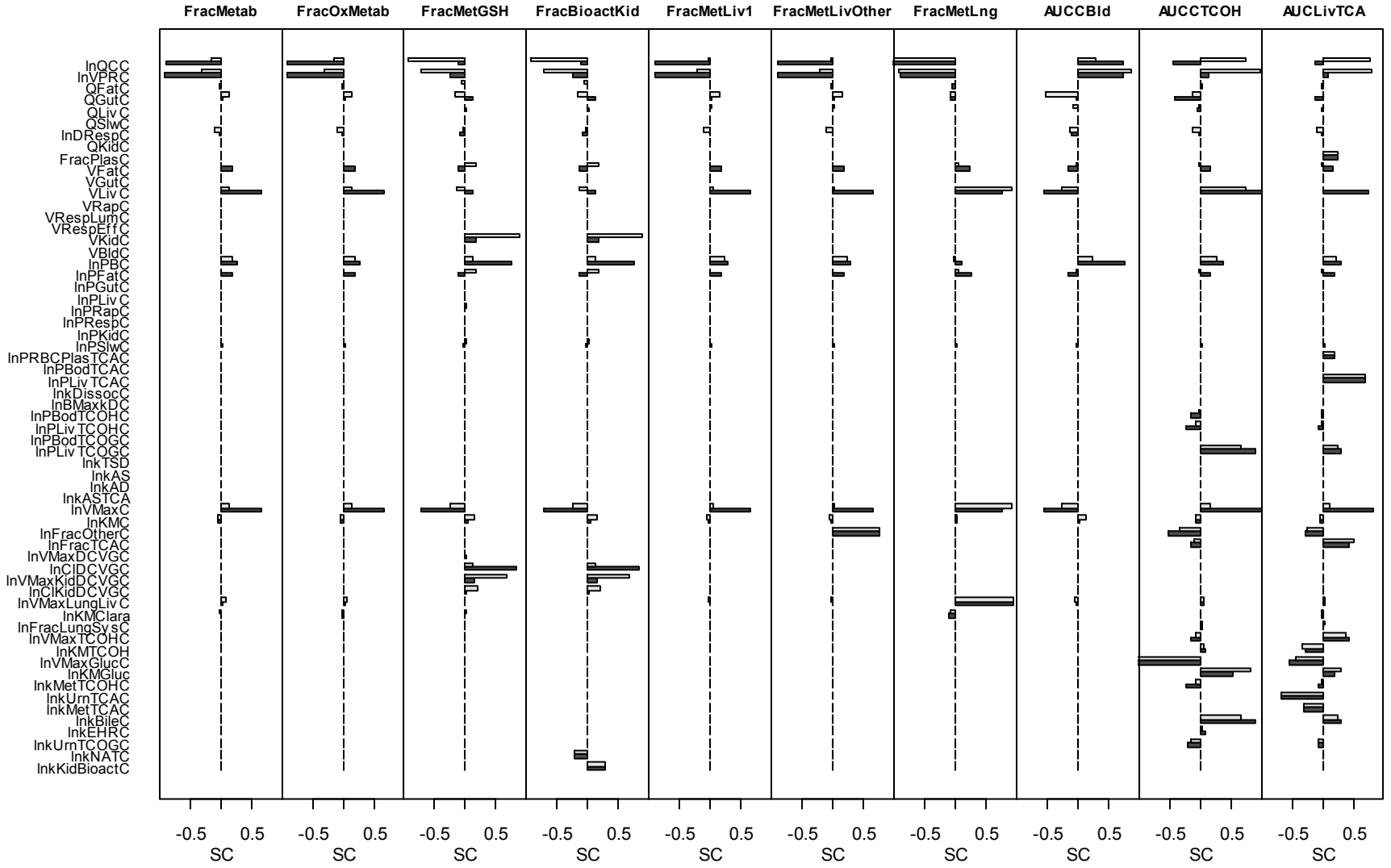


Figure 3-28. Sensitivity analysis results: SC for rat scaling parameters with respect to dose-metrics following 100 ppm (light bars) and 600 ppm (dark bars), 7 hours/day, 5 days/week inhalation exposures.

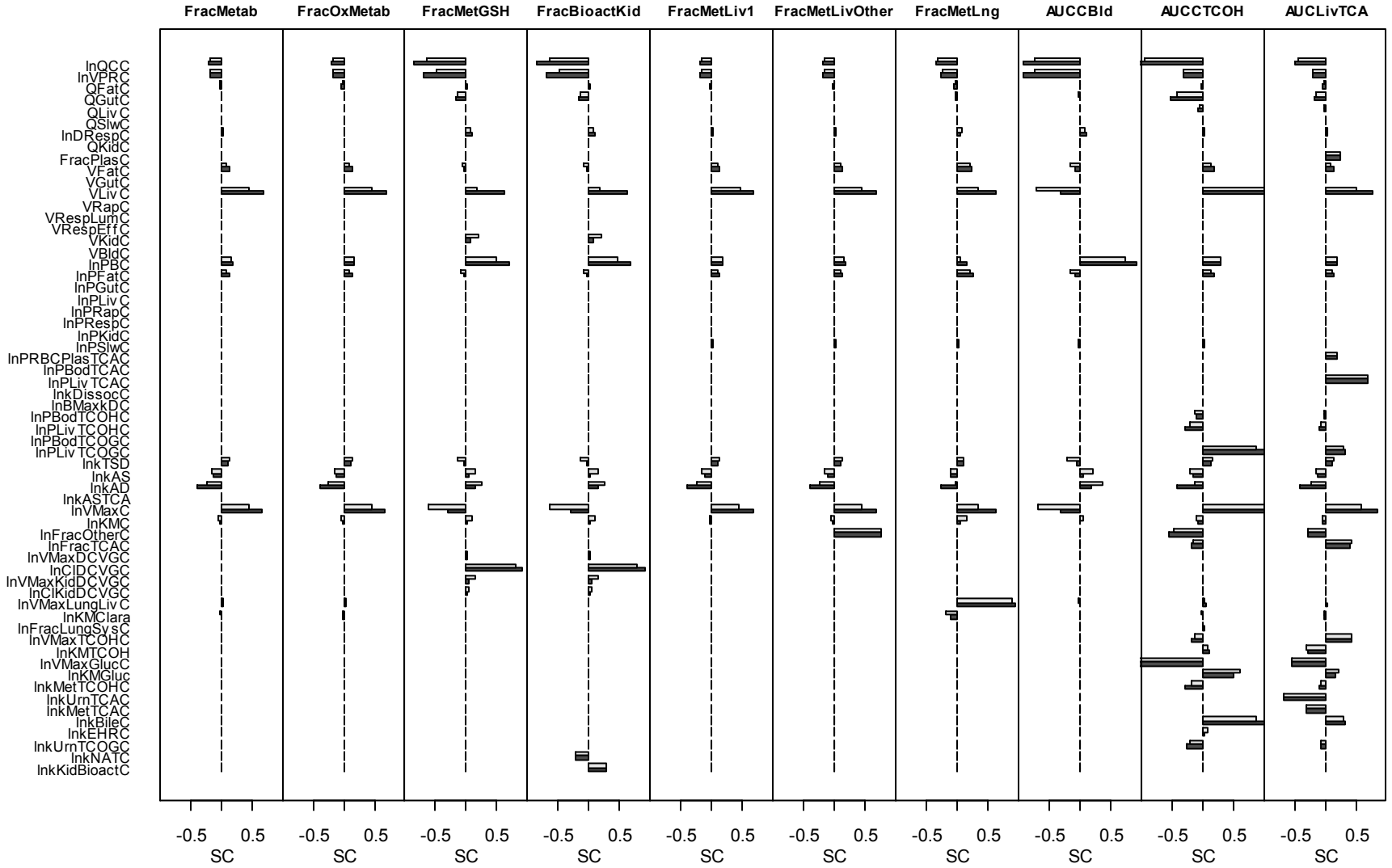


Figure 3-29. Sensitivity analysis results: SC for rat scaling parameters with respect to dose-metrics following 300 mg/kg-day (light bars) and 1,000 mg/kg-day (dark bars), 5 days/week gavage exposures.

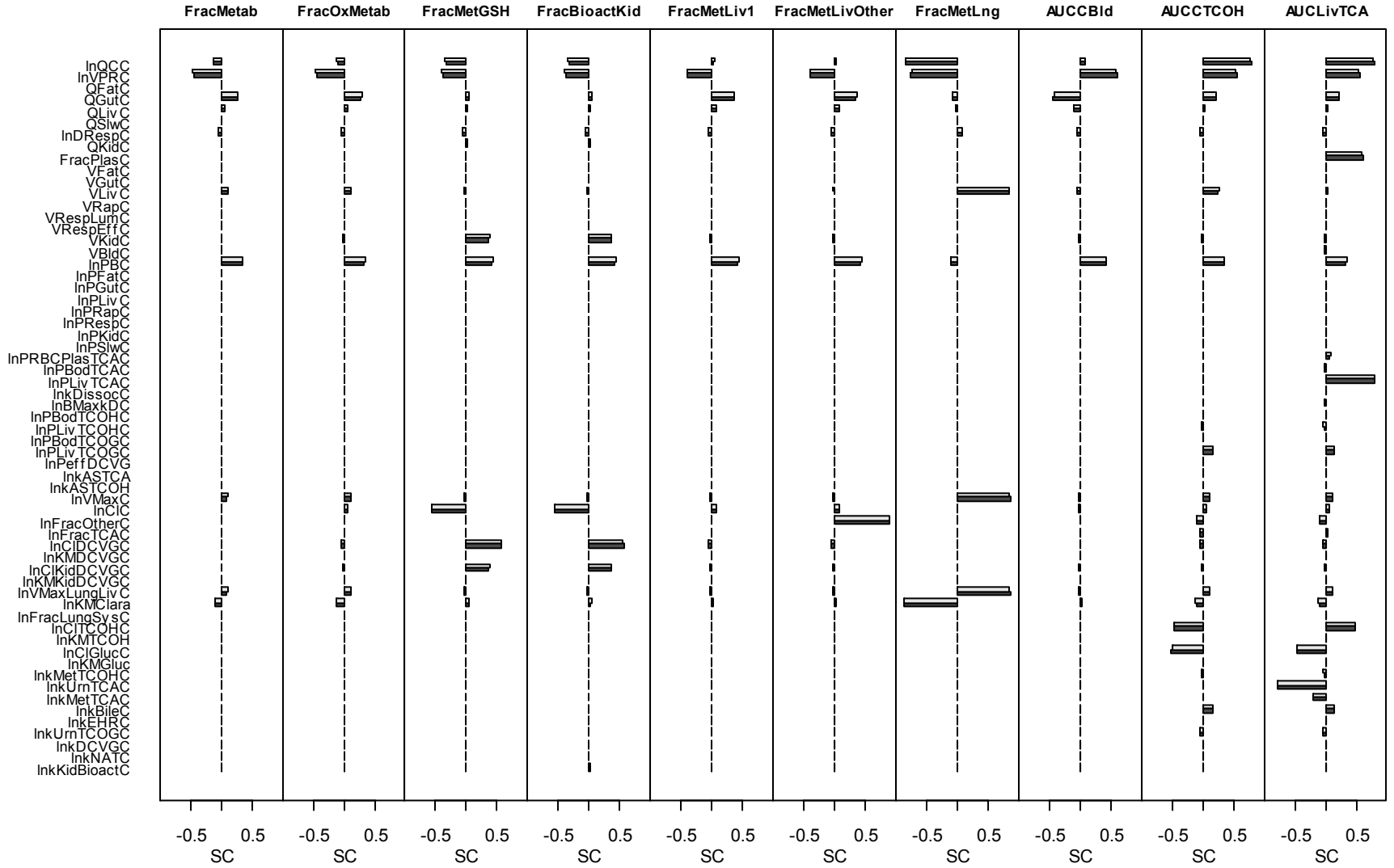


Figure 3-30. Sensitivity analysis results: SC for female (light bars) and male (dark bars) human scaling parameters with respect to dose-metrics following 0.001 ppm continuous inhalation exposures.

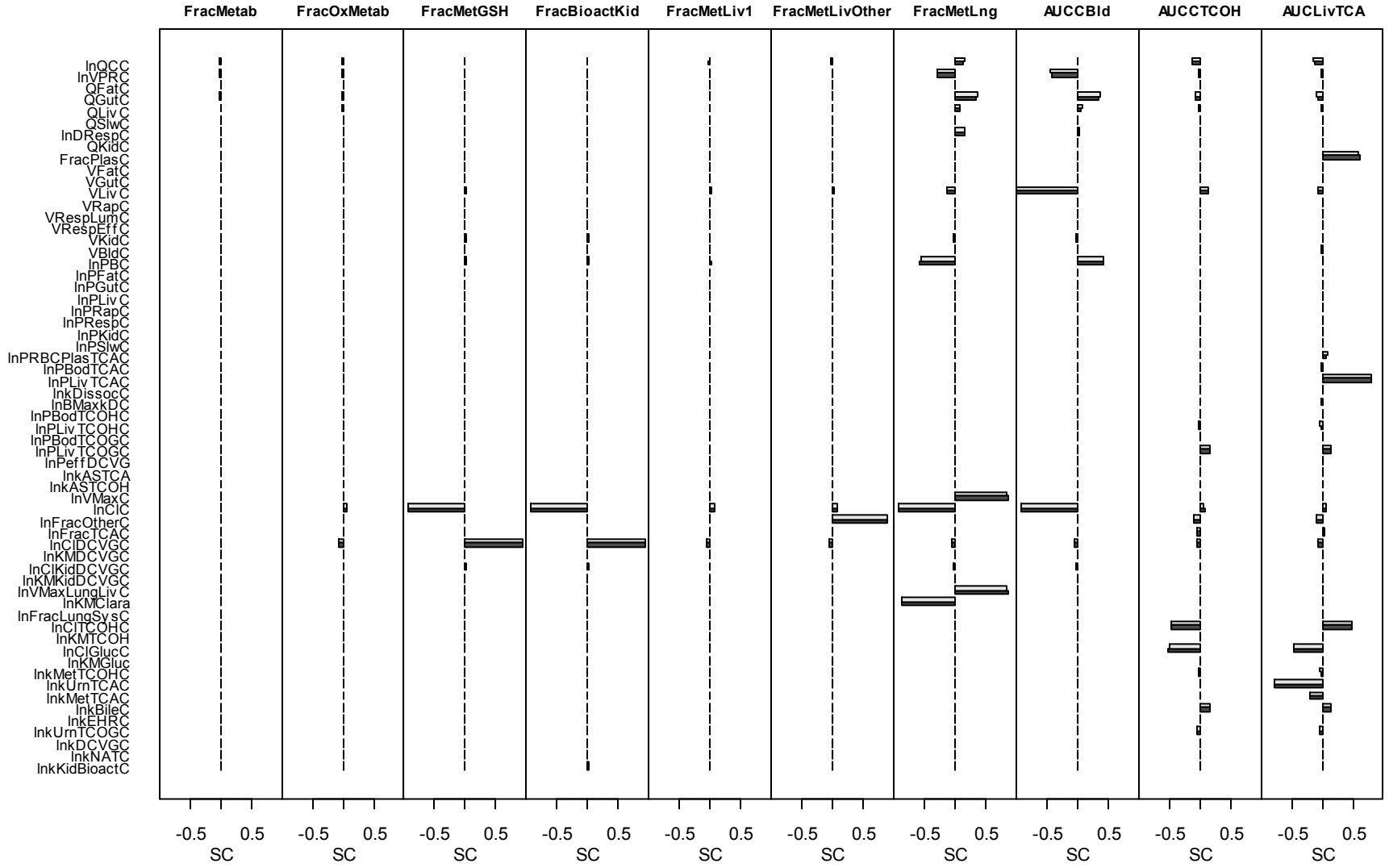


Figure 3-31. Sensitivity analysis results: SC for female (light bars) and male (dark bars) human scaling parameters with respect to dose-metrics following 0.001 mg/kg-day continuous oral exposures.

3.5.7.3. Implications for the Population Pharmacokinetics of TCE

3.5.7.3.1. Results

The overall uncertainty and variability in key toxicokinetic predictions, as a function of dose and species, is shown in Figures 3-17–3-25. As expected, TCE that is inhaled or ingested is substantially metabolized in all species, predominantly by oxidation (see Figures 3-17–3-18). At higher exposures, metabolism becomes saturated and the fraction metabolized declines. Mice, on average, have a greater capacity to oxidize TCE than rats or humans, and this is reflected in the predictions at the two highest levels for each route. The uncertainty in the predictions for the population means for total and oxidative metabolism is relatively modest; therefore, the wide CI for combined uncertainty and variability largely reflects intersubject variability. Of particular note is the high variability in oxidative metabolism at low doses in humans, with the 95% CIs spanning 0.1–0.7 for inhalation and 0.2–1.0 for ingestion.

Predictions of GSH conjugation and renal bioactivation of DCVC are highly uncertain in rodents, spanning >1,000-fold in mice and 100-fold in rats (see Figures 3-19–3-20). In both mice and rats, the uncertainty in the population mean virtually overlaps with the combined uncertainty and variability. The uncertainty in mice reflects the lack of GSH-conjugate specific data in that species, and is, therefore, based on overall mass balance only. The somewhat smaller uncertainty in rats reflects the fact that, in addition to overall mass balance, urinary NAcDCVC excretion data are available in that species. However, while the lower bound of GSH conjugation is informed by NAcDCVC excretion data, the upper bound for GSH conjugation and the amount of DCVC bioactivation are still indirectly estimated from data on other clearance pathways. In humans, however, overall GSH conjugation is strongly constrained by the blood concentrations of DCVG from Lash et al. (1999b), with 95% CIs on the population mean spanning only about threefold. DCVC bioactivation is still indirectly estimated, derived from the difference between overall GSH conjugation flux and NAcDCVC excretion data from Bernauer et al. (1996). However, substantial variability is predicted (reflecting variability in the measurements of Lash et al., (1999b), since the error bars for the population mean are substantially smaller than those for overall uncertainty and variability. Of particular note is the prediction of 1 or 2 orders of magnitude more GSH conjugation and DCVC bioactivation, on average, in humans than in rats, although importantly, the 95% CIs for the predicted population means do overlap. However, as discussed above in Section 3.3.3.2.1, there are uncertainties as to the accuracy of analytical method used by Lash et al. (1999b) in the measurement of DCVG in blood. Because these data are so influential, the analytical uncertainties contribute substantially to the overall uncertainty in the estimates of the overall GSH conjugation flux, and may be greater than the statistical uncertainties calculated using the model.

Predictions for respiratory tract oxidative metabolism were, as expected, greatest in mice, followed by rats and then humans (see Figure 3-21). In addition, due to the ―presystemic" nature of the respiratory tract metabolism model as well as the hepatic first-pass effect, substantially

more metabolism was predicted from inhalation exposures as compared to oral exposures. Interestingly, the population means appeared to be fairly well constrained despite the lack of direct data, suggesting that overall mass balance is an important constraint for the presystemic respiratory tract metabolism modeled here.

Some constraints were also placed on "other" hepatic oxidation (i.e., through a pathway that does not result in chloral formation and subsequent formation of TCA and TCOH, see Figure 3-22). The 95% CI for overall uncertainty and variability spanned about 100-fold, a large fraction of that due to uncertainty in the population mean. Interestingly, a higher rate per kg tissue was predicted for rats than for mice or humans, although importantly, the 95% CIs for the population means overlap among all three species.

The AUC of TCE in blood (see Figure 3-23) showed the expected nonlinear behavior with increasing dose, with the nonlinearity more pronounced with oral exposure, as would be expected by hepatic first-pass. Notably, the predicted AUC of TCE in blood from inhalation exposures corresponds closely with cross-species ppm-equivalence, as is assumed for Category 3 gases for which the blood:air partition coefficient in laboratory animals is greater than that in humans (U.S. EPA, 1994b). For low oral exposures (≤1 mg/kg-day), cross-species mg/kg-day equivalence appears to be fairly accurate (within twofold), implying the usual assumption of $mg/kg^{3/4}$-day equivalence would be somewhat less accurate, at least for humans. Interestingly, the AUC of TCOH in blood (see Figure 3-24) was relatively constant with dose, reflecting the parallel saturation of both TCE oxidation and TCOH glucuronidation. In fact, in humans, the mean AUC for TCOH in blood increases up to 100 ppm or 100 mg/kg-day, due to saturation of TCOH glucuronidation, before decreasing at 1,000 ppm or 1,000 mg/kg-day, due to saturation of TCE oxidation.

The predictions for the AUC for TCA in the liver showed some interesting features (see Figure 3-25). The predictions for all three species with within an order of magnitude of each other, with a relatively modest uncertainty in the population mean (reflecting the substantial amount of data on TCA). The shape of the curves, however, differs substantially, with humans showing saturation at much lower doses than rodents, especially for oral exposures. In fact, the ratio between the liver TCA AUC and the rate of TCA production, although differing between species, is relatively constant as a function of dose within species (not shown). Therefore, the shape of the curves largely reflect saturation in the production of TCA from TCOH, *not* in the oxidation of TCE itself, for which saturation is predicted at higher doses, particularly via the oral route (see Figure 3-18). In addition, while for the same exposure (ppm or mg/kg-day TCE), more TCA (on a mg/kg-day basis) is produced in mice relative to rats and humans, humans and rats have longer TCA half-lives even though plasma protein binding of TCA is, on average, greater.

3.5.7.3.2. Discussion

This analysis substantially informs four of the major areas of pharmacokinetic uncertainty previously identified in numerous reports (reviewed in Chiu et al., 2006b): GSH conjugation pathway, respiratory tract metabolism, alternative pathways of TCE oxidation including DCA formation, and the impact of plasma binding on TCA kinetics, particularly in the liver. In addition, the analysis helps identify data that have the potential to further reduce the uncertainties in TCE toxicokinetics and risk assessment.

With respect to the first, previous estimates of the degree of TCE GSH conjugation and subsequent bioactivation of DCVC in humans were based on urinary excretion data alone (Bernauer et al., 1996; Birner et al., 1993). For instance, Bloemen et al. (2001) concluded that due to the low yield of identified urinary metabolites through this pathway (<0.05% as compared to 20–30% in urinary metabolites of TCE oxidation), GSH conjugation of TCE is likely of minor importance. However, as noted by Lash et al. (2000a; 2000b), urinary excretion is a poor quantitative marker of flux through the GSH pathway because it only accounts for the portion detoxified, and not the portion bioactivated (a limitation acknowledged by Bloemen et al., 2001).

A reexamination of the available in vitro data on GSH conjugation by Chiu et al. (2006b) suggested that the difference in flux between TCE oxidation and GSH conjugation may not be as large as suggested by urinary excretion data. For example, the formation rate of DCVG from TCE in freshly isolated hepatocytes was similar in order of magnitude to the rate measured for oxidative metabolites (Lash et al., 1999a; Lipscomb et al., 1998b). A closer examination of the only other available human in vivo data on GSH conjugation, the DCVG blood levels reported in Lash et al. (1999b), also suggests a substantially greater flux through this pathway than inferred from urinary data. In particular, the peak DCVG blood levels reported in this study were comparable on a molar basis to peak blood levels of TCOH, the major oxidative metabolite, in the same subjects, as previously reported by Fisher et al. (1998). A lower bound estimate of the GSH conjugation flux can be derived as follows. The reported mean peak blood DCVG concentrations of 46 μM in males exposed to 100 ppm TCE for 4 hours (Lash et al., 1999b), multiplied by a typical blood volume of 5 L (ICRP, 2003), yields a peak amount of DCVG in blood of 0.23 mmoles. In comparison, the retained dose from 100 ppm exposure for 4 hours is 4.4 mmol, assuming retention of about 50% (Monster et al., 1976) and minute-volume of 9 L/minute (ICRP, 2003). Thus, in these subjects, about 5% of the retained dose is present in blood as DCVG at the time of peak blood concentration. This is a strong lower bound on the total fraction of retained TCE undergoing GSH conjugation because DCVG clearance is ongoing at the time of peak concentration, and DCVG may be distributed to tissues other than blood. It should be reiterated that only grouped DCVG blood data were available for PBPK model-based analysis; however, this should only result in an underestimation of the degree of *variation* in GSH conjugation. Finally, this hypothesis of a significant flux through the human GSH conjugation pathway is consistent with the limited available total recovery data in humans in

which only 60–70% of the TCE dose is recovered as TCE in breath and excreted urinary metabolites (reviewed in Chiu et al., 2007).

Thus, there is already substantial qualitative and semi-quantitative evidence to suggest a substantially greater flux through the GSH conjugation pathway than previously estimated based on urinary excretion data alone. The scientific utility of applying a combination of PBPK modeling and Bayesian statistical methods to this question comes from being able to systematically integrate these different types of data—in vitro and in vivo, direct (blood DCVG) and indirect (total recovery, urinary excretion)—and quantitatively assess their consistency and implications. For example, the in vitro data discussed above on GSH conjugation were used for developing prior distributions for GSH conjugation rates, and were not used in previous PBPK models for TCE. Then, both the direct and indirect in vivo data were used to the extent possible either in the Bayesian calibration or model evaluation steps.

However, this evidence—both qualitative and quantitative—is highly dependent on the reliability of the human DCVG measurements, both in vitro and in vivo, from Lash et al. (1999a; 1999b). In vitro, Green et al. (1997a) reported much lower rates of DCVG formation in humans using a different analytical method. Similarly, the rates of in vitro DCVG formation in rats have uneven consistency among studies. In male rat liver cytosol, Green et al. (1997a) reported a rate of 0.54 pmol/minute-mg, consistent with the <2 pmol/minute-mg reported by Dekant et al. (1990), but much less than the 121 pmol/minute-mg reported by Lash et al. (1999a). However, in microsomes, Green et al. (1997a) reported no enzymatic formation, whereas Dekant et al. (1990) reported a higher rate (i.e., 2 pmol/minute-mg) and Lash et al. (1999a) reported a much higher rate (i.e., 171 pmol/minute-mg). Differing results in humans may be attributable to true interindividual variation (especially since GSTs are known to be polymorphic). However, this may be less plausible for rats, suggesting that significant uncertainties remain in the quantitative estimation of GSH conjugation flux.

Several other aspects of the predictions related to GSH conjugation of TCE are worthy of note. Predictions for rats and mice remain more uncertain due to their having less direct toxicokinetic data, but are better constrained by total recovery studies. For instance, the total recovery of 60-70% of dose in exhaled breath and oxidative metabolites in human studies is substantially less than the >90% reported in rodent studies (also noted by Goeptar et al., 1995). In addition, it has been suggested that "saturation" of the oxidative pathway for volatiles in general, and TCE in particular, may lead to marked increases in flux through the GSH conjugation pathway (Slikker et al., 2004a, b; Goeptar et al., 1995), but the PBPK model predicts only a modest, at most ~twofold, change in flux. This is because there is evidence that both pathways are saturable in the liver for this substrate at similar exposures and because GSH conjugation also occurs in the kidney. Therefore, the available data are not consistent with toxicokinetics alone causing substantially nonlinearites in TCE kidney toxicity or cancer, or in any other effects associated with GSH conjugation of TCE.

Finally, the present analysis suggests a number of areas where additional data can further reduce uncertainty in and better characterize the TCE GSH conjugation pathway. The Bayesian analysis predicts a relatively low distribution volume for DCVG in humans, a hypothesis that could be tested experimentally. In addition, in vivo measurements of DCVG in blood via a different, validated analytical method, in humans with known exposures to TCE, would be highly influential in either corroborating the DCVG blood levels reported in Lash et al. (1999b) or providing evidence that those reported DCVG blood levels are too high due to analytical issues. Moreover, it would be useful in such studies to be able to match individuals with respect to toxicokinetic data on oxidative and GSH conjugation metabolites so as to better characterize variability. A consistent picture as to which GST isozymes are involved in TCE GSH conjugation, along with data on variability in isozyme polymorphisms and activity levels, can further inform the extent of human variability. In rodents, more direct data on GSH metabolites, such as reliably-determined DCVG blood concentrations, preferably coupled with simultaneous data on oxidative metabolites, would greatly enhance the assessment of GSH conjugation flux in laboratory animals. Given the large apparent variability in humans, data on interstrain variability in rodents may also be useful.

With respect to oxidative metabolism, as expected, the liver is the major site of oxidative metabolism in all three species, especially after oral exposure, where >85% of total metabolism is oxidation in the liver in all three species. However, after inhalation exposure, the model predicts a greater proportion of metabolism via the respiratory tract than previous models for TCE. This is primarily because previous models for TCE respiratory tract metabolism (Hack et al., 2006; Clewell et al., 2000) were essentially flow-limited—i.e., the amount of respiratory tract metabolism (particularly in mice) was determined primarily by the (relatively small) blood flow to the tracheobronchial region. However, the respiratory tract structure used in the present model is more biologically plausible, is more consistent with that of other volatile organics metabolized in the respiratory tract (e.g., styrene), and leads to a substantially better fit to closed-chamber data in mice.

Consistent with the qualitative suggestions from in vitro data, the analysis here predicts that mice have a greater rate of respiratory tract oxidative metabolism as compared to rats and humans. However, the predicted difference of about 50-fold on average between mice and humans is not as great as the 600-fold suggested by previous reports (NRC, 2006; Green, 2000; Green et al., 1997b). The suggested factor of 600-fold was based on multiplying the Green et al. (1997b) data on TCE oxidation in lung microsomes from rats vs. mice (23-fold lower) by a factor for the total CYP content of human lung compared to rat lung (27-fold lower) (incorrectly cited as being from Raunio et al., 1998; Wheeler and Guenthner, 1990). However, because of the isozyme-specificity of TCE oxidation, and the differing proportions of different isozymes across species, total CYP content may not be the best measure of interspecies differences in TCE respiratory tract oxidative metabolism. Wheeler et al. (1992) reported that CYP2E1 content of

human lung microsomes is about 10-fold lower than that of human liver microsomes. Given that Green et al. (1997b) report that TCE oxidation by human liver microsomes is about threefold lower than that in mouse lung microsomes, this suggests that the mouse-to-human comparison TCE oxidation in lung microsomes would be about 30-fold. Moreover, the predicted amount of metabolism corresponds to about the detection limit reported by Green et al. (1997b) in their experiments with human lung microsomes, suggesting overall consistency in the various results. Therefore, the 50-fold factor predicted by our analysis is biologically plausible given the available in vitro data. More direct in vivo measures of respiratory tract metabolism would be especially beneficial to reduce its uncertainty as well as better characterize its human variability.

TCA dosimetry is another uncertainty that was addressed in this analysis. In particular, the predicted interspecies differences in liver TCA AUC are modest, with a range of about 10-fold across species, due to the combined effects of interspecies differences in the yield of TCA from TCE, plasma protein binding, and elimination half-life. This result is in contrast to previous analyses that did not include TCA protein binding (Clewell et al., 2000; Fisher, 2000), which predicted significantly more than an order of magnitude difference in TCA AUC across species. In addition, in order to be consistent with available data, the model requires some metabolism or other clearance of TCA in addition to urinary excretion. That urinary excretion does not represent 100% of TCA clearance is evident empirically, as urinary recovery after TCA dosing is not complete even in rodents (Yu et al., 2000; Abbas et al., 1997). Additional investigation into possible mechanisms, including metabolism to DCA or enterohepatic recirculation with fecal excretion, would be beneficial to provide a stronger biological basis for this empirical finding.

With respect to ~~"u~~untracked" oxidative metabolism, this pathway appears to be a relatively small contribution to total oxidative metabolism. While it is tempting to use this pathway as a surrogate for DCA production through from the TCE epoxide (Cai and Guengerich, 1999), one should be reminded that DCA may be formed through multiple pathways (see Section 3.3). Therefore, this pathway at best represents a lower bound on DCA production. In addition, better quantitative markers of oxidative metabolism through the TCE epoxide pathway (e.g., dichloroacetyl lysine protein adducts, as reported in [e.g., dichloroacetyl lysine protein adducts, as reported in Forkert et al. (2006)] are needed in order to more confidently characterize its flux.

In a situation such as TCE in which there is large database of studies coupled with complex toxicokinetics, the Bayesian approach provides a systematic method of simultaneously estimating model parameters and characterizing their uncertainty and variability. While such an approach is not necessarily needed for all applications, such as route-to-route extrapolation (Chiu and White, 2006), as discussed in Barton et al. (2007), characterization of uncertainty and variability is increasingly recognized as important for risk assessment while representing a continuing challenge for both PBPK modelers and users. If there is sufficient reason to characterize uncertainty and variability in a highly transparent and objective manner, there is no

reason why our approach could not be applied to other chemicals. However, such an endeavor is clearly not trivial, though the high level of effort for TCE is partially due to the complexity of its metabolism and the extent of its toxicokinetic database.

It is notable that, with experience, the methodology for the Bayesian approach to PBPK modeling of TCE has evolved significantly from that of Bois (2000b, a), to Hack et al. (2006), to the present analysis. Part of this evolution has been a more refined specification of the problem being addressed, showing the importance of "problem formulation" in risk assessment applications of PBPK modeling. The particular hierarchical population model for each species was specified based on the intended use of the model predictions, so that relevant data can be selected for analysis (e.g., excluding most grouped human data in favor of individual human data) and data can be appropriately grouped (e.g., in rodent data, grouping by sex and strain within a particular study). Thus, the predictions from the population model in rodents are the "average" for a particular "lot" of rodents of a particular species, strain, and sex. This is in contrast to the Hack et al. (2006) model, in which each dose group was treated as a separate subject. As discussed above, this previous population model structure led to the unlikely result that different dose groups within a closed-chamber study had significantly different V_{MAX} values. In humans, however, interindividual variability is of interest, and furthermore, substantial individual data are available in humans. Hack et al. (2006) mixed individual- and group-level data, depending on the availability from the published study, but this approach likely underestimates population variability due to group means being treated as individuals. In addition, in some studies, the same individual was exposed more than once, and in Hack et al. (2006), these were treated as different "individuals." In this case, actual interindividual variability may be either over- or underestimated, depending on the degree of interoccasion variability. While it is technically feasible to include interoccasion variability, it would have added substantially to the computational burden and reduced parameter identifiability. In addition, a primary interest for this risk assessment is chronic exposure, so the predictions from the population model in humans are the "average" across different occasions for a particular individual (adult).

The second aspect of this evolution is the drive towards increased objectivity and transparency. For instance, available information, or the lack thereof, is formally codified and explicit either in prior distributions or in the data used to generate posterior distributions, and not both. Methods at minimizing subjectivity (and hence improving reproducibility) in parameter estimation include: (1) clear separation between the in vitro or physiologic data used to develop prior distributions and the in vivo data used to generate posterior distributions; (2) use of noninformative distributions, first updated using a probabilistic model of interspecies-scaling that allows for prediction error, for parameters lacking in prior information; and (3) use of a more comprehensive database of physiologic data, in vitro measurements, and in vivo data for parameter calibration or for out-of-sample evaluation ("validation"). These measures increase

the confidence that the approach employed also provides adequate characterization of the uncertainty in metabolic pathways for which available data was sparse or relatively indirect, such as GSH conjugation in rodents and respiratory tract metabolism. Moreover, this approach yields more confident insights into what additional data can reduce these uncertainties than approaches that rely on more subjective methods.

3.5.7.4. Key Limitations and Potential Implications of Violating Key Assumptions

Like all analyses, this one has a number of limitations and opportunities for refinement, both biological and statistical. Of course, the modeling results are highly dependent on the assumed PBPK model structure. However, most of the elements of the model structure are well established for volatile, lipophilic chemicals such as TCE, and, thus, these assumptions are unlikely to introduce much bias or inaccuracy. In terms of the statistical model, a key assumption is the choice of prior and population distributions—particularly the choice of unimodal distributions for population variability. While reasonable as a first approximation, especially without data to suggest otherwise, this assumption may introduce inaccuracies in the predictions of population variability. For example, if there were an underlying bimodal distribution, then fitting using a unimodal population distribution would lead to a high estimate for the variance, and potentially overestimate the degree of population variability. In some cases in the human model where larger population variance distributions are estimated, this may be the underlying cause. However, only in the case of GSH conjugation in humans do the larger estimates of population variability impact the dose-metric predictions used in the dose-response assessment, so the impact of this assumption is limited for this assessment.

In addition, certain sources of variability, such as between-animal variability in rodents and between-occasion variability in humans were not included in the hierarchical model, but were aggregated with other sources of variability in a "residual" error term. Based on the posterior predictions, it does not appear that this assumption has introduced significant bias in the estimates because the residuals between predictions and data do not overall appear systematically high or low. However, this could be verified by addressing between-animal variability in rodents [requiring a more rigorous treatment of aggregated data, e.g., Chiu and Bois (2007)] and incorporation of interoccasion variability in humans (e.g., Bernillon and Bois, 2000).

Some key potential refinements are as follows. First would be the inclusion of a CH submodel, so that pharmacokinetic data, such as that recently published by Merdink et al. (2008), could be incorporated. In addition, the current analysis is still dependent on a model structure substantially informed by deterministic analyses that test alternative model structures (Evans et al., 2009), as probabilistic methods for discrimination or selection among complex, nonlinear models such as that for TCE toxicokinetics have not yet been widely accepted. Therefore, additional refinement of the respiratory tract model may be possible, though more direct in vivo data would likely be necessary to strongly discriminating among models. In terms of validation,

application of more sophisticated methods such as cross-validation, may be useful in further assessing the robustness of the modeling. Finally, additional model changes that may be of utility to risk assessment, such as development of models for different lifestages (including childhood and pregnancy), would likely require additional in vivo or in vitro data, particularly as to metabolism, to ensure model identifiability.

3.5.7.5. Overall Evaluation of PBPK Model-Based Internal Dose Predictions

The utility of the PBPK model developed here for making predictions of internal dose can be evaluated based on four different components: (1) the degree to which the simulations have converged to the true posterior distribution; (2) the degree of overall uncertainty and variability; (3) for humans, the degree of uncertainty in the population; and (4) the degree to which the model predictions are consistent with in vivo data that are informative to a particular dose-metric. Table 3-51 summarizes these considerations for each dose-metric prediction. Note that this evaluation does not consider in any way the extent to which a dose-metric may be the appropriate choice for a particular toxic endpoint.

Table 3-51. Degree of variance in dose-metric predictions due to incomplete convergence (columns 2–4), combined uncertainty and population variability (columns 5–7), uncertainty in particular human population percentiles (columns 8–10), model fits to in vivo data (column 11); the GSD is a "fold-change" from the central tendency

Dose-metric abbreviation	Convergence: *R* for generic scenarios			GSD for combined uncertainty and variability			GSD for uncertainty in human population percentiles			Comments regarding model fits to in vivo data
	Mouse	Rat	Human	Mouse	Rat	Human	1~5%	25~75%	95~99%	
ABioactDCVCBW34, ABioactDCVCKid	–	≤1.016	≤1.015	–	≤3.92	≤3.77	≤2.08	≤1.64	≤1.30	Good fits to urinary NAcDCVC and blood DCVG.
AMetGSHBW34	≤1.011	≤1.024	≤1.015	≤9.09	≤3.28	≤3.73	≤2.08	≤1.64	≤1.29	Good fits to urinary NAcDCVC and blood DCVG.
AMetLiv1BW34	≤1.000	≤1.003	≤1.004	≤2.02	≤1.84	≤1.97	≤1.82	≤1.16	≤1.16	Good fits to oxidative metabolites.
AMetLivOtherBW34, AMetLivOtherLiv	≤1.004	≤1.151	≤1.012	≤3.65	≤3.36	≤3.97	≤2.63	≤1.92	≤2.05	No direct in vivo data.
AMetLngBW34, AMetLngResp	≤1.001	≤1.003	≤1.002	≤4.65	≤4.91	≤10.4	≤4.02	≤2.34	≤1.83	No direct in vivo data, but good fits to closed-chamber.
AUCCBld	≤1.001	≤1.004	≤1.005	≤3.04	≤3.16	≤3.32	≤1.20	≤1.43	≤1.49	Generally good fits, but poor fit to a few mouse and human studies.
AUCCTCOH	≤1.001	≤1.029	≤1.002	≤3.35	≤8.78	≤5.84	≤1.73	≤1.20	≤1.23	Good fits across all three species.
AUCLivTCA	≤1.000	≤1.005	≤1.002	≤2.29	≤3.18	≤2.90	≤1.65	≤1.30	≤1.40	Good fits to rodent data.
TotMetabBW34	≤1.001	≤1.004	≤1.004	≤1.92	≤1.82	≤1.81	≤1.13	≤1.12	≤1.18	Good fits to closed-chamber.
TotOxMetabBW34	≤1.001	≤1.003	≤1.004	≤1.94	≤1.85	≤1.96	≤1.77	≤1.15	≤1.20	Good fits to closed-chamber and oxidative metabolites.
TotTCAInBW	≤1.002	≤1.002	≤1.001	≤1.96	≤2.69	≤2.30	≤1.68	≤1.19	≤1.19	Good fits to TCA data.

Overall, the least uncertain dose-metrics are the fluxes of total metabolism (TotMetabBW34), total oxidative metabolism (TotOxMetabBW34), and hepatic oxidation (AMetLiv1BW34). These all have excellent posterior convergence (*R* diagnostic ≤1.01), relatively low uncertainty and variability (GSD <2), and relatively low uncertainty in human population variability (GSD for population percentiles <2). In addition, the PBPK model predictions compare well with the available in vivo pharmacokinetic data.

Predictions for TCE in blood (AUCCBld) are somewhat more uncertain. Although convergence was excellent across species ($R \leq 1.01$), overall uncertainty and variability was about threefold. In humans, the uncertainty in human population variability was relatively low (GSD for population percentiles <1.5). TCE blood level predictions were somewhat high in comparison to the Chiu et al. (2007) study at 1 ppm, though the predictions were better for most of the other studies at higher exposure levels. In mice, TCE blood levels were somewhat overpredicted in open-chamber inhalation studies. In both mice and rats, there were some cases in which fits were inconsistent across dose groups if the same parameters were used across dose groups, indicating unaccounted-for dose-related effects or intrastudy variability. However, in both rats and humans, TCE blood (humans and rats) and tissue (rats only) concentrations from studies not used for calibration (i.e., saved for "out-of-sample" evaluation/"validation") were well simulated, adding confidence to the parent compound dose-metric predictions.

For the TCA dose-metric predictions (TotTCAInBW, AUCLivTCA) convergence in all three species was excellent ($R \leq 1.01$). Overall uncertainty and variability was intermediate between dose-metrics for metabolism and that for TCE in blood, with GSDs of about two to threefold. Uncertainty in human population percentiles was relatively low (GSD of 1.2–1.7). While liver TCA levels were generally well fit, the data was relatively sparse. Plasma and blood TCA levels were generally well fit, though in mice, there were again some cases in which fits were inconsistent across dose groups if the same parameters were used across dose groups, indicating unaccounted-for dose-related effects or intrastudy variability. In humans, the accurate predictions for, TCA blood and urine concentrations from studies used for "out of sample" evaluation lends further confidence to dose-metrics involving TCA.

The evaluation of TCOH in blood followed a similar pattern. Convergence in all three species was good, though the rat model had slightly worse convergence ($R \sim 1.03$) than the mouse and humans ($R \leq 1.01$). In mice, overall uncertainty and variability was slightly more than for TCE in blood. There was much higher overall uncertainty and variability in the rat predictions (GSD of almost 9), which likely reflects true interstudy variability. The population-generated predictions for TCOH and TCOG in blood and urine were quite wide, with some in vivo data at both the upper and lower ends of the range of predictions. In humans, the overall uncertainty and variability was intermediate between mice and rats (GSD = 5.8). As with the rats, this likely reflects true population heterogeneity, as the uncertainty in human population percentiles was relatively low (GSD of around 1.2~1.7-fold). For all three species, fits to in vivo

data are generally good. In mice, however, there were again some cases in which fits were inconsistent across dose groups if the same parameters were used across dose groups, indicating unaccounted-for, dose-related effects or intrastudy variability. In humans, the accurate predictions for TCOH blood and urine concentrations from studies used for "out of sample" evaluation lends further confidence to those dose-metrics involving TCOH.

GSH metabolism dose-metrics (ABioactDCVCBW34, ABioactDCVCKid, AMetGSHBW34) had the greatest overall uncertainty in mice but was fairly well characterized in rats and humans. In mice, there were no in vivo data informing this pathway except for the indirect constraint of overall mass balance. So although convergence was adequate ($R < 1.02$), the uncertainty/variability was very large, with a GSD of ninefold for the overall flux (the amount of bioactivation was not characterized because there are no data constraining downstream GSH pathways). For rats, there were additional constraints from (well-fit) urinary NAcDCVC data, which reduced the overall uncertainty and variability substantially (GSD less than fourfold). In humans, in addition to urinary NAcDCVC data, DCVG blood concentration data was available, though only at the group level. These data, both of which were well fit, in addition to the greater amount of in vitro metabolism data, allowed for the flux through the GSH pathway and the rate of DCVC bioactivation to be fairly well constrained, with overall uncertainty and variability having GSD less than fourfold, and uncertainty in population percentiles no more than about twofold. However, these predictions may need to be interpreted with caution, given potential analytical issues with quantifying DCVG either in vitro or in vivo (see Section 3.3.3.2). Thus, the substantial inconsistencies across studies and methods in the quantification of DCVG following TCE exposure suggest lower confidence in the accuracy of these predictions.

The final two dose-metrics, respiratory metabolism (AMetLngBW34, AMetLngResp) and "other" oxidative metabolism (AMetLivOtherBW34, AMetLivOtherLiv), also lacked direct in vivo data and were predicted largely on the basis of mass balance and physiological constraints. Respiratory metabolism had good convergence ($R < 1.01$), helped by the availability of closed-chamber data in rodents. In rats and mice, overall uncertainty and variability was rather uncertain (GSD of 4~5-fold), but the overall uncertainty and variability was much greater in humans, with a GSD of about 10-fold. This largely reflects the significant variability across individuals as well as substantial uncertainty in the low population percentiles (GSD of fourfold). However, the middle (i.e., "typical" individuals) and upper percentiles (i.e., the individuals at highest risk) are fairly well constrained with a GSD of around twofold. For the "other" oxidative metabolism dose-metric, convergence was good in mice and humans ($R < 1.02$), but less than ideal in rats ($R \sim 1.15$). In rodents, the overall uncertainty and variability were moderate, with a GSD around 3.5-fold, slightly higher than that for TCE in blood. The overall uncertainty and variability in this metric in humans had a GSD of about fourfold, slightly higher than for GSH

conjugation metrics. However, uncertainty in the middle and upper population percentiles had GSDs of only about twofold, similar to that for respiratory metabolism.

Overall, as shown in Table 3-51, the updated PBPK model appears to be most reliable for the fluxes of total, oxidative, and hepatic oxidative metabolism. In addition, dose-metrics related to blood levels of TCE and oxidative metabolites, TCOH and TCA, had only modest uncertainty. In the case of TCE in blood, for some data sets, model predictions overpredicted the in vivo data, and, in the case of TCOH in rats, substantial interstudy variability was evident. For GSH metabolism, dose-metric predictions for rats and humans had only slightly greater uncertainty than the TCE and metabolism metrics. Predictions for mice were much more uncertain, reflecting the lack of GSD-specific in vivo data. Finally, for "other" oxidative metabolism and respiratory oxidative metabolism, predictions also had somewhat more uncertainty than the TCE and metabolism metrics, though uncertainty in middle and upper human population percentiles was modest.

4. HAZARD CHARACTERIZATION

This section presents the hazard characterization of TCE health effects. Because of the number of studies and their relevance to multiple endpoints, the evaluation of epidemiologic studies of cancer and TCE is summarized in Section 4.1 (endpoint-specific results are presented in subsequent sections). Genotoxicity data are discussed in Section 4.2. Due to the large number of endpoints and studies in the toxicity database, subsequent sections (see Sections 4.3–4.10) are organized by tissue/organ system. Each section is further organized by noncancer and cancer endpoints, discussing data from human epidemiologic and laboratory experimental studies. In cases where there is adequate information, the role of metabolism in toxicity, comparisons of toxicity between TCE and its metabolites, and carcinogenic mode of action are also discussed. Finally, Section 4.11 summarizes the overall hazard characterization and the weight of evidence for noncancer and carcinogenic effects.

4.1. EPIDEMIOLOGIC STUDIES ON CANCER AND TCE—METHODOLOGICAL OVERVIEW

This brief overview of the epidemiologic studies on cancer and TCE below provides background to the discussion contained in Sections 4.4–4.10. Over 50 epidemiologic studies on cancer and TCE exposure (see Tables 4-1 through 4-3) were examined to assess their ability to inform weight-of-evidence evaluation (i.e., to inform the cancer hazard from TCE exposure) according to 15 standards of study design (see Table 4-4), conduct, and analysis. The analysis of epidemiologic studies on cancer and TCE serves to document essential design features, exposure assessment approaches, statistical analyses, and potential sources of confounding and bias. This analysis, furthermore, supports the discussion of site-specific cancer observations in Sections 4.4–4.9. In those sections, study findings are presented with an assessment and discussion of their observations according to a study‘s weight of evidence and the potential for alternative explanations, including bias and confounding. Tables containing observed findings for site-specific cancers are also found in Sections 4.4–4.9. Full details of the weight-of evidence-review to identify a cancer hazard and study selections for meta-analysis may be found in Appendix B.

Table 4-1. Description of epidemiologic cohort and proportionate mortality ratio (PMR) studies assessing cancer and TCE exposure

Reference	Description	Study group (*N*) comparison group (*N*)	Exposure assessment and other information
Aircraft and aerospace workers			
Radican et al. (2008); Blair et al. (1998)	Civilian aircraft-maintenance workers with at least 1 yr in 1952–1956 at Hill Air Force Base, Utah. VS to 1990 (Blair et al., 1998) or 2000 (Radican et al., 2008); cancer incidence 1973–1990 (Blair et al., 1998)	14,457 (7,204 ever exposed to TCE). Incidence (Blair et al., 1998) and mortality rates (Radican et al., 2008; Blair et al., 1998) of nonchemical exposed subjects.	Most subjects (n = 10,718) with potential exposure to 1–25 solvents. Cumulative TCE assigned to individual subjects using JEM. Exposure-response patterns assessed using cumulative exposure, continuous or intermittent exposures, and peak exposure. TCE replaced in 1968 with 1,1,1-trichloroethane and was discontinued in 1978 in vapor degreasing activities. **Median TCE exposures were about 10 ppm for rag and bucket; 100–200 ppm for vapor degreasing.** Poisson regression analyses controlled for age, calendar time, sex (Blair et al., 1998), or Cox proportional hazard model for age and race.
Krishnadasan et al. (2007)	Nested case-control study within a cohort of 7,618 workers employed between 1950 and 1992, or who had started employment before 1980 at Boeing/Rockwell/ Rocketdyne [SSFL, the UCLA cohort of Morgenstern (1997)]. Cancer incidence 1988–1999.	326 prostate cancer cases, 1,805 controls. Response rate: cases, 69%; controls, 60%.	JEM for TCE, hydrazine, PAHs, benzene, and mineral oil constructed from company records, walk-through, or interviews. Lifestyle factors obtained from living subjects through mail and telephone surveys. Conditional logistic regression controlled for cohort, age at diagnosis, physical activity, SES, and other occupational exposure (benzene, PAHs, mineral oil, hydrazine).

Table 4-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) comparison group (*N*)	Exposure assessment and other information
Ritz et al. (1999); Zhao et al. (2005)	Aerospace workers with ≥2 yrs of employment at Rockwell/ Rocketdyne (now Boeing) and who worked at SSFL, Ventura, California, from 1950 to 1993 [the UCLA cohort of (Morgenstern et al., 1997)]. Cancer mortality as of December 31, 2001. Cancer incidence 1988–2000 for subjects alive as of 1988.	6,044 (2,689 with high cumulative exposure to TCE). Mortality rates of subjects in lowest TCE exposure category. 5,049 (2,227 with high cumulative exposure to TCE). Incidence rates of subjects in lowest TCE exposure category.	JEM for TCE, hydrazine, PAHs, mineral oil, and benzene. IH ranked each job title ranked for presumptive TCE exposure as high (3), medium (2), low (1), or no (0) exposure for three time periods (1951–1969, 1970–1979, 1980–1989). Cumulative TCE score: low (<3), medium (>3–12), high (>12) assigned to individual subjects using JEM. Cox proportional hazard, controlled for time, since 1st employment, SES, age at diagnosis, and hydrazine exposure.
Boice et al. (2006b)	Aerospace workers with ≥6 mo employment at Rockwell/ Rocketdyne (SSFL and nearby facilities) from 1948 to 1999 [IEI cohort (IEI, 2005)]. VS to 1999.	41,351, 1,642 male hourly test stand mechanics (1,111 with potential TCE exposure). Mortality rates of U.S. population and California population. Internal referent groups including male hourly nonadministrative Rocketdyne workers; male hourly, nonadministrative SSFL workers; and test stand mechanics with no potential exposure to TCE.	Potential TCE exposure assigned to test stands workers only whose tasks included the cleaning or flushing of rocket engines (engine flush) (n = 639) or for general utility cleaning (n = 472); potential for exposure to large quantities of TCE was much greater during engine flush than when TCE used as a utility solvent. JEM for TCE and hydrazine without semiquantitative intensity estimates. Exposure to other solvents not evaluated due to low potential for confounding (few exposed, low exposure intensity, or not carcinogenic). Exposure metrics included employment duration, employment decade, year worked with potential TCE exposure, and year worked with potential TCE exposure via engine cleaning, weighted by number of tests. Lifetable (SMR); Cox proportional hazard controlling for birth year, hire year, and hydrazine exposure.
Boice et al. (1999)	Aircraft-manufacturing workers with at least 1 yr ≥1960 at Lockheed Martin (Burbank, California). VS to 1996.	77,965 (2,267 with potential routine TCE exposures and 3,016 with routine or intermittent TCE exposure). Mortality rates of U.S. population (routine TCE exposed subjects) and nonexposed internal referents (routine and intermittent TCE exposed subjects).	12% with potential routine mixed solvent exposure and 30% with route or intermittent solvent exposure. JEM for potential TCE exposure on (1) routine basis or (2) intermittent or routine basis without semiquantitative intensity estimate. Exposure-response patterns assessed by any exposure or duration of exposure and internal control group. Vapor degreasing with TCE before 1966 and perchloroethylene, afterwards. Lifetable analyses; Poisson regression analysis adjusting for birth date, starting employment date, finishing employment date, sex, and race.

Table 4-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) comparison group (*N*)	Exposure assessment and other information
Morgan et al. (1998)	Aerospace workers with ≥6 mo 1950–1985 at Hughes (Tucson, Arizona). VS to 1993.	20,508 (4,733 with TCE exposures). Mortality rates of U.S. population for overall TCE exposure; mortality rates of all-other cohort subjects (internal referents).	TCE exposure intensity assigned using JEM. Exposure-response patterns assessed using cumulative exposure (low vs. high) and job with highest TCE exposure rating (peak, medium/high exposure vs. no/low exposure). **—High exposure" job classification defined as >50 ppm.** Vapor degreasing with TCE 1952–1977, but limited IH data <1975. Limited IH data before 1975 and medium/low rankings likely misclassified given temporal changes in exposure intensity not fully considered (NRC, 2006).
Costa et al. (1989)	Aircraft manufacturing workers employed 1954–1981 at plant in Italy. VS to 1981.	8,626 subjects Mortality rates of the Italian population.	No exposure assessment to TCE and job titles grouped into one of four categories: blue- and white-collar workers, technical staff, and administrative clerks. Lifetable (SMR).
Garabrant et al. (1988)	Aircraft manufacturing workers ≥4 yrs employment and who had worked at least 1 d at San Diego, California, plant 1958–1982. VS to 1982.	14,067 Mortality rates of U.S. population.	TCE exposure assessment for 70 of 14,067 subjects; 14 cases of esophageal cancer and 56 matched controls. For these 70 subjects, company work records identified 37% with job title with potential TCE exposure without quantitative estimates. Lifetable (SMR).
Cohorts identified from biological monitoring (U-TCA)			
Hansen et al. (2001)	Workers biological monitored using U-TCA and air-TCE, 1947–1989. Cancer incidence from 1964 to 1996.	803 total. Cancer incidence rates of the Danish population.	712 with U-TCA, 89 with air-TCE measurement records, two with records of both types. U-TCA from 1947 to 1989; air TCE measurements from 1974. Historic median exposures estimated from the U-TCA concentrations were: 9 ppm for 1947–1964, 5 ppm for 1965–1973, 4 ppm for 1974–1979, and 0.7 ppm for 1980–1989. Air TCE measurements from 1974 onward were 19 ppm (mean) and 5 ppm (median). **Overall, median TCE exposure to cohort as extrapolated from air TCE and U-TCA measurements was 4 ppm (arithmetic mean, 12 ppm)**. Exposure metrics: year 1st employed, employment duration, mean exposure, cumulative exposure. Exposure metrics: employment duration, average TCE intensity, cumulative TCE, period 1st employment. Lifetable analysis (SIR).
Anttila et al. (1995)	Workers biological monitored using U-TCA, 1965–1982. VS 1965–1991 and cancer incidence 1967–1992.	3,974 total (3,089 with U-TCA measurements). Mortality and cancer incidence rates of the Finnish population.	Median U-TCA, 63 µmol/L for females and 48 µmol/L for males; mean U-TCA was 100 µmol/L. Average 2.5 U-TCA measurements per individual. Using the Ikeda et al. (1972) relationship for TCE exposure to U-TCA, **TCE exposures were roughly 4 ppm (median) and 6 ppm (mean).** Exposure metrics: year since 1st measurement. Lifetable analysis (SMR, SIR).

Table 4-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) comparison group (*N*)	Exposure assessment and other information
Axelson et al. (1994)	Workers biological monitored using U-TCA, 1955–1975. VS to 1986 and cancer incidence 1958–1987.	1,421 males. Mortality and cancer incidence rates of Swedish male population.	Biological monitoring for U-TCA from 1955 and 1975. **Roughly ¾ of cohort had U-TCA concentrations equivalent to <20 ppm TCE.** Exposure metrics: duration exposure, mean U-TCA. Lifetable analysis (SMR, SIR).
Other cohorts			
Clapp and Hoffman (2008)	Deaths between 1969 and 2001 among employees ≥5 yrs employment duration at an IBM facility (Endicott, New York).	360 deaths. Proportion of deaths among New York residents during 1979–1998.	No exposure assessment to TCE. PMR analysis.
Sung et al. (2008; 2007)	Female workers 1st employed 1973–1997 at an electronics (RCA) manufacturing factory (Taoyuan, Taiwan). Cancer incidence 1979–2001 (Sung et al., 2007). Childhood leukemia 1979–2001 among first born of female subjects in Sung et al. (2008).	63,982 females and 40,647 females with 1st live born offspring. Cancer incidence rates of Taiwan population (Sung et al., 2007). Childhood leukemia incidence rates of first born live births of Taiwan population (Sung et al., 2008).	No exposure assessment. Chlorinated solvents including TCE and perchloroethylene found in soil and groundwater at factory site. Company records indicated TCE not used 1975–1991 and perchloroethylene 1975–1991 and perchloroethylene after 1981. No information for other time periods. Exposure-response using employment duration. Lifetable analysis (SMR, SIR) (Sung et al., 2007; Chang et al., 2005; Chang et al., 2003) or Poisson regression adjusting for maternal age, education, sex, and birth year (Sung et al., 2008).
Chang et al. (2003; 2005)	Male and female workers employed 1978–1997 at electronics factory as studied by Sung et al. (2007). VS from 1985 to 1997 and cancer incidence 1979 to 1997.	86,868 total. Incidence (Chang et al., 2005) mortality (Chang et al., 2003) rates Taiwan population.	
ATSDR (2004a)	Workers 1952–1980 at the View-Master factory (Beaverton, Oregon).	616 deaths 1989–2001. Proportion of deaths between 1989 and 2001 in Oregon population.	No exposure information on individual subjects. TCE and other VOCs detected in well water at the time of the plant closure in 1998 were TCE, 1,220–1,670 µg/L; 1,1-DCE, up to 33 µg/L; and, perchloroethylene up to 56 µg/L. PMR analysis.
Raaschou-Nielsen et al. (2003)	Blue-collar workers employed >1968 at 347 Danish TCE-using companies. Cancer incidence through 1997.	40,049 total (14,360 with presumably higher level exposure to TCE). Cancer incidence rates of the Danish population.	Employers had documented TCE usage. Blue-collar vs. white-collar workers and companies with <200 workers were variables identified as increasing the likelihood for TCE exposure. Subjects from iron and metal, electronics, painting, printing, chemical, and dry cleaning industries. **Median exposures to TCE were 40–60 ppm for the year before 1970, 10–20 ppm for 1970–1979, and approximately 4 ppm for 1980–1989.** Exposure metrics: employment duration, year 1st employed, and # employees in company. Lifetable (SIR).

Table 4-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) comparison group (*N*)	Exposure assessment and other information
Ritz (1999a)	Male uranium-processing plant workers ≥3 mo employment 1951–1972 at DOE facility (Fernald, Ohio). VS 1951–1989, cancer.	3,814 white males monitored for radiation (2,971with potential TCE exposure). Mortality rates of the U.S. population; non-TCE exposed internal controls for TCE exposure-response analyses.	JEM for TCE, cutting fluids, kerosene, and radiation generated by employees and industrial hygienists. Subjects assigned potential TCE according to intensity: light (2,792 subjects), moderate (179 subjects), heavy (no subjects). Lifetable (SMR) and conditional logistic regression adjusted for pay status, date first hire, radiation.
Henschler et al. (1995)	Male workers ≥1 yr 1956–1975 at cardboard factory (Arnsberg region, Germany). VS to 1992.	169 exposed; 190 unexposed. Mortality rates from German Democratic Republic (broad categories) or RCC incidence rates from Danish population, German Democratic, or non-TCE exposed subjects.	Walk-through surveys and employee interviews used to identify work areas with TCE exposure. TCE exposure assigned to renal cancer cases using workman‘s compensation files. Lifetable (SMR, SIR) or Mantel-Haenszel.
Greenland et al. (1994)	Cancer deaths, 1969–1984, among pensioned workers employed <1984 at GE transformer manufacturing plant (Pittsfield, Massachusetts), and who had job history record; controls were noncancer deaths among pensioned workers.	512 cases, 1,202 controls. Response rate: cases, 69%; controls, 60%.	IH assessment from interviews and position descriptions. TCE (no/any exposure) assigned to individual subjects using JEM. Logistic regression.
Sinks et al. (1992)	Workers employed 1957–1980 at a paperboard container manufacturing and printing plant (Newnan, Georgia). VS to 1988. Kidney and bladder cancer incidence through 1990.	2,050 total. Mortality rates of the U.S. population, bladder and kidney cancer incidence rates from the Atlanta-SEER registry for the years 1973–1977.	No exposure assessment to TCE; analyses of all plant employees including white- and blue-collar employees. Assignment of work department in case-control study based upon work history; Material Safety Data Sheets identified chemical usage by department. Lifetable (SMR, SIR) or conditional logistic regression adjusted for hire date and age at hire, and using 5- and 10-yr lagged employment duration.
Blair et al. (1989)	Workers employed 1942–1970 in U.S. Coast. VS to 1980.	3,781 males of whom 1,767 were marine inspectors (48%). Mortality rates of the U.S. population. Mortality rates of marine inspectors also compared to that of noninspectors.	No exposure assessment to TCE. Marine inspectors worked in confined spaces and had exposure potential to multiple chemicals. TCE was identified as one of 10 potential chemical exposures. Lifetable (SMR) and directly adjusted RRs.

Table 4-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) comparison group (*N*)	Exposure assessment and other information
Shannon et al. (1988)	Workers employed ≥6 mo at GE lamp manufacturing plant, 1960–1975. Cancer incidence from 1964 to 1982.	1,870 males and females, 249 (13%) in coiling and wire-drawing area. Cancer incidence rates from Ontario Cancer Registry.	No exposure assessment to TCE. Workers in CWD had potential exposure to many chemicals including metals and solvents. A 1955-dated engineering instruction sheet identified TCE used as degreasing solvent in CWD. Lifetable (SMR).
Shindell and Ulrich (1985)	Workers employed ≥3 mo at a TCE manufacturing plant 1957–1983. VS to 1983.	2,646 males and females. Mortality rates of the U.S. population.	No exposure assessment to TCE; job titles categorized as either white- or blue-collar. Lifetable analysis (SMR).
Wilcosky et al. (1984)	Respiratory, stomach, prostate, lymphosarcoma, and lymphatic leukemia cancer deaths 1964–1972 among 6,678 active and retired production workers at a rubber plant (Akron, Ohio); controls were a 20% age-stratified random sample of the cohort.	183 cases (101 respiratory, 33 prostate, 30 stomach, 9 lymphosarcoma, and 10 lymphatic leukemia cancer deaths).	JEM without quantitative intensity estimates for 20 exposures including TCE. Exposure metric: ever held job with potential TCE exposure.

CWD = coiling and wire drawing; DOE = U.S. Department of Energy; GE = General Electric; IBM = International Business Machines Corporation; IEI = International Epidemiology Institute; IH = industrial hygienist; JEM = job-exposure matrix; PAH = polycyclic aromatic hydrocarbon; RCC = renal cell carcinoma; RR = relative risk; SES = socioeconomic status; SIR = standardized incidence ratio; SMR = standardized mortality ratio; SSFL = Santa Susanna Field Laboratory; U-TCA = urinary TCA; UCLA = University of California, Los Angeles; VS = vital status

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
Bladder			
Pesch et al. (2000a)	Histologically confirmed urothelial cancer (bladder, ureter, renal pelvis) cases from German hospitals (five regions) in 1991–1995; controls randomly selected from residency registries matched on region, sex, and age.	1,035 cases. 4,298 controls. Cases, 84%; controls, 71%.	Occupational history using job title or self-reported exposure. JEM and JTEM to assign exposure potential to metals and solvents (chlorinated solvents, TCE, perchloroethylene). Lifetime exposure to TCE exposure examined as 30^{th}, 60^{th}, and 90^{th} percentiles (medium, high, and substantial) of exposed control exposure index. Duration used to examine occupational title and job task duties and defined as 30^{th}, 60^{th}, and 90^{th} percentiles (medium, long, and very long) of exposed control durations. Logistic regression with covariates for age, study center, and smoking.
Siemiatycki et al. (1994); (1991)	Male bladder cancer cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	484 cases. 533 population controls; 740 other cancer controls. Cases, 78%; controls, 72%.	JEM to assign 294 exposures including TCE on semiquantitative scales categorized as any or substantial exposure. Other exposure metrics included exposure duration in occupation or job title. Logistic regression adjusted for age, ethnic origin, SES status, smoking, coffee consumption, and respondent status (occupation or job title) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, and respondent status (TCE).
Brain			
DeRoos et al. (2001); Olshan et al. (1999)	Neuroblastoma cases in children of <19 yrs selected from Children's Cancer Group and Pediatric Oncology Group with diagnosis in 1992–1994; population controls (random digit dialing) matched to control on birth date.	504 cases. 504 controls. Cases, 73%; controls, 74%.	Telephone interview with parent using questionnaire to assess parental occupation and self-reported exposure history and judgment-based attribution of exposure to chemical classes (halogenated solvents) and specific solvents (TCE). Exposure metric was any potential exposure. Logistic regression with covariate for child's age and material race, age, and education.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
Heineman et al. (1994)	White, male cases, age ≥30 yrs, identified from death certificates in 1978–1981; controls identified from death certificates and matched for age, year of death and study area.	300 cases. 386 controls. Cases, 74%; controls, 63%.	In-person interview with next-of-kin; questionnaire assessing lifetime occupational history using job title and JEM of Gomez et al. (1994). Cumulative exposure metric (low, medium, or high) based on weighted probability and duration. Logistic regression with covariates for age and study area.
Colon and rectum			
Goldberg et al. (2001); Siemiatycki (1991)	Male colon cancer cases, 35–75 yrs, from 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	497 cases. 533 population controls and 740 cancer controls. Cases, 82%; controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, ethnic origin, birthplace, education, income, parent‘s occupation, smoking, alcohol consumption, tea consumption, respondent status, heating source SES status, smoking, coffee consumption, and respondent status (occupation, some chemical agents) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, and respondent status (TCE).
Dumas et al. (2000); Simeiatycki (1991)	Male rectal cancer cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	292 cases. 533 population controls and 740 other cancer controls. Cases, 78%; controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, respondent status, cigarette smoking, beer consumption and BMI (TCE) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, ethnic origin, and beer consumption (TCE).
Fredriksson et al. (1989)	Colon cancer cases aged 30–75 yrs identified through the Swedish Cancer Registry among patients diagnosed in 1980–1983; population-based controls were frequency-matched on age and sex and were randomly selected from a population register.	329 cases. 658 controls. Not available.	Mailed questionnaire assessing occupational history with telephone interview follow-up. Self-reported exposure to TCE defined as any exposure. Mantel-Haenszel stratified on age, sex, and physical activity.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
Esophagus			
Parent et al. (2000b); Siemiatycki (1991)	Male esophageal cancer cases, 35–75 yrs, diagnosed in 19 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	292 cases. 533 population controls; 740 subjects with other cancers. Cases, 78%; controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, respondent status, cigarette smoking, beer consumption and BMI (solvents) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, ethnic origin, and beer consumption (TCE).
Lymphoma			
Purdue et al. (2011)	Cases aged 20–74 with histologically-confirmed NHL (B-cell diffuse and follicular, T-cell, lymphoreticular) without HIV in 1998–2000 and identified from four SEER areas (Los Angeles County and Detroit metropolitan area, random sample; Seattle_Puget Sound and Iowa, all consecutive cases); population controls aged 20–74 with no previous diagnosis of HIV infection or NHL, identified through (1) if >65 yrs of age, random digit dialing, or (2) if ≥65 yrs, identified from Medicare eligibility files and stratified on geographic area, age, and race.	1,321 cases. 1,057 controls. Cases, 76%; controls, 78%.	In-person interview using questionnaire or computer-assisted personal interview questionnaire specific for jobs held for >1 yr since the age of 16 yrs, hobbies, and medical and family history. For occupational history, 32 job- or industry-specific interview modules asked for detailed information on individual jobs and focused on solvents exposure, including TCE, assessment by expert industrial hygienist blinded to case and control status by levels of probability, frequency, and intensity. Exposure metric of overall exposure, average weekly exposure, year exposed, average exposure intensity, and cumulative exposure. Logistic regression adjusted for sex, age, race, education and SEER site.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
Gold et al. (2011)	Cases aged 35–74 with histologically-confirmed multiple myeloma in 2000–2002 and identified from Seer areas (Detroit, Seattle-Puget Sound); population controls.	181 cases. 481 controls. Cases, 71%; controls, 52%.	In-person interview using computer-assisted personal interview questionnaire for jobs held ≥1 yr since 1941 (cases) or 1946 (controls) and since age 18 yrs. For occupational history, 20 occupations, job- or industry-specific interview modules asked for detailed information on individual jobs held at least 2 yrs and focused on solvents exposure, including TCE, assessment by expert industrial hygienist blinded to case and control status by levels of probability, duration, and cumulative exposure. Logistic regression adjusted for sex, age, race, education, and SEER site.
Cocco et al. (2010)	Histologically or cytologically confirmed cases aged ≥17 yrs with lymphoma (B-cell, T-cell, CLL, multiple myeloma, Hodgkin) in 1998–2004 and residents of referral areas from seven European countries (Czech Republic, Finland, France, Germany, Ireland, Italy, and Spain); hospital (four participating countries) or population controls (all others); controls from: (1) Germany and Italy selected by random digit dialing from general population and matched (individually in German and group-based in Italy) to cases by sex, age, and residence area and (2) for all other countries, matched hospital controls with diagnoses other than cancer, infectious diseases, and immundeficient diseases (individually in Czech Republic group-based in all other countries).	2,348 cases. 2,462 controls. Cases, 88%; controls, 81% hospital and 52% population.	In-person interviews using same structured questionnaire translated to the local language for information on sociodemographic factors, lifestyle, health history, and all full-time job held ≥1 yr. Assessment by industrial hygienists in each participating center to 43 agents, including TCE, by confidence, exposure intensity, and exposure frequency. Exposure metric of overall TCE exposure and cumulative TCE exposure for subjects assessed with high degree of confidence. Logistic regression adjusted for age, gender, education, and study center.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
German centers: Seidler et al. (2007); Mester et al. (2006); Becker et al. (2004)	NHL and Hodgkin lymphoma cases aged 18–80 yrs identified through all hospitals and ambulatory physicians in six regions of Germany between 1998 and 2003; population controls were identified from population registers and matched on age, sex, and region.	710 cases. 710 controls. Cases, 87%; controls, 44%.	In-person interview using questionnaire assessing personal characteristics, lifestyle, medical history, UV light exposure, and occupational history of all jobs held for ≥1 yr. Exposure of a prior interest were assessed using job task-specific supplementary questionnaires. JEM used to assign cumulative quantitative TCE exposure metric, categorized according to the distribution among the control persons (50^{th} and 90^{th} percentile of the exposed controls). Conditional logistic regression adjusted for age, sex, region, smoking, and alcohol consumption.
Wang et al. (2009)	Cases among females aged 21 and 84 yrs with NHL in 1996–2000 and identified from Connecticut Cancer Registry; population-based female controls: (1) if <65 yrs of age, having Connecticut address stratified by 5-yr age groups identified from random digit dialing or (2) ≥65 yrs of age, by random selection from Centers for Medicare and Medicaid Service files.	601 cases. 717 controls. Cases, 72%; controls, 69% (<65 yrs), 47% (≥65 yrs)	In-person interview using questionnaire assessment specific for jobs held for >1 yr. Intensity and probability of exposure to broad category of organic solvents and to individual solvents, including TCE, estimated using JEM (Dosemeci et al., 1994; Gómez et al., 1994) and assigned blinded. Exposure metric of any exposure, exposure intensity (low, medium/high), and exposure probability (low, medium/high). Logistic regression adjusted for age, family history of hematopoietic cancer, alcohol consumption, and race.
Costantini et al. (2008); Miligi et al. (2006)	Cases aged 20–74 with NHL, including CLL, all forms of leukemia, or MM in 1991–1993 and identified through surveys of hospital and pathology departments in study areas and in specialized hematology centers in eight areas in Italy; population-based controls stratified by 5-yr age groups and by sex selected through random sampling of demographic or of National Health Service files.	1,428 NHL + CLL, 586 Leukemia, 263, MM. 1,278 controls (leukemia analysis). 1,100 controls (MM analysis). Cases, 83%; controls, 73%.	In-person interview primarily at interviewee‘s home (not blinded) using questionnaire assessing specific jobs, extra occupational exposure to solvents and pesticides, residential history, and medical history. Occupational exposure assessed by job-specific or industry-specific questionnaires. JEM used to assign TCE exposure and assessed using intensity (two categories) and exposure duration (two categories). All NHL diagnoses and 20% sample of all cases confirmed by panel of three pathologists. Logistic regression with covariates for sex, age, region, and education. Logistic regression for specific NHL included an additional covariate for smoking.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
Persson and Fredriksson (1999); combined analysis of NHL cases in Persson et al. (1993; 1989)	Histologically confirmed cases of B-cell NHL, age 20–79 yrs, identified in two hospitals in Sweden: Oreboro in 1964–1986 (Persson et al., 1989) and in Linkoping between 1975 and 1984 (Persson et al., 1993); controls were identified from previous studies and were randomly selected from population registers.	NHL cases, 199. 479 controls. Cases, 96% (Oreboro), 90% (Linkoping); controls, not reported.	Mailed questionnaire to assess self reported occupational exposures to TCE and other solvents. Unadjusted Mantel-Haenszel χ^2.
Nordstrom et al. (1998)	Histologically-confirmed cases in males of hairy-cell leukemia reported to Swedish Cancer Registry in 1987–1992 (includes one case latter identified with an incorrect diagnosis date); population-based controls identified from the National Population Registry and matched (1:4 ratio) to cases for age and county.	111 cases. 400 controls. Cases, 91%; controls, 83%.	Mailed questionnaire to assess self reported working history, specific exposure, and leisure time activities. Univariate analysis for chemical-specific exposures (any TCE exposure).
Fritschi and Siemiatycki (1996b); Siemiatycki (1991)	Male NHL cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	215 cases. 533 population controls (Group 1) and 1,900 subjects with other cancers (Group 2). Cases, 83%; controls, 71%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales). Exposure metric defined as any or substantial exposure. Logistic regression adjusted for age, proxy status, income, and ethnicity (solvents) or Mantel-Haenszel stratified by age, BMI, and cigarette smoking (TCE).

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
Hardell et al. (1994; 1981)	Histologically-confirmed cases of NHL in males, age 25–85 yrs, admitted to Swedish (Umea) hospital between 1974 and 1978; living controls (1:2 ratio) from the National Population Register, matched to living cases on sex, age, and place of residence; deceased controls from the National Registry for Causes of Death, matched (1:2 ratio) to dead cases on sex, age, place of residence, and year of death.	105 cases. 335 controls. Response rate not available.	Self-administered questionnaire assessing self-reported solvent exposure; phone follow-up with subject, if necessary. Unadjusted Mantel-Haenszel χ^2.
Persson et al. (1993; 1989)	Histologically confirmed cases of Hodgkin lymphoma, age 20–80 yrs, identified in two hospitals in Sweden: Oreboro in 1964–1986 (Persson et al., 1989) and in Linkoping between 1975 and 1984 (Persson et al., 1993); controls randomly selected from population registers.	54 cases (1989 study); 31 cases (1993 study). 275 controls (1989 study); 204 controls (1993 study). Response rate not available.	Mailed questionnaire to assess self reported occupational exposures to TCE and other solvents. Logistic regression with adjustment for age and other exposure; unadjusted Mantel-Haenszel χ^2.
Childhood leukemia			
Shu et al. (2004; 1999)	Childhood leukemia cases, <15 yrs, diagnosed between 1989 and 1993 by a Children's Cancer Group member or affiliated institute; population controls (random digit dialing), matched for age, race, and telephone area code and exchange.	1,842 cases. 1,986 controls. Cases, 92%; controls, 77%.	Telephone interview with mothers, and whenever available, fathers, using questionnaire to assess occupation using job-industry title and self-reported exposure history. Questionnaire included questions specific for solvent, degreaser, or cleaning agent exposures. Logistic regression with adjustment for maternal or paternal education, race, and family income. Analyses of paternal exposure also included age and sex of the index child.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
Costas et al. (2002); MDPH (1997c, b)	Childhood leukemia (<19 yrs age) diagnosed in 1969–1989 and who were resident of Woburn, Massachusetts; controls randomly selected from Woburn public school records, matched for age.	19 cases. 37 controls. Cases, 91%; controls, not available.	Questionnaire administered to parents separately assessing demographic and lifestyle characteristics, medical history information, environmental and occupational exposure, and use of public drinking water in the home. Hydraulic mixing model used to infer delivery of TCE and other solvents water to residence. Logistic regression with composite covariate, a weighted variable of individual covariates.
McKinney et al. (1991)	Incident childhood leukemia and NHL cases, 1974–1988, ages not identified, from three geographical areas in England; controls randomly selected from children of residents in the three areas and matched for sex and birth health district.	109 cases. 206 controls. Cases, 72%; controls, 77%.	In-person interview with questionnaire with mother to assess maternal occupational exposure history, and with father and mother, as surrogate, to assess paternal occupational exposure history. No information provided in paper whether interviewer was blinded as to case and control status. Matched pair design using logistic regression for univariate and multivariate analysis.
Lowengart et al. (1987)	Childhood leukemia cases aged ≤10 yrs and identified from the Los Angeles (California) Cancer Surveillance Program in 1980–1984; controls selected from random digit dialing or from friends of cases and matched on age, sex, and race.	123 cases. 123 controls. Cases, 79%; controls, not available.	Telephone interview with questionnaire to assess parental occupational and self-reported exposure history. Matched (discordant) pair analysis.
Melanoma			
Fritschi and Siemiatycki (1996b); Siemiatycki (1991)	Male melanoma cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	103 cases. 533 population controls and 533 other cancer controls. Cases, 78%; controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, and ethnic origin (TCE) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, and ethnic origin (TCE).

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
Pancreas			
Kernan et al. (1999)	Pancreatic cancer deaths from 1984 to 1993 in 24 U.S. states; age-, sex-, race-, and state-matched noncancer deaths, excluding other pancreatic diseases and pancreatitis, controls.	63,097 cases. 252,386 population controls. Response rates not identified.	Exposure surrogate assigned for 111 chlorinated hydrocarbons, including TCE, and two broad chemical categories using usual occupation on death certificate and job-exposure-matrix of Gomez et al. (1994). Race and sex-specific mortality ORs from logistic regression analysis adjusted for age, marital status, metropolitan area, and residential status.
Prostate			
Aronson et al. (1996); Siemiatycki (1991)	Male prostate cancer cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	449 cases. 533 population controls (Group 1) and other cancer cases from same study (Group 2). Cases, 81%; controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales). Logistic regression adjusted for age, ethnic origin, SES status, Quetlet, and respondent status (occupation) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, ethnic origin, and respondent status (TCE).
Renal cell			
Moore et al. (2010)	Cases aged 20–74 yrs from four European countries (Russia, Romania, Poland, Czech Republic) with histologically confirmed kidney cancer in 1999–2003; hospital controls with diagnoses unrelated to smoking or genitourinary disorders in 1998–2003 and frequency matched by sex, age, and study center.	1,097 cases (825 RCCs). 1,184 controls. Cases, 90–99%; controls, 90.3–96%.	In-person interview using questionnaire for information on lifestyle habits, smoking, anthropometric measures, personal and family medical history, and occupational history. Specialized job-specific questionnaire for specific jobs or industries of interest focused on solvents exposure, including TCE, with exposure assignment by expert blinded to case and control status by frequency, intensity, and confidence of TCE exposure. Exposure metric of overall exposure, duration (total hr, yr), and cumulative exposure. Logistic regression adjusted for sex, age, and study center. BMI, hypertension, smoking, residence location also included in initial models but did not alter ORs by >10%.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
Charbotel et al. (2009; 2006)	Cases from Arve Valley region in France identified from local urologists files and from area teaching hospitals; age- and sex-matched controls chosen from file of same urologist as who treated case or recruited among the patients of the case's general practitioner.	87 cases. 316 controls. Cases, 74%; controls, 78%.	Telephone interview with case or control, or, if deceased, with next-of-kin (22% cases, 2% controls). Questionnaire assessing occupational history, particularly, employment in the screw cutting jobs, and medical history. Semiquantitative TCE exposure assigned to subjects using a task/TCE-Exposure Matrix designed using information obtained from questionnaires and routine atmospheric monitoring of workshops or biological monitoring (U-TCA) of workers carried out since the 1960s. Cumulative exposure, cumulative exposure with peaks, and TWA. Conditional logistic regression with covariates for tobacco smoking and BMI.
Brüning et al. (2003)	Histologically-confirmed cases 1992–2000 from German hospitals (Arnsberg); hospital controls (urology department) serving area, and local geriatric department, for older controls, matched by sex and age.	134 cases. 401 controls. Cases, 83%; controls, not available.	In-person interviews with case or next-of-kin; questionnaire assessing occupational history using job title. Exposure metrics included longest job held, JEM of Pannett et al. (1985) to assign cumulative exposure to TCE and perchloroethylene, and exposure duration. Logistic regression with covariates for age, sex, and smoking.
Pesch et al. (2000b)	Histologically-confirmed cases from German hospitals (five regions) in 1991–1995; controls randomly selected from residency registries matched on region, sex, and age.	935 cases. 4,298 controls. Cases, 88%; controls, 71%.	In-person interview with case or next-of-kin; questionnaire assessing occupational history using job title (JEM approach), self-reported exposure, or job task (JTEM approach) to assign TCE and other exposures. Logistic regression with covariates for age, study center, and smoking.
Parent et al. (2000a); Siemiatycki (1991)	Male RCC cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	142 cases. 533 population controls (Group 1) and other cancer controls (excluding lung and bladder cancers) (Group 2). Cases, 82%; controls, 71%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (about 300 exposures on semiquantitative scales); TCE defined as any or substantial exposure. Mantel-Haenszel stratified by age, BMI, and cigarette smoking (TCE) or logistic regression adjusted for respondent status, age, smoking, and BMI (occupation, job title).

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) comparison group (*N*) response rates	Exposure assessment and other information
Dosemeci et al. (1999)	Histologically-confirmed cases, 1988–1990, white males and females, 20–85 yrs, from Minnesota Cancer Registry; controls stratified for age and sex using random digit dialing, 21–64 yrs, or from HCFA records, 64–85 yrs.	438 cases. 687 controls. Cases, 87%; controls, 86%.	In-person interviews with case or next-of-kin; questionnaire assessing occupational history of TCE using job title and JEM of Gomez et al. (1994). Exposure metric was any TCE exposure. Logistic regression with covariates for age, smoking, hypertension, and BMI.
Vamvakas et al. (1998)	Cases who underwent nephrectomy in 1987–1992 in a hospital in Arnsberg region of Germany; controls selected accident wards from nearby hospital in 1992.	58 cases. 84 controls. Cases, 83%; controls, 75%.	In-person interview with case or next-of-kin; questionnaire assessing occupational history using job title or self-reported exposure to assign TCE and perchloroethylene exposure. Logistic regression with covariates for age, smoking, BMI, hypertension, and diuretic intake.
Multiple or other sites			
Lee et al. (2003)	Liver, lung, stomach, colorectal cancer deaths in males and females between 1966 and 1997 from two villages in Taiwan; controls were cardiovascular and cerebral-vascular disease deaths from same underlying area as cases.	53 liver, 39 stomach, 26 colorectal, 41 lung cancer cases. 286 controls. Response rate not reported.	Residence as recorded on death certificate. Mantel-Haenszel stratified by age, sex, and time period.
Siemiatycki (1991)	Male cancer cases, 1979–1985, 35–75 yrs, diagnosed in 16 Montreal-area hospitals, histologically confirmed; cancer controls identified concurrently; age-matched, population-based controls identified from electoral lists and random digit dialing.	857 lung and 117 pancreatic cancer cases. 533 population controls (Group 1) and other cancer cases from same study (Group 2). Cases, 79% (lung), 71% (pancreas); controls, 72%.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); TCE defined as any or substantial exposure. Mantel-Haenszel stratified on age, income, index for cigarette smoking, ethnic origin, and respondent status (lung cancer) and age, income, index for cigarette smoking, and respondent status (pancreatic cancer).

Bolded study(ies) carried forward for consideration in dose-response assessment (see Chapter 5).

BMI = body mass index; CLL = chronic lymphocytic leukemia; HCFA = Health Care Financing Administration; JTEM = job-task exposure matrix; MM = multiple myeloma; NCI = National Cancer Institute; NHL = non-Hodgkin lymphoma; OR = odds ratio; UV = ultra-violet

Table 4-3. Geographic-based studies assessing cancer and TCE exposure

Reference	Description	Analysis approach	Exposure assessment
Broome County, New York studies			
ATSDR (2006a), (2008b)	Total, 22 site-specific, and childhood cancer incidence from 1980 to 2001 among residents in two areas in Endicott, New York.	SIR among all subjects (ATSDR, 2006a) or among white subjects only (ATSDR, 2008b) with expected numbers of cancers derived using age-specific cancer incidence rates for New York State, excluding New York City. Limited assessment of smoking and occupation using medical and other records in lung and kidney cancer subjects (ATSDR, 2008b).	Two study areas, Eastern and Western study areas, identified based on potential for soil vapor intrusion exposures as defined by the extent of likely soil vapor contamination. Contour lines of modeled VOC soil vapor contamination levels based on exposure model using GIS mapping and soil vapor sampling results taken in 2003. The study areas were defined by 2000 Census block boundaries to conform to model predicted areas of soil vapor contamination. TCE was the most commonly found contaminant in indoor air in Eastern study area at levels ranging from 0.18 to 140 µg/m^3, with tetrachloroethylene, cis-1,2-dichloroethene, 1,1,1-trichloroethane, 1,1-DCE, 1,1-dichloroethane, and Freon 113 detected at lower levels. Perchloroethylene was most common contaminant in indoor air in Western study area with other VOCs detected at lower levels.
Maricopa County, Arizona studies			
Aickin et al. (1992); Aickin (2004)	Cancer deaths, including leukemia, 1966–1986, and childhood (≤19 yrs old) leukemia incident cases (1965–1986), Maricopa County, Arizona.	Standardized mortality rate ratio from Poisson regression modeling. Childhood leukemia incidence data evaluated using Bayes methods and Poisson regression modeling.	Location of residency in Maricopa County, Arizona, at the time of death as surrogate for exposure. Some analyses examined residency in West Central Phoenix and cancer. Exposure information is limited to TCE concentration in two drinking water wells in 1982.
Pima County, Arizona studies			
ADHS (1995, 1990)	Cancer incidence in children (≤19 yrs old) and testicular cancer in 1970–1986 and 1987–1991, Pima County, Arizona.	Standardized incidence RR from Poisson regression modeling using method of Aickin et al. (Aickin et al., 1992). Analysis compares incidence in Tucson Airport Area to rate for rest of Pima County.	Location of residency in Pima, County, Arizona, at the time of diagnosis or death as surrogate for exposure. Exposure information is limited to monitoring since 1981 and include VOCs in soil gas samples (TCE, perchloroethylene, 1,1-DCE, 1,1,1-trichloroacetic acid); PCBs in soil samples, and TCE in municipal water supply wells.

Table 4-3. Geographic-based studies assessing cancer and TCE exposure (continued)

Reference	Description	Analysis approach	Exposure assessment
Other			
Coyle et al. (2005)	Incident breast cancer cases among men and women, 1995–2000, reported to Texas Cancer Registry.	Correlation study using rank order statistics of mean average annual breast cancer rate among women and men and atmospheric release of 12 hazardous air pollutants.	Reporting to EPA Toxic Release Inventory the number of pounds released for 12 hazardous air pollutants, (carbon tetrachloride, formaldehyde, methylene chloride, styrene, tetrachloroethylene, TCE, arsenic, cadmium, chromium, cobalt, copper, and nickel).
Morgan and Cassady (2002)	Incident cancer cases, 1988–1989, among residents of 13 census tracts in Redlands area, San Bernardino County, California.	SIR for all cancer sites and 16 site-specific cancers; expected numbers using incidence rates of site-specific cancer of a four-county region between 1988 and 1992.	TCE and perchlorate detected in some county wells; no information on location of wells to residents, distribution of contaminated water, or TCE exposure potential to individual residents in studied census tracts.
Vartiainen et al. (1993)	Total cancer and site-specific cancer cases (lymphoma sites and liver) from 1953 to 1991 in two Finnish municipalities.	SIR with expected number of cancers and site-specific cancers derived from incidence of the Finnish population.	Monitoring data from 1992 indicated presence of TCE, tetrachloroethylene and 1,1,1,-trichloroethane in drinking water supplies in largest towns in municipalities. Residence in town used to infer exposure to TCE.
Cohn et al. (1994b); Fagliano et al. (1990)	Incident leukemia and NHL cases, 1979–1987, from 75 municipalities and identified from the New Jersey State Cancer Registry. Histological type classified using WHO scheme and the classification of NIH Working Formulation Group for grading NHL.	Logistic regression modeling adjusted for age.	Monitoring data from 1984 to 1985 on TCE, trihalomethanes, and VOCs concentrations in public water supplies, and historical monitoring data conducted in 1978–1984.
Mallin (1990)	Incident bladder cancer cases and deaths, 1978–1985, among residents of nine northwestern Illinois counties.	SIR and SMR by county of residence and zip code; expected numbers of bladder cancers using age-race-sex specific incidence rates from SEER or bladder cancer mortality rates of the U.S. population from 1978 to 1985.	Exposure data are lacking for the study population with the exception of noting one of two zip code areas with observed elevated bladder cancer rates also had groundwater supplies contaminated with TCE, perchloroethylene, and other solvents.
Isacson et al. (1985)	Incident bladder, breast, prostate, colon, lung and rectal cancer cases reported to Iowa cancer registry between 1969 and 1981.	Age-adjusted site-specific cancer incidence in Iowa towns with populations of 1,000–10,000 and who were serviced by a public drinking water supply.	Monitoring data of drinking water at treatment plant in each Iowa municipality with populations of 1,000–10,000 used to infer TCE and other VOC concentrations in finished drinking water supplies.

GIS = geographic information system; NIH = National Institutes of Health; PCB = polychlorinated biphenyl; SEER = Surveillance, Epidemiology, and End Results; WHO = World Health Organization

Table 4-4. Standards of epidemiologic study design and analysis use for identifying cancer hazard and TCE exposure

Category A: STUDY DESIGN
Clear articulation of study objectives or hypothesis. The ideal is a clearly stated hypothesis or study objectives and the study is designed to achieve the identified objectives.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls (case-control studies) is adequate. The ideal is for selection of cohort and referents from the same underlying population and differences between these groups to be due to TCE exposure or level of TCE exposure and not to physiological, health status, or lifestyle factors. Controls or referents are assumed to lack or to have background exposure to TCE. These factors may lead to a downward bias including one of which is known as ―healthy worker bias," often introduced in analyses when mortality or incidence rates from a large population such as the U.S. population are used to derive expected numbers of events. The ideal in case-control studies is cases and controls are derived from the same population and are representative of all cases and controls in that population. Any differences between controls and cases are due to exposure to TCE itself and not to confounding factors related to both TCE exposure and disease. Additionally, the ideal is for controls to be free of any disease related to TCE exposure. In this latter case, potential bias is toward the null hypothesis.
Category B: ENDPOINT MEASURED
Levels of health outcome assessed. Three levels of health outcomes are considered in assessing the human health risks associated with exposure to TCE: biomarkers of effects and susceptibility, morbidity, and mortality. Both morbidity, as enumerated by incidence, and mortality, as identified from death certificates, are useful indicators in risk assessment for hazard identification. The ideal is for accurate and predictive indicator of disease. Incidence rates are generally considered to provide an accurate indication of disease in a population and cancer incidence is generally enumerated with a high degree of accuracy in cancer registries. Death certifications are readily available and have complete national coverage but diagnostic accuracy is reduced and can vary by specific diagnosis. Furthermore, diagnostic inaccuracies can contribute to death certificates as a poor surrogate for disease incidence. Incidence, when obtained from population-based cancer registries, is preferred for identifying cancer hazards.
Changes in diagnostic coding systems for lymphoma, particularly NHL. Classification of lymphomas today is based on morphologic, immunophenotypic, genotypic, and clinical features using the WHO classification, introduced in 2001, and incorporation of WHO terminology into International Classification of Disease (ICD)-0-3. ICD Versions 7 and earlier had rubrics for general types of lymphatic and hematopoietic cancer, but no categories for distinguishing specific types of cancers, such as acute leukemia. Epidemiologic studies based on causes of deaths as coded using these older ICD classifications typically grouped together lymphatic neoplasms instead of examining individual types of cancer or specific cell types. Before the use of immunophenotyping, these grouping of ambiguous diseases such as NHL and Hodgkin lymphoma may be have misclassified. With the introduction of ICD-10 in 1990, lymphatic tumors coding, starting in 1994 with the introduction of the Revised European-American Lymphoma classification, the basis of the current WHO classification, was more similar to that presently used. Misclassification of specific types of cancer, if unrelated to exposure, would have attenuated estimate of RR and reduced statistical power to detect associations. When the outcome was mortality, rather than incidence, misclassification would be greater because of the errors in the coding of underlying causes of death on death certificates (IOM, 2003). Older studies that combined all lymphatic and hematopoietic neoplasms must be interpreted with care.

Table 4-4. Standards of epidemiologic study design and analysis use for identifying cancer hazard and TCE exposure (continued)

Category C: TCE-EXPOSURE CRITERIA
Adequate characterization of exposure. The ideal is for TCE exposure potential known for each subject and quantitative assessment (job-exposure-matrix approach) of TCE exposure assessment for each subject as a function of job title, year exposed, duration, and intensity. The assessment approach is accurate for assigning TCE intensity (TCE concentration or a TWA) to individual study subjects and estimates of TCE intensity are validated using monitoring data from the time period. For the purpose of this report, the objective for cohort and case-controls studies is to differentiate TCE-exposed subjects from subjects with little or no TCE exposure. A variety of dose-metrics may be used to quantify or classify exposures for an epidemiologic study. They include precise summaries of quantitative exposure, concentrations of biomarkers, cumulative exposure, and simple qualitative assessments of whether exposure occurred (yes or no). Each method has implicit assumptions and potential problems that may lead to misclassification. Studies in which it was unclear that the study population was actually exposed to TCE are excluded from analysis.
Category D: FOLLOW-UP (COHORT)
Loss to follow-up. The ideal is complete follow-up of all subjects; however, this is not achievable in practice, but it seems reasonable to expect loss to follow-up not to exceed 10%. The bias from loss to follow-up is indeterminate. Random loss may have less effect than if subjects who are not followed have some significant characteristics in common.
Follow-up period allows full latency period for over 50% of the cohort. The ideal to follow all study subjects until death. Short of the ideal, a sufficient follow-up period to allow for cancer induction period or latency over 15 or 20 yrs is desired for a large percentage of cohort subjects.
Category E: INTERVIEW TYPE (CASE-CONTROL)
Interview approach. The ideal interviewing technique is face-to-face by trained interviewers with >90% of interviews with cases and control subjects conduced face-to-face. The effect on the quality of information from other types of data collection is unclear, but telephone interviews and mail-in questionnaires probably increase the rate of misclassification of subject information. The bias is toward the null hypothesis if the proportion of interview by type is the same for case and control, and of indeterminate direction otherwise.
Blinded interviewer. The ideal is for the interviewer to be unaware whether the subject is among the cases or controls and the subject to be unaware of the purpose and intended use of the information collected. Although desirable for case-control studies, blinding is usually not possible to fully accomplish because subject responses during the interview provide clues as to subject status. The potential for bias from face-to-face interviews is probably less than with mail-in interviews. Some studies have assigned exposure status in a blinded manner using a JEM and information collected in the unblinded interview. The potential for bias in this situation is probably less with this approach than for nonblinded assignment of exposure status.

Table 4-4. Standards of epidemiologic study design and analysis use for identifying cancer hazard and TCE exposure (continued)

Category F: PROXY RESPONDENTS
Proxy respondents. The ideal is for data to be supplied by the subject because the subject generally would be expected to be the most reliable source; <10% of either total cases or total controls for case-control studies. A subject may be either deceased or too ill to participate, however, making the use of proxy responses unavoidable if those subjects are to be included in the study. The direction and magnitude of bias from use of proxies is unclear, and may be inconsistent across studies.
Category G: SAMPLE SIZE
The ideal is for the sample size is large enough to provide sufficient statistical power to ensure that any elevation of effect in the exposure group, if present, would be found, and to ensure that the confidence bounds placed on RR estimates can be well characterized.
Category H: ANALYSIS ISSUES
Control for potentially confounding factors of importance in analysis. The ideal in cohort studies is to derive expected numbers of cases based on age-sex- and time-specific cancer rates in the referent population and in case-control studies by matching on age and sex in the design and then adjusting for age in the analysis of data. Age and sex are likely correlated with exposure and are also risk factors for cancer development. Similarly, other factors such as cigarette smoking and alcohol consumption are risk factors for several site-specific cancers reported as associative with TCE exposure. To be a confounder of TCE, exposure to the other factor must be correlated, and the association of the factor with the site-specific cancer must be causal. The expected effect from controlling for confounders is to move the estimated RR estimate closer to the true value.
Statistical methods are appropriate. The ideal is that conclusions are drawn from the application of statistical methods that are appropriate to the problem and accurately interpreted.
Evaluation of exposure-response. The ideal is an examination of a linear exposure-response as assessed with a quantitative exposure metric such as cumulative exposure. Some studies, absent quantitative exposure metrics, examine exposure response relationships using a semiquantitative exposure metric or by duration of exposure. A positive dose-response relationship is usually more convincing of an association as causal than a simple excess of disease using TCE dose-metric. However, a number of reasons have been identified for a lack of linear exposure-response finding and the failure to find such a relationship mean little from an etiological viewpoint.
Documentation of results. The ideal is for analysis observations to be completely and clearly documented and discussed in the published paper, or provided in supplementary materials accompanying publication.

Twenty-four of the studies identified in a systematic review were selected for inclusion in the meta-analysis through use of the following meta-analysis inclusion criteria: (1) cohort or0020case-control designs; (2) evaluation of incidence or mortality; (3) adequate selection in cohort studies of exposure and control groups and of cases and controls in case-control studies; (4) TCE exposure potential inferred to each subject and quantitative assessment of TCE exposure assessment for each subject by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, job-exposure matrices (JEMs), water distribution models, or obtained from subjects using questionnaire (case-control studies); and (5) relative risk (RR) estimates for kidney cancer, liver cancer, or non-Hodgkin lymphoma (NHL) adjusted, at minimum, for possible confounding of age, sex, and race (see Table 4-5). This evaluation is summarized below, separately for cohort and case-control studies. Appendix C contains a full discussion of the meta-analysis, its analytical methodology, including sensitivity analyses, and findings. The meta-analysis focuses on kidney cancer, liver cancer, and NHL, as most studies reported RRs for these sites. Fewer numbers of studies reported RRs for other site-specific cancers and TCE exposure and examination of these site-specific cancers and TCE exposure using meta-analysis was not attempted.

Table 4-5. Summary of criteria for meta-analysis study selection

Decision outcome	Studies	Primary reason(s)
Studies recommended for meta-analysis:		
	Axelson et al. (1994); Greenland et al. (1994); Hardell et al. (1994); Siemiatycki (1991); Anttila et al. (1995); Morgan et al. (1998); Nordstrom et al. (1998); Boice et al. (1999); Boice et al. (2006b); Dosemeci et al. (1999); Persson and Fredriksson (1999); Pesch et al. (2000b); Hansen et al. (2001); Brüning et al. (2003); Raaschou-Nielsen et al. (2003); Zhao et al. (2005); Miligi et al. (2006); Charbotel et al. (2006); Blair et al. (1998); its follow-up Radican et al. (2008); Wang et al. (2009); Cocco et al. (2010); Moore et al. (2010); Purdue et al. (2011)	Analytical study designs of cohort or case-control; evaluation of incidence or mortality; adequate selection in cohort studies of exposure and control groups and of cases and controls in case-control studies; TCE exposure potential inferred to each subject and quantitative assessment of TCE exposure assessment for each subject by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, JEMs, water distribution models, or obtained from subjects using questionnaire (case-control studies); RR estimates for kidney cancer, liver cancer, or NHL adjusted, at minimum, for possible confounding of relevant risk factors (e.g., age, sex, and race).

Table 4-5. Summary of criteria for meta-analysis study selection (continued)

Decision outcome	Studies	Primary reason(s)
Studies not recommended for meta-analysis:		
	Clapp and Hoffman (2008); ATSDR (2004a; Cohn et al., 1994b)	Weakness with respect to analytical study design (i.e., geographic-based, ecological or PMR design).
	Garabrant et al. (1988); Isacson et al.(1985); Shindell and Ulrich (1985); Wilcosky et al. (1984); Shannon et al. (1988); Blair et al. (1989); Costa et al. (1989); (ADHS, 1995, 1990); Mallin (1990); Aickin et al. (1992); Sinks et al. (1992); Vartiainen et al. (1993); Morgan and Cassady (2002); Lee et al. (2003); Aickin (2004); Chang et al. (2005; Chang et al., 2003); Coyle et al. (2005); ATSDR (2006a); ATSDR (2008b); Sung et al. (2008; 2007)	TCE exposure potential not assigned to individual subjects using JEM, individual biomarkers, water distribution models, or industrial hygiene data from other process indicating a high probability of TCE use (cohort studies).
	Lowengart et al. (1987); Fredriksson et al. (1989); McKinney et al. (1991); Heineman et al. (1994); Siemiatycki et al. (1994); Aronson et al. (1996); Fritschi and Siemiatycki (1996b); Dumas et al. (2000); Kernan et al. (1999); Shu et al. (2004; 1999); Parent et al. (2000b); Pesch et al. (2000a); DeRoos et al. (2001); Goldberg et al. (2001); Costas et al. (2002); Krishnadasan et al. (2007) Costantini et al. (2008); Gold et al. (2011)	Cancer incidence or mortality reported for cancers other than kidney, liver, or NHL.
	Ritz (1999a)	Subjects monitored for radiation exposure with likelihood for potential confounding; cancer mortality and TCE exposure not reported for kidney cancer and all hemato- and lymphopoietic cancer reported as broad category.
	Henschler et al. (1995)	Incomplete identification of cohort and index kidney cancer cases included in case series.

The cohort studies (Clapp and Hoffman, 2008; Radican et al., 2008; Sung et al., 2008; Krishnadasan et al., 2007; Sung et al., 2007; Boice et al., 2006b; Chang et al., 2005; Zhao et al., 2005; ATSDR, 2004a; Chang et al., 2003; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Henschler et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Sinks et al., 1992; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Shannon et al., 1988; Shindell and Ulrich, 1985; Wilcosky et al., 1984) (see Table 4-1), with data on the incidence or morality of site-specific cancer in relation to TCE exposure, range in size 803 (Hansen et al., 2001) to 86,868 (Chang et al., 2005; Chang et al., 2003), and were conducted in Denmark, Sweden, Finland, Germany, Taiwan, and the United States (see Table 4-1). Three case-control studies nested within cohorts (Krishnadasan et al., 2007; Greenland et al., 1994; Wilcosky et al., 1984) are considered as cohort studies because the summary risk estimate from a nested case-control study, the odds ratio (OR), was estimated from incidence density sampling. This is considered an unbiased estimate of the hazard ratio, similar to a RR estimate from a cohort study, if, as is the

case for these studies, controls are selected from the same source population as the cases, the sampling rate is independent of exposure status, and the selection probability is proportional to time-at-risk (IOM, 2003). Cohort and nested case-control study designs are analytical epidemiologic studies and are generally relied on for identifying a causal association between human exposure and adverse health effects (U.S. EPA, 2005b).

While all of these cohort studies are considered in the overall weight of evidence, 11 of them met all meta-analysis inclusion criteria: the cohorts of Blair et al. (1998) and its follow-up by Radican et al. (2008); Morgan et al. (1998), Boice et al. (Boice et al., 2006b; 1999), and Zhao et al.(2005), of aerospace workers or aircraft mechanics; and Axelson et al. (1994), Anttila et al. (1995), Hansen et al. (2001), and Raaschou-Nielsen et al. (2003) of Nordic workers in multiple industries with TCE exposure; and Greenland et al. (1994) of electrical manufacturing workers. Subjects or cases and controls in these studies are considered to sufficiently represent the underlying population, and the bias associated with selection of referent populations is considered minimal. The exposure-assessment approaches included detailed JEM, biomonitoring data, or use of industrial hygiene data on TCE exposure patterns and factors that affect such exposure, with high probability of TCE exposure potential to individual subjects. The statistical analyses methods were appropriate and well documented, the measured endpoint was an accurate indicator of disease, and the follow-up was sufficient for cancer latency. These studies are also considered as strong studies for identifying kidney, liver, and NHL cancer hazard. The remaining cohort studies less satisfactorily meet identified criteria or standards of epidemiologic design and analysis, having deficiencies in multiple criteria (Clapp and Hoffman, 2008; Sung et al., 2008; Sung et al., 2007; Chang et al., 2005; ATSDR, 2004a; Chang et al., 2003; Ritz, 1999a; Henschler et al., 1995; Sinks et al., 1992; Costa et al., 1989; Garabrant et al., 1988; Shindell and Ulrich, 1985; Wilcosky et al., 1984). Krishnandansen et al. (2007), who reported on prostate cancer, met four of the five meta-analysis inclusion criteria except that for reporting an RR estimate cancer of the kidney, liver, or NHL, the site-specific cancers examined using meta-analysis.

The case-control studies on TCE exposure are of several site-specific cancers, including bladder (Pesch et al., 2000a; Siemiatycki et al., 1994; Siemiatycki, 1991); brain (De Roos et al., 2001; Heineman et al., 1994); childhood lymphoma or leukemia (Shu et al., 2004; Costas et al., 2002; Shu et al., 1999; McKinney et al., 1991; Lowengart et al., 1987); colon cancer (Goldberg et al., 2001; Siemiatycki, 1991); esophageal cancer (Parent et al., 2000b; Siemiatycki, 1991); liver cancer (Lee et al., 2003); lung cancer (Siemiatycki, 1991); adult lymphoma or leukemia (Hardell et al., 1994) [NHL, Hodgkin lymphoma]; (Fritschi and Siemiatycki, 1996a; Siemiatycki, 1991) [NHL]; (Nordström et al., 1998) [hairy cell leukemia]; (Persson and Fredrikson, 1999) [NHL]; (Miligi et al., 2006) [NHL and chronic lymphocytic leukemia (CLL)]; (Seidler et al., 2007) [NHL, Hodgkin lymphoma and subjects included in (Cocco et al., 2010; Costantini et al., 2008) [leukemia types, CLL included with NHL] (Wang et al., 2009; Miligi et al., 2006) [NHL];

(Cocco et al., 2010) [B-cell including CLL and multiple myeloma, T-cell, and Hodgkin lymphomas]; (Purdue et al., 2011) [NHL]; Gold et al. (2011) [multiple myeloma]); melanoma (Fritschi and Siemiatycki, 1996b; Siemiatycki, 1991); rectal cancer (Dumas et al., 2000; Siemiatycki, 1991); renal cell carcinoma (RCC), a form of kidney cancer (Moore et al., 2010; Charbotel et al., 2006; Brüning et al., 2003; Parent et al., 2000a; Pesch et al., 2000b; Dosemeci et al., 1999; Vamvakas et al., 1998; Siemiatycki, 1991); pancreatic cancer (Siemiatycki, 1991); and prostate cancer (Aronson et al., 1996; Siemiatycki, 1991) (see Table 4-2). No case-control studies of reproductive cancers (breast or cervix) and TCE exposure were found in the peer-reviewed literature.

While all of these case-control studies are considered in the overall weight of evidence, 13 of them met the meta-analysis inclusion criteria identified in Section B.2.9 (Purdue et al., 2011; Cocco et al., 2010; Moore et al., 2010; Wang et al., 2009; Charbotel et al., 2006; Miligi et al., 2006; Brüning et al., 2003; Pesch et al., 2000b; Dosemeci et al., 1999; Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994; Siemiatycki, 1991). They were of analytical study design, cases and controls were considered to represent underlying populations and selected with minimal potential for bias; exposure assessment approaches included assignment of TCE exposure potential to individual subjects using information obtained from face-to-face, mailed, or telephone interviews; analyses methods were appropriate, well-documented, included adjustment for potential confounding exposures, with RR estimates and associated CIs reported for kidney cancer, liver cancer or NHL.

These studies were also considered, to varying degrees, as strong studies for weight-of evidence characterization of hazard. Both Brüning et al. (2003) and Charbotel et al. (2006) had a priori hypotheses for examining RCC and TCE exposure. Strengths of both studies are in their examination of populations with potential for high exposure intensity and in areas with high frequency of TCE usage and their assessment of TCE potential. An important feature of the exposure assessment approach of Charbotel et al. (2006) is their use of a large number of studies on biological monitoring of workers in the screw-cutting industry, a predominant industry with documented TCE exposures, as support. Charbotel et al. (2006) is preferred to Charbotel et al. (2009), who examined kidney cancer risk and TCE exposure at the existing French occupational exposure limit for cases and controls from their earlier publication (Charbotel et al., 2009); the earlier publication contained more extensive analyses and included exposure-response analyses using several exposure metrics and multiple exposure categories. Other studies were either large multiple-center studies (Purdue et al., 2011; Cocco et al., 2010; Moore et al., 2010; Wang et al., 2009; Miligi et al., 2006; Pesch et al., 2000b) or reporting from one location of a larger international study (Seidler et al., 2007; Dosemeci et al., 1999). Cocco et al. (2010) includes subjects in Seidler et al. (2007) and is preferred because of the larger number of subjects from four other European countries. In contrast to Brüning et al. (2003) and Charbotel et al. (2006), two studies conducted in geographical areas with widespread TCE usage and potential for

exposure to higher intensity; in these other studies, a lower exposure prevalence to TCE is found [any TCE exposure: 15% of cases (Dosemeci et al., 1999); 6% of cases (Miligi et al., 2006); 13% of cases (Wang et al., 2009); 4% of cases (Cocco et al., 2010)], and most subjects were identified as exposed to TCE probably had minimal contact (3% of cases with moderate/high TCE exposure (Miligi et al., 2006); 2% of cases with high intensity, but of low probability of TCE exposure (Wang et al., 2009). This pattern of lower exposure prevalence and intensity is common to community-based, population case-control studies (Teschke et al., 2002).

Fourteen case-control studies did not meet specific meta-analysis inclusion criterion (Gold et al., 2011; Shu et al., 2004; Lee et al., 2003; Costas et al., 2002; Goldberg et al., 2001; Dumas et al., 2000; Parent et al., 2000b; Pesch et al., 2000a; Kernan et al., 1999; Shu et al., 1999; Vamvakas et al., 1998; Aronson et al., 1996; Fritschi and Siemiatycki, 1996b; Siemiatycki et al., 1994). Twelve studies reported RR estimates for site-specific cancers other than kidney, liver, and NHL (Gold et al., 2011; Shu et al., 2004; Costas et al., 2002; Goldberg et al., 2001; Dumas et al., 2000; Parent et al., 2000b; Pesch et al., 2000a; Kernan et al., 1999; Shu et al., 1999; Aronson et al., 1996; Fritschi and Siemiatycki, 1996b; Siemiatycki et al., 1994). Vamvakas et al. (1998) has been the subject of considerable controversy (Cherrie et al., 2001; Mandel, 2001; Green and Lash, 1999; McLaughlin and Blot, 1997; Bloemen and Tomenson, 1995; Swaen, 1995) with questions raised on the potential for selection bias related to the study's controls. This study was deficient in the criterion for adequacy of case and control selection. Brüning et al. (2003), a study from the same region as Vamvakas et al. (1998), is considered a stronger study for identifying cancer hazard since it addresses many of the deficiencies of Vamvakas et al. (1998) Lee et al. (2003), in their study of hepatocellular cancer, assigns one level of exposure to all subjects in a geographic area, an inherent measurement error and misclassification bias because not all subjects are exposed uniformly. Additionally, statistical analyses in this study did not control for hepatitis viral infection, a known risk factor for hepatocellular cancer and of high prevalence in the study area.

The geographic-based studies (ATSDR, 2008b, 2006a; Aickin, 2004; Morgan and Cassady, 2002; ADHS, 1995; Cohn et al., 1994b; Vartiainen et al., 1993; Aickin et al., 1992; ADHS, 1990; Mallin, 1990; Isacson et al., 1985) with data on cancer incidence are correlation studies to examine cancer outcomes of residents in communities with TCE and other chemicals detected in groundwater wells or in municipal drinking water supplies (see Table 4-3). These studies did not meet all five meta-analysis inclusion criteria. The geographic-base studies are not of analytical designs such as cohort and case-control designs. Another deficiency in all studies is their low level of detail to individual subjects for TCE. One level of exposure to all subjects in a geographic area is assigned without consideration of water distribution networks, which may influence TCE concentrations delivered to a home, or a subject's ingestion rate to estimate TCE exposure to individual study subjects. Some inherent measurement error and misclassification bias is likely in these studies because not all subjects are exposed uniformly. Additionally, in

contrast to case-control studies, the geographic-based studies, including the Agency for Toxic Substances and Disease Registry (ATSDR, 2008b), had limited accounting for other potential risk factors. These studies are of low sensitivity for weight-of evidence characterization of hazard compared to other cohort and case-control studies.

4.2. GENETIC TOXICITY

This section discusses the genotoxic potential of TCE and its metabolites. A summary is provided at the end of each section for TCE or its metabolite for their mutagenic potential in addition to an overall synthesis summary at the end of the genotoxicity section. The liver and kidney are subjects of study for the genotoxic potential of TCE and its metabolites, and are discussed more in-depth in Sections 4.4.3, 4.4.7, 4.5.6.2.7, 4.5.7, E.2.3, and E.2.4.

The application of genotoxicity data to predict potential carcinogenicity is based on the principle that genetic alterations are found in all cancers. Genotoxicity is the ability of chemicals to alter the genetic material in a manner that permits changes to be transmitted during cell division. Although most tests for mutagenicity detect changes in DNA or chromosomes, some specific modifications of the epigenome including proteins associated with DNA or RNA, can also cause transmissible changes. Changes that occur due to the modifications in the epigenome are discussed in endpoint-specific Sections 4.3–4.9 as well as Sections E.3.1–E.3.4.

Genetic alterations can occur through a variety of mechanisms including gene mutations, insertions, deletions, translocations, or amplification; evidence of mutagenesis provides mechanistic support for the inference of potential for carcinogenicity in humans.

Evaluation of genotoxicity data entails a weight-of-evidence approach that includes consideration of the various types of genetic damage that can occur. In acknowledging that genotoxicity tests are by design complementary evaluations of different mechanisms of genotoxicity, a recent International Programme on Chemical Safety (IPCS) publication (Eastmond et al., 2009) notes that "multiple negative results may not be sufficient to remove concern for mutagenicity raised by a clear positive result in a single mutagenicity assay." These considerations inform the present approach. In addition, consistent with U.S. EPA's *Guidelines on Carcinogenic Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005c, b), the approach does not address relative potency (e.g., among TCE metabolites, or of such metabolites with other known genotoxic carcinogens) per se, nor does it consider quantitative issues related to the probable production of these metabolites in vivo. Instead, the analysis of genetic toxicity data presented here focuses on the identification of a genotoxic hazard of these metabolites; a quantitative analysis of TCE metabolism to reactive intermediates, via PBPK modeling, is presented in Section 3.5.

TCE and its known metabolites, TCA, DCA, CH, TCOH, DCVC, and DCVG, have been studied to varying degrees for their genotoxic potential. The following section summarizes

available data on genotoxicity for both TCE and its metabolites for each potential genotoxic endpoints, when available, in different organisms.

4.2.1. TCE

4.2.1.1. DNA Binding Studies

Covalent binding of TCE to DNA and protein in cell-free systems has been studied by several investigators. Incubation of [^{14}C]-TCE with salmon sperm DNA in the presence of microsomal preparations from B6C3F$_1$ mice resulted in dose-related covalent binding of TCE to DNA. The binding was enhanced when the microsomes were taken from mice pretreated with phenobarbital, which induces CYP enzymes, suggesting that the binding may be related to an oxidative metabolite, or when 1,2-epoxy-3,3,3-trichloropropane, an inhibitor of epoxide hydrolase, was added to the incubations (Banerjee and Van Duuren, 1978). In addition, covalent binding of [^{14}C]-TCE with microsomal proteins was detected after incubation with microsomal preparations from mouse lung, liver, stomach, and kidney, and rat liver (Banerjee and Van Duuren, 1978). Furthermore, incubation of [^{14}C]-TCE with calf thymus DNA in the presence of hepatic microsomes from phenobarbital-pretreated rats yielded significant covalent binding (DiRenzo et al., 1982).

A number of studies have also examined the role of TCE metabolism in covalent binding to DNA and proteins. Miller and Guengerich (1983) used liver microsomes from control, b-naphthoflavone- and phenobarbital-induced B6C3F$_1$ mice, Osborne-Mendel rats, and human liver microsomes. Significant covalent binding of TCE metabolites to calf thymus DNA and proteins was observed in all experiments. Phenobarbital treatment increased the formation of chloral and TCE oxide formation, DNA, and protein adducts. In contrast, b-naphthoflavone treatment did not induce the formation of any microsomal metabolite, suggesting that the forms of CYP induced by phenobarbital are primarily involved in TCE metabolism while the b-naphthoflavone-inducible forms of CYP have only a minor role in TCE metabolism. TCE metabolism (based on TCE-epoxide and DNA-adduct formation) was 2.5–3-fold higher in mouse than in rat microsomes due to differences in rates and clearance of metabolism (discussed in Section 3.3.3.1). The levels of DNA and protein adducts formed in human liver microsomal system approximated those observed in liver microsomes prepared from untreated rats. It was also shown that whole hepatocytes of both untreated mice and phenobarbital-induced rats and mice could activate TCE into metabolites able to covalently bind extracellular DNA. A study by Cai and Guengerich (2001a) postulates that TCE oxide (an intermediate in the oxidative metabolism of TCE in rat and mouse liver microsomes) is responsible for the covalent binding of TCE with protein, and to a lesser extent, DNA. Mass spectrometry was used to analyze the reaction of TCE oxide (synthesized by m-chloroperbenzoic acid treatment of TCE) with nucleosides, oligonucleotides, and protein to understand the transient nature of the inhibition of enzymes in the context of adduct formation. Protein amino acid adducts were observed during

the reaction of TCE oxide with the model peptides. The majority of these adducts were unstable under physiological conditions. Results using other peptides also indicate that adducts formed from the reaction of TCE oxide with macromolecules and their biological effects are likely to be relatively short-lived.

Studies have been conducted using in vitro and in vivo systems to understand the DNA and protein binding capacity of TCE. In a study in male mice, after repeated i.p. injections of [^{14}C]-TCE, radioactivity was detected in the DNA and RNA of all organs studied (kidney, liver, lung, spleen, pancreas, brain, and testis) (Bergman, 1983). However, in vivo labeling was shown to be due to metabolic incorporation of C1 fragments, particularly in guanine and adenine, rather than to DNA-adduct formation. In another study (Stott et al., 1982), following i.p. injection of [^{14}C]-TCE in male Sprague-Dawley rats (10–100 mg/kg) and B6C3F$_1$ mice (10–250 mg/kg), high liver protein labeling was observed while very low DNA labeling was detected. Stott et al. (1982) also observed very low levels of DNA binding (0.62 ± 0.43 alkylation/10^6 nucleotides) in mice administered 1,200 mg/kg of TCE. In addition, a dose-dependent binding of TCE to hepatic DNA and protein at low doses in mice was demonstrated by Kautiainen et al. (1997). In their dose-response study (doses between 2 µg/kg and 200 mg/kg body weight), the highest level of protein binding (2.4 ng/g protein) was observed 1 hour after the treatment followed by a rapid decline, indicating pronounced instability of the adducts and/or rapid turnover of liver proteins. Highest binding of DNA (120 pg/g DNA) was found between 24 and 72 hours following treatment. Dose-response curves were linear for both protein and DNA binding. In this study, the data suggest that TCE does bind to DNA and proteins in a dose-dependent fashion; however, the type and structure of adducts were not determined.

Mazzullo et al. (1992) reported that TCE was covalently bound in vivo to DNA, RNA, and proteins of rat and mouse organs 22 hours after i.p. injection. Labeling of proteins from various organs of both species was higher than that of DNA. Bioactivation of TCE to its intermediates using various microsomal fractions was dependent on CYP enzyme induction and the capacity of these intermediates to bind to DNA. It appeared that mouse lung microsomes were more efficient in forming the intermediates than rat lung microsomes, although no other species specific differences were found (Mazzullo et al., 1992). This also supports the results described by Miller and Guengerich (1983). The authors suggest some binding ability of TCE to interact covalently with DNA (Mazzullo et al., 1992).

In summary, studies report that TCE exposure in vivo can lead to binding to nucleic acids and proteins, and some authors have suggested that such binding is likely due to conversion to one or more reactive metabolites.

4.2.1.2. Bacterial Systems—Gene Mutations

Gene mutation studies (Ames assay) in various *Salmonella typhimurium* strains of bacteria exposed to TCE both in the presence and absence of stabilizing agent have been

conducted by different laboratories (McGregor et al., 1989; Mortelmans et al., 1986; Shimada et al., 1985; Crebelli et al., 1982; Baden et al., 1979; Waskell, 1978; Henschler et al., 1977; Simmon et al., 1977) (see Table 4-6). It should be noted that these studies have tested TCE samples of different purities using various experimental protocols. In all in vitro assays, volatilization is a concern when TCE is directly administered.

Waskell (1978) studied the mutagenicity of several anesthetics and their metabolites. Included in their study was TCE (and its metabolites) using the Ames assay. The study was conducted both in the presence and absence of a metabolic activation system, S9, and caution was exercised to perform the experiment under proper conditions (incubation of reaction mixture in sealed dessicator vials). This study was performed in both TA98 and TA100 *S. typhimurium* strains at a dose range of 0.5–10% between 4 and 48 hours. No change in revertant colonies was observed in any of the doses or time courses tested. No information either on the presence or absence of stabilizers in TCE obtained commercially nor its effect on cytotoxicity was provided in the study.

In other studies, highly purified, epoxide-free TCE samples were not mutagenic in experiments with and without exogenous metabolic activation by S9 in *S. typhimurium* strain TA100 using the plate incorporation assay (Henschler et al., 1977). Furthermore, no mutagenic activity was found in several other strains including TA1535, TA1537, TA97, TA98, and TA100 using the preincubation protocol (Mortelmans et al., 1986). Simmon et al. (1977) observed a less than twofold but reproducible and dose-related increase in *his* + revertants in plates inoculated with *S. typhimurium* TA100 and exposed to a purified, epoxide-free TCE sample. The authors observed no mutagenic response in strain TA1535 with S9 mix and in either TA1535 or TA100 without rat or mouse liver S9. Similar results were obtained by Baden et al. (1979), Bartsch et al. (1979), and Crebelli et al. (1982). In all of these studies, purified, epoxide-free TCE samples induced slight but reproducible and dose-related increases in *his* + revertants in *S. typhimurium* TA100 only in the presence of S9. No mutagenic activity was detected without exogenous metabolic activation or when liver S9 from naïve rats, mice, and hamsters (Crebelli et al., 1982) was used for activation. Therefore, a number of these studies showed positive results in TA100 with metabolic activation, but not in other strains or without metabolic activation.

Table 4-6. TCE genotoxicity: bacterial assays

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
S. typhimurium (TA100)	0.1–10 µL (epoxide-free)	–	–	Plate incorporation assay	Henschler et al. (1977)
S. typhimurium (TA1535, TA100)	1–2.5% (epoxide-free)	+ (TA100) – (TA1535)			Simmon et al. (1977)
S. typhimurium (TA98, TA100)	0.5–10%	–	–	The study was conducted in sealed dessicator vials	Waskell (1978)
S. typhimurium (TA100, TA1535)	1–3% (epoxide-free)	+ (TA100) ± (TA1535)	–		Baden et al. (1979)
S. typhimurium (TA100)	5–20% (v/v)	–	–	Negative under normal conditions, but twofold increase in mutations in a preincubation assay	Bartsch et al. (1979)
	0.33–1.33% (epoxide-free)	+	–		Crebelli et al. (1982)
S. typhimurium (TA1535, TA100)	1–5% (higher and lower purity)	– (higher purity) + (lower purity)	–	Extensive cytotoxicity	Shimada et al. (1985)
S. typhimurium (TA98, TA100, TA1535, TA1537, TA97)	10–1,000 µL/plate	–	–	Preincubation protocol	Mortelmans et al. (1986)
S. typhimurium (TA98, TA100, TA1535)	≤10,000 µg/plate (unstabilized)	–	Not determined	Vapor assay	McGregor et al. (1989)
	≤10,000 µg/plate (oxirane-stabilized)	+	+	Vapor assay	McGregor et al. (1989)
S. typhimurium	≤10,000 µg/plate (epoxybutane stabilized)	Not determined	+	Preincubation assay	McGregor et al. (1989)
	≤10,000 µg/plate (epichlorohydrin stabilized)	Not determined	+	Vapor assay	McGregor et al. (1989)
S. typhimurium (YG7108)	1.000–3.000 µg/plate	Not determined	+	Microcolony assay/revertants	Emmert et al. (2006)
Escherichia coli (K12)	0.9 mM (analytical grade)	+	–	Revertants at arg56 but not nad113 or other loci	Greim et al. (1975)

Shimada et al. (1985) tested a low-stabilized, highly purified TCE sample in an Ames reversion test, modified to use vapor exposure, in *S. typhimurium* TA1535 and TA100. No mutagenic activity was observed—either in the presence or absence of S9 mix. However, at the same concentrations (1, 2.5, and 5%), a sample of lower purity, containing undefined stabilizers, was directly mutagenic in TA100 (>5-fold) and TA1535 (>38-fold) at 5% concentration regardless of the presence of S9. It should be noted that the doses used in this study resulted in extensive killing of bacterial population, particularly at 5% concentration; >95% toxicity was observed.

A series of studies evaluating TCE (with and without stabilizers) were conducted by McGregor et al. (1989). The authors tested high-purity and oxirane-stabilized TCE samples for their mutagenic potential in *S. typhimurium* strains TA1535, TA98, and TA100. Preincubation protocol was used to test stabilized TCE (up to 10,000 µg/plate). Mutagenic response was not observed either in the presence or absence of metabolic activation. When TCE was tested in a vapor delivery system without the oxirane stabilizers, the authors did not observe any mutagenic activity. However, TA1535 and TA100 produced a mutagenic response both in the presence and absence of S9 when exposed to TCE containing 0.5–0.6% 1,2-epoxybutane. Furthermore, exposure to epichlorohydrin also increased the frequency of mutants.

Emmert et al. (2006) used a CYP2E1-competent bacterial strain (*S. typhimurium* containing YG7108pin3ERb$_5$ plasmid) in their experiments. TCE was among several other compounds investigated and was tested at concentrations of 1,000–3,000 µg/plate. TCE induced toxicity and microcolonies ≥1,000 µg per plate. A study on *Escherichia coli* K12 strain was conducted by Greim et al. (1975) using analytical-grade TCE samples. Revertants were scored at two loci: arg_{56}, sensitive to base-pair substitution and nad_{113}, reverted by frameshift mutagens. In addition, forward mutations to 5-methyltryptophan resistance and galactose fermentation were selected. Approximately twofold increase in *arg* + colonies was observed. No change in other sites was observed. No definitive conclusion can be drawn from this study due to lack of information on reproducibility and dose-response.

In addition to the above studies, the ability of TCE to induce gene mutations in bacterial strains has been reviewed and summarized by several authors (Clewell and Andersen, 2004; Moore and Harrington-Brock, 2000; Douglas et al., 1999; Fahrig et al., 1995; Crebelli and Carere, 1989). In summary, TCE, in its pure form as a parent compound, is unlikely to induce point mutations in most bacterial strains. It is possible that some mutations observed in response to exposure to technical-grade TCE may be contributed by the contaminants/impurities such as 1,2-epoxybutane and epichlorohydrin, which are known bacterial mutagens. However, several studies of TCE reported low, but positive responses in the TA100 strain in the presence of S9 metabolic activation, even when genotoxic stabilizers were not present.

4.2.1.3. Fungal and Yeast Systems—Gene Mutations, Conversions, and Recombination

Gene mutations, conversions, and recombinations have been studied to identify the effect of TCE in fungi and yeast systems (see Table 4-7).

Crebelli et al. (1985) studied the mutagenicity of TCE in *Aspergillus nidulans* both for gene mutations and mitotic segregation. No increase in mutation frequency was observed when *A. nidulans* was plated on selective medium and then exposed to TCE vapors. A small but statistically significant increase in mutations was observed when conidia of cultures were grown in the presence of TCE vapors and then plated on selective media. Since TCE required actively growing cells to exerts its genotoxic activity and previous studies (Bignami et al., 1980) have shown activity in the induction of *methG1* suppressors by TCOH and CH, it is possible that endogenous metabolic conversion of TCE into TCOH or CH may have been responsible for the positive response.

To understand the CYP mediated genotoxic activity of TCE, Callen et al. (1980) conducted a study in two yeast strains (D7 and D4) CYP. The D7 strain in it log-phase had a CYP concentration up to 5 times higher than a similar cell suspension of D4 strain. Two different concentrations (15 and 22 mM) at two different time points (1 and 4 hours) were studied. A significant increase in frequencies of mitotic gene conversion and recombination was observed at 15 mM concentrations at 1-hour exposure period in the D7 strain; however, the 22 mM concentration was highly cytotoxic (only 0.3% of the total number of colonies survived). No changes were seen in D4 strain, suggesting that metabolic activation via CYP played an important role in both genotoxicity and cytotoxicity. However, marginal or no genotoxic activity was observed when incubation of cells and test compounds were continued for 4 hours in either strain, possibly because of increased cytotoxicity, or a destruction of the metabolic system.

Koch et al. (1988) studied the genotoxic effects of chlorinated ethylenes including TCE in various yeast *Saccharomyces cerevisiae* strains. Strain D7 was tested (11.1, 16.6, and 22.2 mM TCE) in stationary-phase cells without S9, stationary-phase cells with S9, and logarithmic-phase cells using different concentrations. No significant change in mitotic gene conversion or reverse mutation was observed in either the absence or presence of S9. In addition, there was a considerable increase in the induction of mitotic aneuploidy in strain D61.M, though no statistical analysis was performed.

Table 4-7. TCE genotoxicity: fungal and yeast systems

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Gene conversions					
Saccharomyces cerevisiae D7 and D4	15 and 22 mM; 1 and 4 hrs	Not determined	+ at 1 hr, D7 strain; – at 4 hrs, both D7 and D4	Gene conversion; CYP content fivefold greater in D7 strain; high cytotoxicity at 22 mM	Callen et al. (1980)
S. cerevisiae D7	11.1, 16.6, and 22.2 mM	–	–	Both stationary and log phase/production of phototropic colonies	Koch et al. (1988)
Schizosaccharomyces pombe	0.2–200 mM (—pre" and technical-grade)	–	–	Forward mutation, different experiments with different doses and time	Rossi et al. (1983)
S. cerevisiae D7		+	–		Bronzetti et al. (1980)
A. nidulans		No data	+	Forward mutation	Crebelli et al. (1985)
Recombination					
S. cerevisiae		+	–	Gene conversion	Bronzetti et al. (1980)
S. cerevisiae D7 and D4	15 and 22 mM; 1 and 4 hrs	Not determined	+		Callen et al. (1980)
A. nidulans		Not determined	+	Gene cross over	Crebelli et al. (1985)
Mitotic aneuploidy					
S. cerevisiae D61.M	5.5, 11.1, and 16.6 mM	+	+	Loss of dominant color homolog	Koch et al. (1988)

Rossi et al. (1983) studied the effect of TCE on yeast species *Schizosaccharomyces pombe* both using in vitro and host-mediated mutagenicity studies and the effect of two stabilizers, epichlorohydrin and 1,2-epoxybutane, that are contained in technical-grade TCE. The main goal of this study was to evaluate the genotoxic activity of TCE samples of different purity and determine whether the effect was due to the additives present in the TCE or TCE itself. Forward mutations at five loci (*ade 1, 3, 4, 5, 9*) of the adenine pathway in the yeast, strain P1 was evaluated. The stationary-phase cells were exposed to 25 mM concentration of TCE for 2, 4, and 8 hours in the presence and absence of S9. No change in mutation frequency was observed either in pure- or technical-grade samples either in the presence or absence of S9 at any of the time-points tested. Interestingly, this suggests that the stabilizers used in technical-grade TCE are not genotoxic in yeast. In a follow-up experiment, the same authors studied the effect of different concentrations (0.22, 2.2, and 22.0 mM) in a host-mediated assay using liver microsome preparations obtained from untreated mice, from phenobarbital- and naphthoflavone-pretreated mice and rats, which also suggested that stabilizers were not genotoxic in yeast. This experiment is described in more detail in Section 4.2.1.4.1.

Furthermore, TCE was tested for its ability to induce both point mutation and mitotic gene conversion in diploid strain of yeast *S. cerevisiae* (strain D7) both with and without a mammalian microsomal activation system. In a suspension test with D7, TCE was active only with microsomal activation (Bronzetti et al., 1980).

These studies are consistent with those of bacterial systems in indicating that pure TCE as a parent compound is not likely to cause mutations, gene conversions, or recombinations in fungal or yeast systems. In addition, the data suggest that contaminants used as stabilizers in technical-grade TCE are not genotoxic in these systems, and that the observed genotoxic activity in these systems is predominantly mediated by TCE metabolites.

4.2.1.4. Mammalian Systems Including Human Studies

4.2.1.4.1. Gene mutations (bacterial, fungal, or yeast with a mammalian host)

Very few studies have been conducted to identify the effect of TCE, particularly on gene (point) mutations using mammalian systems (see Table 4-8). An overall summary of different endpoints using mammalian systems will be provided at the end of this section. In order to assess the potential mutagenicity of TCE and its possible contaminants, Rossi et al. (1983) performed genotoxicity tests using two different host-mediated assays with pure- and technical-grade TCE. Male mice were administered with one dose of 2 g/kg of pure or technical-grade TCE by gavage. Following the dosing, for the i.p. host-mediated assay, yeast cell suspensions (2 × 109 cells/mL) were inoculated into the peritoneal cavity of the animals. Following 16 hours, animals were sacrificed and yeast cells were recovered to detect the induction of forward mutations at five loci (*ade 1, 2, 4, 5, 9*) of the adenine pathway. A second host-mediated assay was performed by exposing the animals to 2 g/kg of pure or technical-grade TCE and

inoculating the cells into the blood system. Yeast cells were recovered from livers following 4 hours of exposure. Forward mutations in the five loci (*ade 1,2,4,5,9*) were not observed in host-mediated assay either with pure or technical-grade TCE. Genotoxic activity was not detected when the mutagenic epoxide stabilizers were tested for mutagenicity independently or in combination. To confirm the sensitivity of the assay, the authors tested a positive control, *N*-nitroso-dimethyl-nitrosamine (1 mg/kg), and found a mutation frequency of >20 times the spontaneous level. The authors suggested that the negative result could have been due to an inadequate incubation time of the sample with the yeast cells.

Male and female transgenic *lac Z* mice were exposed by inhalation to an actual concentrations of 0, 203, 1,153, and 3,141 ppm TCE, 6 hours/day for 12 days (Douglas et al., 1999). Following 14 and 60 days of last exposure, animals were sacrificed and the mutation frequencies were determined in various organs such as bone marrow, kidney, spleen, liver, lung, and testicular germ cells. No statistically significant increases in base-changes or small-deletions were observed at any of the doses tested in male or female lung, liver, bone marrow, spleen, and kidney, or in male testicular germ cells when the animals were sampled 60 days after exposure. In addition, statistically significantly increased gene mutations were not observed in the lungs at 14 days after the end of exposure (Douglas et al., 1999). The authors acknowledged that *lacZ* bacteriophage transgenic assay does not detect large deletions. The authors also acknowledged that their hypothesis does not readily explain the increases in small deletions and base-change mutations found in the von Hippel-Lindau (*VHL*) tumor suppressor gene in RCCs of the TCE-exposed population. DCA, a TCE metabolite has been shown to increase *lacI* mutations in transgenic mouse liver, however, only after 60-weeks-of-exposure to high concentration (>1,000 ppm) in drinking water (Leavitt et al., 1997). DCA induced relatively small increase in *lac I* mutations when the animals were exposed for 60 weeks, a significantly longer duration than the TCE exposure in the Douglas et al. (1999) study (<2 weeks). Because a relatively small fraction of TCE is metabolized to DCA (see Section 3.3), the mutagenic effect of DCA is unlikely to have been detected in the experiments in Douglas et al. (1999). GSH conjugation, which leads to the production of genotoxic metabolites (see Section 4.2.5), constitutes a relatively small (and relatively uncertain) portion of TCE metabolism in mice, with little data on the extent of renal DCVC bioactivation vs. detoxification in mice (see Sections 3.3 and 3.5). In addition, statistically significantly increased kidney tumors have not been reported in mice with TCE treatment, and the increased incidence of kidney tumors in rats, while considered biologically significant, are quite low and not always statistically significant (see Section 4.4). Therefore, although Douglas et al. (1999) did not detect increased mutations in the kidney, these results are not highly informative as to the role of mutagenicity in TCE-induced kidney tumors, given the uncertainties in the production in genotoxic GSH conjugation metabolites in mice and the low carcinogenic potency of TCE for kidney tumors in rodents relative to what is detectable in experimental bioassays.

Table 4-8. TCE genotoxicity: mammalian systems—gene mutations and chromosome aberrations

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Gene mutations (forward mutations)					
Schizosaccharomyces pombe	2 g/kg, 4 and 16 hrs	Not determined	–	Host-mediated: i.v. and i.p. injections of yeast cells	Rossi et al. (1983)
Gene mutations (mutations frequency)					
lac Z transgenic mice	0, 203, 1,153, or 3,141 ppm	No base changes or small deletions	No base changes or small deletions	Lung, liver, bone marrow, spleen, kidney, testicular germ cells used	Douglas et al. (1999)
Chromosomal aberrations[a]					
Chinese hamster ovary	745–14,900 µg/mL	Not determined	–	8–14 hrs	Galloway et al. (1987)
	499–14,900 µg/mL	–	Not determined	2 hrs exposure	Galloway et al. (1987)
C57BL/6J mice	5, 50, 500, or 5,000 ppm (6 hrs)	_	Not applicable	Splenocytes	Kligerman et al. (1994)
Sprague-Dawley rats	5, 50, 500, or 5,000 ppm (6 hrs, single and 4-d exposure)	_	Not applicable	Peripheral blood lymphocytes	Kligerman et al. (1994)

[a]It should be noted that results of most chromosomal aberration assays report the combined incidence of multiple effects, including chromatid breaks, isochromatid or chromosome breaks, chromatid exchanges, dicentric chromosomes, ring chromosomes, and other aberrations.

4.2.1.4.2. *VHL* gene mutations

Studies have been conducted to determine the role of *VHL* gene mutations in RCC, with and without TCE exposure, and are summarized here. Most of these studies are epidemiologic, comparing *VHL* mutation frequencies of TCE-exposed to nonexposed cases from RCC case-control studies, or to background mutation rates among other RCC case series (described in Section 4.4.3). Inactivation of the *VHL* gene through mutations, loss of heterozygosity, and imprinting has been observed in about 70% of renal clear cell carcinomas (Alimov et al., 2000; Kenck et al., 1996). Recent studies have also examined the role of other genes or pathways in RCC subtypes, including c-Myc activation and vascular endothelial growth factor (VEGF) (Toma et al., 2008; Furge et al., 2007).

Several studies have examined the role of *VHL* gene inactivation in RCC, including a recent study that measured not only mutations but also promoter hypermethylation (Nickerson et al., 2008). This study focused on kidney cancer regardless of cause, and found that 91% of cc-RCC exhibited alterations of the *VHL* gene, suggesting a role for *VHL* mutations as an early event in clear cell-RCC. A recent analysis of current epidemiological studies of renal cell cancer suggests *VHL* gene alterations as a marker of clear cell-RCC, but that limitations of previous studies may make the results difficult to interpret (Chow and Devesa, 2008). Conflicting results have been reported in epidemiological studies of *VHL* mutations in TCE-exposed cases and are described in detail in Section 4.2.7. Both Brüning et al. (1997b) and Brauch et al. (2004; 1999) associated increased *VHL* mutation frequency in TCE-exposed RCC cases. The two other available studies of Schraml et al. (1999) and Charbotel et al. (2007), because of their limitations and lower mutation detection rate in the case of Charbotel et al. (2007) neither add nor detract to the conclusions from the earlier studies. Additional discussion of these data is provided in Section 4.4.3.

Limited animal studies have examined the role of TCE and *VHL* mutations, although Mally et al. (2006) have recently conducted both in vitro and in vivo studies using the Eker rat model (see Section 4.4.6.1.1). The Eker rat model (*Tsc-2*$^{\pm}$) is at increased risk for the development of spontaneous RCC and as such, has been used to understand the mechanisms of renal carcinogenesis (Stemmer et al., 2007; Wolf et al., 2000). One study has demonstrated similar pathway activation in Eker rats as that seen in humans with *VHL* mutations leading to RCC, suggesting that *Tsc-2* inactivation is analogous to inactivation of *VHL* in human RCC (Liu et al., 2003). In Mally et al. (2006), male rats carrying the Eker mutation were exposed to TCE (0, 100, 250, 500, or 1,000 mg/kg body weight by gavage, 5 days/week) for 13 weeks to determine the renal effects (additional data from this study on in vitro DCVC exposure are discussed below, Section 4.2.5). A significant increase in labeling index in kidney tubule cells was observed; however, no enhancement of preneoplastic lesions or tumor incidence was found in Eker rat kidneys compared to controls. In addition, no *VHL* gene mutations in exons 1–3 were

detected in tumors obtained from either control or TCE-exposed Eker rats. Although no other published studies have directly examined *VHL* mutations following exposure to TCE, two studies performed mutational analysis of archived formalin-fixed paraffin embedded tissues from renal carcinomas from previous rat studies. These carcinomas were induced by the genotoxic carcinogens potassium bromate (Shiao et al., 2002) or *N*-nitrosodimethylamine (Shiao et al., 1998). Limited mutations in the *VHL* gene were observed in all samples, but, in both studies, these were found only in the clear cell renal carcinomas. Limitations of these two studies include the small number of total samples analyzed, as well as potential technical issues with DNA extraction from archival samples (see Section 4.4.6.6.1). However, analyses of *VHL* mutations in rats may not be informative as to the potential genotoxicity of TCE in humans because the *VHL* gene may not be the target for nephrocarcinogenesis in rats to the extent that it appears to be in humans.

4.2.1.4.3. Chromosomal aberrations

A few studies were conducted to investigate the ability of TCE to induce chromosomal aberrations in mammalian systems (see Table 4-8). Galloway et al. (1987) studied the effect of TCE on chromosome aberrations in Chinese hamster ovary cells. When the cells were exposed to TCE (499–14,900 µg/mL) for 2 hours with metabolic activation, S9, no chromosomal aberrations were observed. Furthermore, without metabolic activation, no changes in chromosomal aberrations were found when the cells were exposed to TCE concentrations of 745–14,900 µg/mL for 8–14 hours. It should be noted that in this study, liquid incubation method was used and the experiment was part of a larger study to understand the genotoxic potential of 108 chemicals.

Three inhalation studies in mice and rats examined if TCE could induce cytogenetic damage (Kligerman et al., 1994). In the first two studies, CD rats or C57Bl/6 mice, were exposed to 0-, 5-, 500-, or 5,000-ppm TCE for 6 hours. Peripheral blood lymphocytes in rats and splenocytes in mice were analyzed for induction of chromosomal aberrations, sister chromatid exchanges (SCEs), and micronucleus formation. The results of micronucleus and SCEs will be discussed in the next sections (see Sections 4.2.1.4.4 and 4.2.1.4.5). No significant increase in chromosomal aberrations was observed in binucleated peripheral blood lymphocytes. In the third study, the authors exposed the same strain of rats for 6 hours/day over 4 consecutive days. No statistically significant concentration-related increases in chromosomal aberrations were observed. The limited results of the above studies have not reported TCE to cause chromosomal aberrations either in in vitro or in vivo mammalian systems.

4.2.1.4.4. Micronucleus induction

The appearance of micronuclei is another endpoint that can demonstrate the genotoxic effect of a chemical. Several studies have been conducted to identify if TCE can cause micronucleus formation (see Table 4-9).

Wang et al. (2001) investigated micronucleus formation by TCE administered as a vapor in Chinese hamster ovary-K1 cells in vitro. Cells were grown in culture media with an inner Petri dish containing TCE that would evaporate into the media containing cells. The concentration of TCE in cultured medium was determined by gas chromatography. The actual concentration of TCE ranged from 0.8 and 1.4 ppm after a 24-hour treatment. A significant dose-dependent increase in micronuclei formation was observed. A dose-dependent decrease in cell growth and cell number was also observed. The authors did not test if the micronuclei formed were due to direct damage to the DNA or spindle formation.

Robbiano et al. (2004) conducted an in vitro study on DNA damage and micronuclei formation in rat and human kidney cells exposed to six carcinogenic chemicals including TCE. The authors examined for the ability of TCE to induce DNA fragmentation and formation of micronuclei in primary cultures of rat and human kidney cells derived from kidney cancer patients with 1–4 mM TCE concentrations. A significant dose-dependent increase in the frequency of micronuclei was obtained in primary kidney cells from both male rats and human of both genders. The authors acknowledged that the significance of the results should be considered in light of the limitations, including: (1) examination of TCE on cells from only three rats; (2) considerable variation in the frequency of DNA lesions induced in the cells; and (3) the possibility that kidney cells derived from kidney cancer patients may be more sensitive to DNA-damaging activity due to a more marked expression of enzymes involved in the metabolic activation of kidney procarcinogens and suppression of DNA repair processes. Nevertheless, this study is important and provides information of the possible genotoxic effects of TCE.

In the same study, Robbiano et al. (2004) administered rats a single oral dose of TCE (3,591 mg/kg) corresponding to ½ $LD_{50,}$ which had been pre-exposed to folic acid for 48 hours and the rats were euthanized 48 hours later following exposure to TCE. The frequency of binucleated cells was taken as an index of kidney cell proliferation. A statistically significant increase in the average frequency of micronucleus was observed.

Hu et al. (2008) studied the effect of TCE on micronuclei frequencies using human hepatoma HepG2 cells. The cells were exposed to 0.5, 1, 2, and 4 mM TCE for 24 hours. TCE caused a significant increase in micronuclei frequencies at all concentrations tested. It is important to note that similar concentrations were used in Robbiano et al. (2004).

Table 4-9. TCE genotoxicity: mammalian systems—micronucleus, sister chromatic exchanges

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Micronucleus					
Human hepatoma HepG2 cells	0.5–4 mM, 24 hrs	Not applicable	+		Hu et al. (2008)
Primary cultures of human and rat kidney cells	1.0, 2.0, or 4.0 mM	Not applicable	+	Dose-dependent significant increase	Robbiano et al. (2004)
Sprague-Dawley rats	3,591 mg/kg	+	–		Robbiano et al. (2004)
Chinese hamster ovary-K1 cells	0.8–1.4 ppm		+	Dose-dependent significant increase	Wang et al. (2001)
Male CD-1 mice	457 mg/kg	+	Not applicable	Bone marrow, correlated with TCOH in urine	Hrelia et al. (1994)
C56BL/6J mice	5, 50, 500, or 5,000 ppm	–	Not applicable	Splenocytes	Kligerman et al. (1994)
Sprague-Dawley rats	5, 50, 500, or 5,000 ppm	+	Not applicable	Dose dependent; peripheral blood lymphocytes	Kligerman et al. (1994)
SCEs					
Chinese hamster ovary	0.17%	–	Not determined	1 hr (vapor)	White et al. (1979)
	17.9–700 µg/mL	Not determined	+	25 hrs (liquid)	Galloway et al. (1987)
	49.7–14,900 µg/mL	+	Not determined	2 hrs	Galloway et al. (1987)
Human lymphocytes	178 µg/mL	Not determined	+		Gu et al. (1981a; 1981b)
Sprague-Dawley rats	5, 50, 500, or 5,000 ppm	–	Not applicable	Peripheral blood lymphocytes	Kligerman et al. (1994)
Peripheral blood lymphocytes from humans occupationally exposed	Occupational exposure	–	Not applicable		Nagaya et al. (1989a)
C57BL/6J mice	5, 50, 500, or 5,000 ppm	–	Not applicable	Splenocytes	Kligerman et al. (1994)

As described in the chromosomal aberration section (see Section 4.2.1.4.3), inhalation studies were performed using male C57BL/6 mice and CD rats (Kligerman et al., 1994) to determine if TCE could induce micronuclei. In the first and second study, rats or mice respectively, were exposed to 0-, 5-, 500-, or 5,000 ppm TCE for 6 hours. Peripheral blood lymphocytes in rats and splenocytes in mice were cultured and analyzed for induction of micronuclei formation. Bone marrow polychromatic erythrocytes (PCEs) were also analyzed for micronuclei. TCE caused a statistically significant increase in micronuclei formation at all concentrations in rat bone marrow PCEs but not in mice. The authors note that TCE was significantly cytotoxic at the highest concentration tested as determined by significant concentration-related decrease in the ratio of PCEs/normochromatic erythrocytes. In the third study, to confirm the results of the first study, the authors exposed rats to one dose of 5,000 ppm for 6 hours. A statistical increase in bone marrow micronuclei-PCEs was observed confirming the results of the first study.

Hrelia et al. (1994) treated male CD-1 mice with TCE (457 mg/kg body weight; i.p.) for 30 hours. Bone marrow cells were harvested for determination of micronuclei frequencies in PCEs. An increase in micronuclei frequency at 30 hours after treatment was observed. Linear regression analysis showed that micronuclei frequency induced by TCE correlated with TCOH concentrations in urine, a marker of TCE oxidative metabolism (Hrelia et al., 1994).

In summary, based on the results of the above studies, TCE is capable of inducing micronuclei in different in vitro and in vivo systems tested. Specific methods were not used that could definitively identify the mechanism of micronuclei formation. These are important findings that indicate TCE has genotoxic potential as measured by the micronucleus formation.

4.2.1.4.5. SCEs

Studies have been conducted to understand the ability of TCE to induce SCEs both in vitro and in vivo systems (see Table 4-9). White et al. (1979) evaluated the possible induction of SCE in Chinese hamster ovary cells using a vapor exposure procedure by exposing the cells to TCE (0.17%) for 1 hour in the presence of S9 metabolic activation. No change in SCE frequencies were observed between the control and the treatment group. However, in another study by Galloway et al. (1987) a dose-related increase in SCE frequency in repeated experiments both with and without metabolic activation was observed. It should be noted that in this study, liquid incubation was used, and the exposure times were 25 hours without metabolic activation at a concentration between 17.9 and 700 µg/mL and 2 hours in the presence of S9 at a concentration of 49.7–14,900 µg/mL. Due to the difference in the dose, length of exposure, and treatment protocol (vapor exposure vs. liquid incubation), no direct comparison can be made. It should also be noted that inadequacy of dose selection and the absence of positive control in the White et al. (1979) makes it difficult to interpret the study. In another study (Gu et al., 1981a), a small but positive response was observed in assays with peripheral lymphocytes.

No statistically significant increase in SCEs was found when male C57Bl/6 mice or CD rats were exposed to TCE at concentrations of 5, 500, or 5,000 ppm for 6 hours (Kligerman et al., 1994). Furthermore, in another study by Nagaya et al. (1989b), lymphocytes of TCE-exposed workers (n = 22) and matched controls (n = 22) were analyzed for SCEs. The workers had constantly used TCE in their jobs, although the exact exposure was not provided. The duration of their employment ranged from 0.7 to 34 years, averaging about 10 years. It should be noted that there were both smokers and nonsmokers among the exposed population. If a subject had not smoked for at least 2 years before the samples were taken, then they were considered as nonsmokers. There were eight nonsmokers in the group. If they were classified as smokers, then they smoked between 10 and 50 cigarettes per day. No significant increase in mean SCE frequencies were found in exposed population compared to controls, though the study is relatively small.

In summary, induction of SCEs has been reported in several, though not all, paradigms of TCE exposure, consistent with the structural damage to DNA/chromosomes indicated by excess micronuclei formation.

4.2.1.4.6. Unscheduled DNA synthesis (UDS)

In vitro studies are briefly described here, with additional discussion of effects related to TCE-induced UDS in the context of the liver in Section E.2.4.1. Perocco and Prodi (1981) studied UDS in human lymphocytes cultured in vitro (see Table 4-10). Three doses of TCE (2.5, 5.0, and 10 µL/mL) were used as final concentrations with and without S9. The results indicate that there was an increase in UDS only in the presence of S9, and in addition, the increase was maximal at the TCE concentration of 5 µL/mL. Three chlorinated ethane and ethylene solvent products were examined for their genotoxicity in hepatocyte primary culture DNA repair assays using vapor-phase exposures. Rat hepatocytes primary cultures were initiated and exposed to low-stabilized or standard stabilized TCE (0.1–2.5%) for 3 or 18 hours. UDS or DNA repair was not observed using either low or standard stabilized TCE, even at vapor phase doses up to those that produced extensive cell killing after 3 or 18 hour exposure (Shimada et al., 1985). Costa and Ivanetich (1984) examined the ability of TCE to induce UDS hepatocytes isolated from phenobarbital treated rats. The UDS was assessed only at the highest concentration that is tolerated by the hepatocytes (2.8 mM TCE).

These results indicate that TCE stimulated UDS in isolated rodent hepatocytes, and, importantly, in human lymphocytes in vitro.

Table 4-10. TCE genotoxicity: mammalian systems—UDS, DNA strand breaks/protein crosslinks, and cell transformation

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
UDS					
Rat primary hepatocytes		Not determined	–		Shimada et al. (1985)
Human lymphocytes	2.5, 5, or 10 µL/mL	±	–	Increase was only in certain doses and maximum at 5 µL/mL concentration	Perocco and Prodi (1981)
Phenobarbital-induced rat hepatocytes	2.8 mM	Not determined	+		Costa and Ivanetich (1984)
DNA strand breaks/protein crosslinks					
Primary rat kidney cells	0.5, 1.0, 2.0, or 4.0 mM	Not applicable	+	Dose-dependent significant increase	Robbiano et al. (2004)
Primary cultures of human kidney cells	1.0, 2.0, or 4.0 mM	Not determined	+	Dose-dependent significant increase	Robbiano et al. (2004)
Sprague-Dawley rats	3,591 mg/kg	+	Not applicable	Single oral administration	Robbiano et al. (2004)
Sprague-Dawley rats	500, 1,000, and 2,000 ppm	–	Not applicable	Comet assay	Clay (2008)
Cell transformation					
BALB/c 3T3 mouse cells	4, 20, 100, or 250 µg/mL	Not applicable	+	Weakly positive compared to other halogenated compounds tested in the same experiment	Tu et al. (1985)
Rat embryo cells		Not applicable	+		Price et al. (1978)
Syrian hamster embryo cells	5, 10, or 25 µg/mL	Not applicable	–		Amacher and Zelljadt (1983)

4.2.1.4.7. DNA strand breaks

DNA damage in response to TCE exposure was studied using comet assay in human hepatoma HepG2 cells (Hu et al., 2008; see Table 4-10). The cells were exposed to 0.5, 1, 2, and 4 mM for 24 hours. TCE increased the DNA migration in a significant dose-dependent manner at all tested concentrations suggesting TCE caused DNA strand breaks and chromosome damage.

TCE (4–10 mmol/kg body weight) were given to male mice by i.p. injection. The induction of single-strand breaks (SSBs) in DNA of liver, kidney, and lung was studied by the DNA unwinding technique. There was a linear increase in the level of SSBs in kidney and liver DNA but not in lung DNA 1 hour after administration (Walles, 1986).

Robbiano et al. (2004) conducted an in vitro study on DNA damage in rat and human kidney cells exposed to six carcinogenic chemicals, including TCE, in the comet assay. The authors examined the ability of TCE to induce DNA fragmentation in primary cultures of rat and human kidney cells with 1–4 mM TCE concentrations. TCE was dissolved in ethanol with a maximum concentration of 0.3% and the rat cultures were exposed to 20 hours. Primary human kidney cells were isolated from fragments of kidney discarded during the course of surgery for carcinoma of both male and female donors with an average age of 64.2 years and were also exposed to 20 hours. Significant dose-dependent increases in the ratio of treated/control tail length (average 4–7 µM compared to control) was observed as measured by comet assay in primary kidney cells from both male rats and human of both genders.

Clay et al. (2008) studied the DNA damage inducing capacity of TCE using the comet assay in rat kidney proximal tubules. Rats were exposed by inhalation to a range of TCE concentrations (500, 1,000, or 2,000 ppm) for 6 hours/day for 5 days. TCE did not induce DNA damage (as measured by tail length and percentage tail DNA and tail movement) in rat kidney proximal tubules in any of the doses tested possibly due to study limitations (small number of animals tested [n = 5] and limited exposure time [6 hours/day for only 5 days]). These results are in contrast to the findings of Robbiano et al. (2004), which showed DNA damage and increased micronuclei in the rat kidney 20 hours following a single dose (3,591 mg/kg body weight) of TCE. The DNA damage reported by comet assay is consistent with results for other markers of chromosomal damage or DNA structural damage such as excess micronuclei formation and SCE induced by TCE exposure.

4.2.1.4.8. DNA damage related to oxidative stress, polymorphisms

A detailed description of studies related to lipid peroxidation of TCE is presented in conjunction with discussion of liver toxicity (see Section 4.5, E.2.4.3, and E.3). A recent study reported on genetic polymorphism in solvent exposed population (Kumar et al., 2009). Normal (n = 220) and solvent-exposed (n = 97) populations were genotyped for CYP1A1, GSTM1, GSTT1 and GSTP1 polymorphisms. No exposure related differences were observed. In addition, the authors also examined TCE-exposed lymphocytes for the presence of chromosomal

aberrations and micronucleus at concentration of 2, 4 or 6 mM TCE. No significant changes in any of the parameters were observed.

4.2.1.4.9. Cell transformation

In vitro cell transformation using BALB/c-3T3 cells was conducted using TCE with concentrations varying from 0 to 250 µg/mL in liquid phase exposed for 72 hours (see Table 4-10). The cytotoxicity of TCE at the concentration tested in the transformation assay was determined by counting cells from duplicate plates of each test conditions at the end of the treatment period. A dose-dependent increase in Type III foci was observed, although no statistical analysis was conducted (Tu et al., 1985). In another study by Amacher and Zelljadt (1983), Syrian hamster embryo cells were exposed to 5, 10, or 25 µg/mL of TCE. In this experiment, two different serums (horse serum and fetal bovine serum) were also tested to understand the importance of serum quality in the transformation assay. A preliminary toxicity assay was performed to select dose levels that had 50–90% cell survival. One week after dosing, the cell colonies were fixed and counted for variability determination and examination of individual colonies for the evidence of morphological transformation. No significant change in morphological cell transformation was obtained. Furthermore, no significant changes were seen in transformed colonies when tested in different serum. However, these studies are of limited use for determining the genotoxic potential of TCE because they did not examine the foci for mutations, for instance in oncogenes or tumor suppressor genes.

4.2.1.5. Summary

Evidence from a number of different analyses and a number of different laboratories using a fairly complete array of endpoints suggests that TCE, following metabolism, has the potential to be genotoxic. A series of carefully controlled studies evaluating TCE itself (without mutagenic stabilizers and without metabolic activation) found it to be incapable of inducing gene mutations in most standard mutation bacterial assays (Mortelmans et al., 1986; Shimada et al., 1985; Crebelli et al., 1982; Baden et al., 1979; Bartsch et al., 1979; Waskell, 1978; Henschler et al., 1977; Simmon et al., 1977). Therefore, it appears that it is unlikely that TCE is a direct-acting mutagen, though TCE has shown potential to affect DNA and chromosomal structure. Low, but positive, responses were observed in the TA100 strain in the presence of S9 metabolic activation, even when genotoxic stabilizers were not present, suggesting that metabolites of TCE are genotoxic. TCE is also positive in some but not all fungal and yeast systems (Koch et al., 1988; Crebelli et al., 1985; Rossi et al., 1983; Callen et al., 1980). Data from human epidemiological studies support the possible mutagenic effect of TCE leading to *VHL* gene damage and subsequent occurrence of RCC. Association of increased *VHL* mutation frequency in TCE-exposed RCC cases has been observed (Brauch et al., 2004; Brauch et al., 1999; Brüning et al., 1997b).

TCE can lead to binding to nucleic acids and proteins (Kautiainen et al., 1997; Mazzullo et al., 1992; Bergman, 1983; Miller and Guengerich, 1983; DiRenzo et al., 1982), and such binding appears to be due to conversion to one or more reactive metabolites. For instance, increased binding was observed in samples bioactivated with mouse and rat microsomal fractions (Mazzullo et al., 1992; Miller and Guengerich, 1983; DiRenzo et al., 1982; Banerjee and Van Duuren, 1978). DNA binding is consistent with the ability to induce DNA and chromosomal perturbations. Several studies report the induction of micronuclei in vitro and in vivo from TCE exposure (Hu et al., 2008; Robbiano et al., 2004; Wang et al., 2001; Hrelia et al., 1994; Kligerman et al., 1994). Reports of SCE induction in some studies are consistent with DNA effects, but require further study (Kligerman et al., 1994; Nagaya et al., 1989b; Gu et al., 1981a; Gu et al., 1981b; White et al., 1979).

Overall, evidence from a number of different analyses and a number of different laboratories using various genetic endpoints indicates that TCE has a potential to induce damage to the structure of the chromosome in a number of targets, but has a more limited ability to induce mutation in bacterial systems.

Below, the genotoxicity data for TCE metabolites, TCA, DCA, TCOH, CH, DCVC, and DCVG, are briefly reviewed. The contributions of these data are twofold. First, to the extent that these metabolites may be formed in the in vitro and in vivo test systems for TCE, they provide insight into what agent or agents may contribute to the limited activity observed with TCE in these genotoxicity assays. Second, because the in vitro systems do not necessarily fully recapitulate in vivo metabolism, the genotoxicity of the known in vivo metabolites themselves provide data as to whether one may expect genotoxicity to contribute to the toxicity of TCE following in vivo exposure.

4.2.2. TCA

The TCE metabolite, TCA, has been studied using a variety of genotoxicity assay for its genotoxic potential (see International Agency for Research on Cancer [IARC, 2004] for additional information). Evaluation of in vitro studies of TCA must consider toxicity and acidification of medium resulting in precipitation of proteins, as TCA is commonly used as a reagent to precipitate proteins.

4.2.2.1. Bacterial Systems—Gene Mutations

TCA has been evaluated in a number of in vitro test systems including the bacterial assays (Ames) using different *S. typhimurium* strains such as TA98, TA100, TA104, TA1535, and RSJ100 (see Table 4-11). The majority of these studies did not report positive findings for genotoxicity ((1976) (1980) (1983) (Kargalioglu et al., 2002; Nelson et al., 2001; DeMarini et al., 1994; Rapson et al., 1980; Waskell, 1978). Waskell (1978) studied the effect of TCA (0.45 mg/plate) on bacterial strains TA98 and TA100 both in the presence and absence of S9.

The author did not find any revertants at the maximum nontoxic dose tested. Following exposure to TCA, Rapson et al. (1980) reported no change in mutagenic activity in strain TA100 in the absence of S9. DeMarini et al. (1994) performed different studies to evaluate the genotoxicity of TCA, including the Microscreen prophage-induction assay (TCA concentrations 0–10 mg/mL) and use of the *S. typhimurium* TA100 strain using bag vaporization technique (TCA concentrations 0–100 ppm), neither of which yielded positive results. Nelson et al. (2001) reported no positive findings with TCA using a *S. typhimurium* microsuspension bioassay (*S. typhimurium* strain TA104) following incubation of TCA for various lengths of time, with or without rat cecal microbiota. Similarly, no activity was observed in a study conducted by Kargalioglu et al. (2002) where *S. typhimurium* strains TA98, TA100, and RSJ100 were exposed to TCA (0.1–100 mM) either in the presence or absence of S9 (Kargalioglu et al., 2002).

Table 4-11. Genotoxicity of TCA—bacterial systems

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference
		With activation	Without activation	
λ Prophage induction, *E. coli* WP2s	10,000	–	–	DeMarini et al. (1994)
SOS chromotest, *E. coli* PQ37	10,000	–	–	Giller et al. (1997)
S. typhimurium TA1535, 1536, 1537, 1538, reverse mutation	20 µg/plate	NT	–	Shirasu et al. (1976)
S. typhimurium TA100, 98, reverse mutation	450 µg/plate	–	–	Waskell (1978)
S. typhimurium TA100, 1535, reverse mutation	4,000 µg/plate	–	–	Nestmann et al. (1980)
S. typhimurium TA1537, 1538, 98, reverse mutation	2,000 µg/plate	–	–	Nestmann et al. (1980)
S. typhimurium TA100, reverse mutation	520 µg/plate	NT	-	Rapson et al. (1980)
S. typhimurium TA100, 98, reverse mutation	5,000 µg/plate	–	–	Moriya et al. (1983)
S. typhimurium TA100, reverse mutation	600 ppm	–	–	DeMarini et al. (1994)
S. typhimurium TA100, reverse mutation, liquid medium	1,750	+	+	Giller et al. (1997)
S. typhimurium TA104, reverse mutation, microsuspension	250 µg/plate	–	–	Nelson et al. (2001)
S. typhimurium TA100, RSJ100, reverse mutation	16,300	–	–	Kargalioglu et al. (2002)
S. typhimurium TA98, reverse mutation	13,100	–	–	Kargalioglu et al. (2002)
S. typhimurium TA1535, SOS DNA repair		+	–	Ono et al. (1991)

[a]LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests unless specified.

+ = positive; – = negative; NT = not tested

Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

TCA was also negative in other bacterial systems. The SOS chromotest (which measures DNA damage and induction of the SOS repair system) in *E. coli* PQ37, ± S9 (Giller et al., 1997) evaluated the genotoxic activity of TCA ranging from 10 to 10,000 µg/mL and did not find any response. Similarly, TCA was not genotoxic in the Microscreen prophage-induction assay in *E. coli* with TCA concentrations ranging from 0 to 10,000 µg/mL, with and without S9 activation (DeMarini et al., 1994).

However, TCA induced a small increase in SOS DNA repair (an inducible error-prone repair system) in *S. typhimurium* strain TA1535 in the presence of S9 (Ono et al., 1991). Furthermore, Giller et al. (1997) reported that TCA demonstrated genotoxic activity in an Ames fluctuation test in *S. typhimurium* TA100 in the absence of S9 at noncytotoxic concentrations ranging from 1,750 to 2,250 µg/mL. The addition of S9 decreased the genotoxic response, with effects observed at 3,000–7,500 µg/mL. Cytotoxic concentrations in the Ames fluctuation assay were 2,500 and 10,000 µg/mL without and with microsomal activation, respectively.

4.2.2.2. Mammalian Systems

4.2.2.2.1. Gene mutations

The mutagenicity of TCA has also been tested in cultured mammalian cells (see Table 4-12). Harrington-Brock et al. (1998) examined the potential of TCA to induce mutations in L5178Y/TK $^{\pm}$ –3.7.2C mouse lymphoma cells. In this study, mouse lymphoma cells were incubated in culture medium treated with TCA concentrations up to 2,150 µg/mL in the presence of S9 metabolic activation and up to 3,400 µg/mL in the absence of S9 mixture. In the presence of S9, a doubling of mutant frequency was seen at concentrations of ≥2,250 µg/mL, including several concentrations with survival >10%. In the absence of S9, TCA increased the mutant frequency by twofold or greater only at concentrations of ≥2,000 µg/mL. These results were obtained at ≤11% survival rates. The authors noted that the mutants included both large- and small-colony mutants. The small-colony mutants are indicative of chromosomal damage. It should be noted that no rigorous statistical evaluation was conducted on these data. Cytotoxic and genotoxic effects of TCA were tested in a microplate-based cytotoxicity test and a HGPRT gene mutation assay using Chinese hamster ovary K1 cells, respectively (Zhang et al., 2010). TCA was the least cytotoxic when compared to six other haloacetic acids. TCA, at concentrations of 0, 200, 1,000, 5,000 and 10,000 µM, induced a visible increase in mutant frequency but did not show any statistically significant increase at any of the doses tested.

Table 4-12. TCA Genotoxicity—mammalian systems (both in vitro and in vivo)

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference
		With activation	Without activation	
Gene mutation, mouse lymphoma L5178Y/TK ± cells, in vitro	3,000	(+)	?	Harrington-Brock et al. (1998)
Gene mutation, Chinese hamster ovary cells in vitro, HGPRT gene mutation assay	10,000 µM	NT	–	Zhang et al. (2010)
DNA strand breaks, $B6C3F_1$ mouse and F344 rat hepatocytes, in vitro	1,630	NT	–	Chang et al. (1992)
DNA strand breaks, human CCRF-CEM lymphoblastic cells, in vitro	1,630	NT	–	Chang et al. (1992)
DNA damage, Chinese hamster ovary cells, in vitro, comet assay	3 mM	NT	–	Plewa et al. (2002)
DNA strand breaks, $B6C3F_1$ mouse liver, in vivo	1.0, oral, × 1	+		Nelson and Bull (1988)
DNA strand breaks, $B6C3F_1$ mouse liver, in vivo	500, oral, × 1	+		Nelson et al. (1989)
DNA strand breaks, $B6C3F_1$ mouse liver, in vivo	500, oral, 10 repeats	–		Nelson et al. (1989)
DNA strand breaks, $B6C3F_1$ mouse liver and epithelial cells from stomach and duodenum, in vivo	1,630, oral, × 1	–		Chang et al. (1992)
DNA strand breaks, male $B6C3F_1$ mice, in vivo	500 (neutralized)	–		Styles et al. (1991)
Micronucleus formation, Swiss mice, in vivo	125, i.p., × 2	+		Bhunya and Behera (1987)
Micronucleus formation, female C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes, in vivo	1,300, i.p., × 2	–		Mackay et al. (1995)
Micronucleus formation, male C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes, in vivo	1,080, i.p., × 2	–		Mackay et al. (1995)
Micronucleus formation, *Pleurodeles waltl* newt larvae peripheral erythrocytes, in vivo	80	+		Giller et al. (1997)
Chromosomal aberrations, Swiss mouse bone-marrow cells in vivo	125, i.p., × 1	+		Bhunya and Behera (1987)
	100, i.p., × 5	+		Bhunya and Behera (1987)
	500, oral, × 1	+		Bhunya and Behera (1987)
Chromosomal aberrations, chicken *Gallus domesticus* bone marrow, in vivo	200, i.p., × 1	+		Bhunya and Jena (1996)

Table 4-12. TCA Genotoxicity—mammalian systems (both in vitro and in vivo) (continued)

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
Chromosomal aberrations, human lymphocytes, in vitro	5,000, (neutralized)	–		Mackay et al. (1995)
Sperm morphology, Swiss mice, in vivo	125, i.p., × 5	+		Bhunya and Behera (1987)

[a]LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests; mg/kg for in vivo tests unless specified.

+ = positive; (+) = weakly positive; – = negative; NT = not tested; ? = inconclusive

Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

4.2.2.2.2. Chromosomal aberrations

Mackay et al. (1995) investigated the ability of TCA to induce chromosomal damage in an in vitro chromosomal aberration assay using cultured human cells. The authors treated the cells with TCA as free acid, both in the presence and absence of metabolic activation. TCA induced chromosomal damage in cultured human peripheral lymphocytes at concentrations (2,000 and 3,500 µg/mL) that significantly reduced the pH of the medium. However, exposure of cells to neutralized TCA did not have any effect even at a cytotoxic concentration of 5,000 µg/mL. It is possible that the reduced pH was responsible for the TCA-induced clastogenicity in this study. To further evaluate the role of pH changes in the induction of chromosome damage, the authors isolated liver-cell nuclei from B6C3F_1 mice and suspended in a buffer at various pH levels. The cells were stained with chromatin-reactive (fluorescein isothiocyanate) and DNA-reactive (propidium iodide) fluorescent dyes. A decrease in chromatin staining intensity was observed with the decrease in pH, suggesting that pH changes, independent of TCA exposure, can alter chromatin conformation. It was concluded by the authors that TCA-induced pH changes are likely to be responsible for the chromosomal damage induced by un-neutralized TCA. In another in vitro study, Plewa et al. (2002) evaluated the induction of DNA strand breaks induced by TCA (1–25 mM) in Chinese hamster ovary cells and did not observe any genotoxicity.

4.2.2.2.3. Micronucleus

Relative genotoxicity of TCA was tested in a mouse in vivo system (see Table 4-12) using three different cytogenetic assay (bone marrow chromosomal aberrations, micronucleus, and sperm-head abnormalities) (Bhunya and Behera, 1987) and for chromosomal aberrations in chicken (Bhunya and Jena, 1996). TCA induced a variety of anomalies including micronucleus in the bone marrow of mice and chicken. A small increase in the frequency of micronucleated erythrocytes at 80 µg/mL in a newt (*Pleurodeles waltl* larvae) micronucleus test was observed in response to TCA exposure (Giller et al., 1997). Mackay et al. (1995) investigated the ability of TCA to induce chromosomal DNA damage in the in vivo bone-marrow micronucleus assay in mice. C57BL mice were given TCA intraperitoneally at doses of 0, 337, 675, or 1,080 mg/kg-day for males and 0, 405, 810, or 1,300 mg/kg-day for females for two consecutive days, and bone-marrow samples were collected 6 and 24 hours after the last dose. The administered doses represented 25, 50, and 80% of the median lethal dose, respectively. No treatment-related increase in micronucleated PCEs was observed.

4.2.2.2.4. Other DNA damage studies

DNA unwinding assays have been used as indicators of SSBs and are discussed in detail in Section E.2.3. Studies were conducted on the ability of TCA to induce SSBs (see Table 4-12)

(Chang et al., 1992; Styles et al., 1991; Nelson et al., 1989; Nelson and Bull, 1988). Nelson and Bull (1988) evaluated the ability of TCA and other compounds to induce single-strand DNA breaks in vivo in Sprague-Dawley rats and B6C3F$_1$ mice. Single oral doses were administered to three groups of three animals, with an additional group as a vehicle control. Animals were sacrificed after 4 hours, and 10% liver suspensions were analyzed for single-strand DNA breaks by the alkaline unwinding assay. Dose-dependent increases in single-strand DNA breaks were induced in both rats and mice, with mice being more susceptible than rats. The lowest dose of TCA that produced significant SSBs was 0.6 mmol/kg (98 mg/kg) in rats but 0.006 mmol/kg (0.98 mg/kg) in mice.

However, in a follow-up study, Nelson et al. (1989) male B6C3F$_1$ mice were treated with 500 mg/kg TCA, and SSBs in whole liver homogenate were examined, and no significant differences from controls were reported. Moreover, in the experiments in the same study with DCA, increased SSBs were reported, but with no dose-response between 10 and 500 mg/kg, raising concerns about the reliability of the DNA unwinding assay used in these studies. For further details, see Section E.2.3. In an additional follow-up experiment with a similar experimental paradigm, Styles et al. (1991) tested TCA for its ability to induce strand breaks in male B6C3F$_1$ mice in the presence and absence of liver growth induction. The test animals were given one, two, or three daily doses of neutralized TCA (500 mg/kg) by gavage and killed 1 hour after the final dose. Additional mice were given a single 500-mg/kg gavage dose and sacrificed 24 hours after treatment. Liver nuclei DNA were isolated, and the induction of SSBs was evaluated using the alkaline unwinding assay. Exposure to TCA did not induce strand breaks under the conditions tested in this assay. In a study by Chang et al. (1992), administration of single oral doses of TCA (1–10 mmol/kg) to B6C3F$_1$ mice did not induce DNA strand breaks in a dose-related manner as determined by the alkaline unwinding assay. No genotoxic activity (evidence for strand breakage) was detected in F344 rats administered by gavage up to 5 mmol/kg (817 mg/kg).

In summary, although Nelson and Bull (1988) report effects on DNA unwinding for TCE and its metabolites with DCA having the highest activity and TCA the lowest, Nelson et al. (1989), using the same assay, reported no effect for TCA and the same effect at 10 and 500 mg/kg for DCA in mice. Moreover, Styles et al. (1991) did not find a positive result for TCA using the same paradigm as Nelson and Bull (1988) and Nelson et al. (1989). Furthermore, Chang et al. (1992) also did not find increased SSBs for TCA exposure in rats. (see Section E.2.4.3).

4.2.2.3. Summary

In summary, TCA has been studied using a variety of genotoxicity assays, including the recommended battery. No mutagenicity was reported in *S. typhimurium* strains in the presence or absence of metabolic activation or in an alternative protocol using a closed system, except in

one study on strain TA100 using a modified protocol in liquid medium. This is largely consistent with the results from TCE, which was negative in most bacterial systems except some studies with the TA100 strain. Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations. Measures of DNA-repair responses in bacterial systems have been inconclusive, with induction of DNA repair reported in *S. typhimurium* but not in *E. coli*. TCA-induced clastogenicity may be secondary to pH changes and not a direct effect of TCA.

4.2.3. DCA

DCA is another metabolite of TCE that has been studied using a variety of genotoxicity assay for its genotoxic potential (see Tables 4-13 and 4-14; see IARC (2004b) for additional information).

4.2.3.1. Bacterial and Fungal Systems—Gene Mutations

Studies were conducted to evaluate mutagenicity of DCA in different *S. typhimurium* and *E. coli* strains (Kargalioglu et al., 2002; Nelson et al., 2001; Giller et al., 1997; Fox et al., 1996a; Fox et al., 1996b; DeMarini et al., 1994; Herbert et al., 1980; Waskell, 1978). DCA was mutagenic in three strains of *S. typhimurium*: strain TA100 in three of five studies, strain RSJ100 in a single study, and strain TA98 in two of three studies. DCA failed to induce point mutations in other strains of *S. typhimurium* (TA104, TA1535, TA1537, and TA1538) or in *E. coli* strain WP2uvrA. In one study, DCA caused a weak induction of SOS repair in *E. coli* strain PQ37 (Giller et al., 1997).

DeMarini et al. (1994), in the same study as described in the TCA section of this section, also studied DCA as one of their compounds for analysis. In the prophage-induction assay using *E. coli*, DCA, in the presence of S9, was genotoxic producing 6.6–7.2 plaque-forming units (PFU)/mM and slightly less than threefold increase in PFU/plate in the absence of S9. In the second set of studies, which involved the evaluation of DCA at concentrations of 0–600 ppm for mutagenicity in *S. typhimurium* TA100 strain, DCA was mutagenic both in the presence and absence of S9, producing 3–5 times increases in the revertants/plate compared to the background. The lowest effective concentrations for DCA without S9 were 100 and 50 ppm in the presence of S9. In the third and most important study, mutation spectra of DCA were determined at the base-substitution allele *hisG46* of *S. typhimurium* TA100. DCA-induced revertants were chosen for further molecular analysis at concentrations that produced mutant yields that were two- to fivefold greater than the background. The mutation spectra of DCA were significantly different from the background mutation spectrum. Thus, despite the modest increase in the mutant yields (3–5 times) produced by DCA, the mutation spectra confirm that DCA is mutagenic. DCA primarily induced GC-AT transitions.

Table 4-13. Genotoxicity of DCA (bacterial systems)

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
λ Prophage induction, *E. coli* WP2s	2,500	+	–	DeMarini et al. (1994)
SOS chromotest, *E. coli* PQ37	500	–	(+)	Giller et al. (1997)
S. typhimurium, DNA repair-deficient strains TS24, TA2322, TA1950	31,000	–	–	Waskell (1978)
S. typhimurium TA100, TA1535, TA1537, TA1538, reverse mutation		–	–	Herbert et al. (1980)
S. typhimurium TA100, reverse mutation	50	+	+	DeMarini et al. (1994)
S. typhimurium TA100,TA1535, TA1537, TA98, reverse mutation	5,000	–	–	Fox et al., (1996b)
S. typhimurium TA100, reverse mutation, liquid medium	100	+	+	Giller et al. (1997)
S. typhimurium RSJ100, reverse mutation	1,935	–	+	Kargalioglu et al. (2002)
S. typhimurium TA104, reverse mutation, microsuspension	150 µg/plate	–	–	Nelson et al. (2001)
S. typhimurium TA98, reverse mutation	10 µg/plate	(+)	–	Herbert et al. (1980)
	5,160	–	+	Kargalioglu et al. (2002)
S. typhimurium TA100, reverse mutation	1,935	+	+	Kargalioglu et al. (2002)
E. coli WP2uvrA, reverse mutation	5,000	–	–	Fox et al., (1996b)

[a]LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests unless specified.

+ = positive; (+) = weakly positive; – = negative

Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

Table 4-14. Genotoxicity of DCA—mammalian systems

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference
		With activation	Without activation	
Gene mutation, mouse lymphoma cell line L5178Y/TK ± in vitro	5,000	–	–	Fox et al., (1996b)
Gene mutation, mouse lymphoma cell line L5178Y/TK ± –3.7.2C in vitro	400	NT	+	Harrington-Brock et al. (1998)
Gene mutation, Chinese hamster ovary cells in vitro, HGPRT gene mutation assay	1,000 µM	NT	+	Zhang et al. (2010)
DNA strand breaks and alkali-labile damage, Chinese hamster ovary cells in vitro (single-cell gel electrophoresis assay)	3,225 µg/mL	NT	–	Plewa et al. (2002)
DNA strand breaks, $B6C3F_1$ mouse hepatocytes in vitro	2,580	NT	–	Chang et al. (1992)
DNA strand breaks, F344 rat hepatocytes in vitro	1,290	NT	–	Chang et al. (1992)
Micronucleus formation, mouse lymphoma L5178Y/TK ± –3.7.2C cell line in vitro	800	NT	–	Harrington-Brock et al. (1998)
Chromosomal aberrations, Chinese hamster ovary in vitro	5,000	–	–	Fox et al., (1996b)
Chromosomal aberrations, mouse lymphoma L5178Y/Tk ± –3.7.2C cell line in vitro	600	NT	+	Harrington-Brock et al. (1998)
Aneuploidy, mouse lymphoma L5178Y/Tk ± –3.7.2C cell line in vitro	800	NT	–	Harrington-Brock et al. (1998)
DNA strand breaks, human CCRF-CEM lymphoblastoid cells in vitro	1,290	NT	–	Chang et al. (1992)
DNA strand breaks, male $B6C3F_1$ mouse liver in vivo	13, oral, × 1	+		Nelson and Bull (1988)
DNA strand breaks, male $B6C3F_1$ mouse liver in vivo	10, oral, × 1	+		Nelson et al. (1989)
DNA strand breaks, male $B6C3F_1$ mouse liver in vivo	1,290, oral, × 1	–		Chang et al. (1992)
DNA strand breaks, male $B6C3F_1$ mouse splenocytes in vivo	1,290, oral, × 1	–		Chang et al. (1992)
DNA strand breaks, male $B6C3F_1$ mouse epithelial cells from stomach and duodenum in vivo	1,290, oral, × 1	–		Chang et al. (1992)
DNA strand breaks, male $B6C3F_1$ mouse liver in vivo	5,000, dw, × 7–14 d	–		Chang et al. (1992)
DNA strand breaks, alkali-labile sites, cross linking, male $B6C3F_1$ mouse blood leukocytes in vivo (single-cell gel electrophoresis assay)	3,500, dw, × 28 d	+		Fuscoe et al. (1996)

Table 4-14. Genotoxicity of DCA—mammalian systems (continued)

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference
		With activation	Without activation	
DNA strand breaks, male Sprague-Dawley rat liver in vivo	30, oral, × 1	+		Nelson and Bull (1988)
DNA strand breaks, male F344 rat liver in vivo	645, oral, × 1	–		Chang et al. (1992)
DNA strand breaks, male F344 rat liver in vivo	2,000, dw, × 30 wks	–		Chang et al. (1992)
Gene mutation, lacI transgenic male B6C3F$_1$ mouse liver assay in vivo	1,000, dw, × 60 wks	+		Leavitt et al. (1997)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes in vivo	3,500, dw, × 9 d	+		Fuscoe et al. (1996)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes in vivo	3,500, dw, × 28 d	–		Fuscoe et al. (1996)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes in vivo	3,500, dw, × 10 wks	+		Fuscoe et al. (1996)
Micronucleus formation, male and female Crl:CD (Sprague-Dawley) BR rat bone-marrow erythrocytes in vivo	1,100, i.v., × 3	–		Fox et al., (1996b)
Micronucleus formation, Pleurodeles waltl newt larvae peripheral erythrocytes in vivo	80 d	–		Giller et al. (1997)

[a]LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests; mg/kg for in vivo tests unless specified; dw = drinking-water (in mg/L).

+ = positive; – = negative; NT = not tested

Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

Kargalioglu et al. (2002) analyzed the cytotoxicity and mutagenicity of the drinking water disinfection byproducts including DCA in *S. typhimurium* strains TA98, TA100, and RSJ100 ± S9. DCA was mutagenic in this test although the response was low when compared to other disinfection byproducts tested in strain TA100. This study was also summarized in a review by Plewa et al. (2002). Nelson et al. (2001) investigated the mutagenicity of DCA using a *S. typhimurium* microsuspension bioassay following incubation of DCA for various lengths of time, with or without rat cecal microbiota. No mutagenic activity was detected for DCA with *S. typhimurium* strain TA104.

Although limited data, it appears that DCA has mutagenic activity in the *S. typhimurium* strains, particularly TA100.

4.2.3.2. Mammalian Systems

4.2.3.2.1. Gene mutations

The mutagenicity of DCA has been tested in mammalian systems, particularly, mouse lymphoma cell lines in vitro (Harrington-Brock et al., 1998; Fox et al., 1996b); and lacI transgenic mice in vivo (Leavitt et al., 1997). Harrington-Brock et al. (1998) evaluated DCA for it mutagenic activity in L5178Y/TK ± (–) 3.7.2C mouse lymphoma cells. A dose-related increase in mutation (and cytotoxic) frequency was observed at concentrations between 100 and 800 µg/mL. Most mutagenic activity of DCA at the Tk locus was due to the production of small-colony Tk mutants (indicating chromosomal mutations). Different pH levels were tested in induction of mutant frequencies and it was determined that the mutagenic effect observed was due to the chemical and not pH effects.

Mutation frequencies were studied in male transgenic $B6C3F_1$ mice harboring the bacterial *lacI* gene administered DCA at either 1.0 or 3.5 g/L in drinking water (Leavitt et al., 1997). No significant difference in mutant frequency was observed after 4 or 10 weeks of treatment in both of the doses tested as compared to control. However, at 60 weeks, mice treated with 1.0 g/L DCA showed a slight increase (1.3-fold) in the mutant frequency over the control, but mice treated with 3.5 g/L DCA had a 2.3-fold increase in the mutant frequency. Mutational spectra analysis revealed that ~33% had G:C-A:T transitions and 21% had G:C–T:A transversions and this mutation spectra was different than that was seen in the untreated animals, indicating that the mutations were likely induced by the DCA treatment. The authors conclude that these results are consistent with the previous observation that the proportion of mutations at T:A sites in codon 61 of the H-ras gene was increased in DCA-induced liver tumors in $B6C3F_1$ mice (Leavitt et al., 1997).

Zhang et al. (2010) tested the cytotoxic and genotoxic effects of DCA in a microplate-based cytotoxicity test and HGPRT gene mutation assay using Chinese hamster ovary K1 cells, respectively. The concentrations at which these tests were conducted were 0, 200, 1,000, 5,000 and 10,000 µM. Two parameters were used to indicate chronic cytotoxicity: the lowest

cytotoxic concentration and the percent C1/2 value. The lowest cytotoxic concentration for DCA was 2.87×10^{-3}M. Statistically significant increase in HGPRT mutant frequency was observed at concentrations ≥1,000 µM.

4.2.3.2.2. Chromosomal aberrations and micronucleus

Harrington-Brock et al. (1998) evaluated DCA for its potential to induce chromosomal aberrations in DCA-treated (0, 600, and 800 µg/mL) mouse lymphoma cells. A clearly positive induction of aberrations was observed at both concentrations tested. No significant increase in micronucleus was observed in DCA-treated (0, 600, and 800 µg/mL) mouse lymphoma cells (Harrington-Brock et al., 1998). However, no chromosomal aberrations were found in Chinese hamster ovary cells exposed to DCA (Fox et al., 1996b)

Fuscoe et al. (1996) investigated in vivo genotoxic potential of DCA in bone marrow and blood leukocytes using the peripheral-blood-erythrocyte micronucleus assay (to detect chromosome breakage and/or malsegregation) and the alkaline single cell gel electrophoresis (comet) assay, respectively. Mice were exposed to DCA in drinking water, available ad libitum, for up to 31 weeks. A statistically significant dose-related increase in the frequency of micronucleated PCEs was observed following subchronic exposure to DCA for 9 days. Similarly, a significant increased was also observed when exposed for ≥10 weeks particularly at the highest dose of DCA tested (3.5 g/L). DNA cross-linking was observed in blood leukocytes in mice exposed to 3.5 g/L DCA for 28 days. These data provide evidence that DCA may have some potential to induce chromosome damage when animals were exposed to concentrations similar to those used in the rodent bioassay.

4.2.3.2.3. Other DNA damage studies

Nelson and Bull (1988) and Nelson et al. (1989) have been described in Sections 4.2.2.2.4 and E.2.3, with positive results for DNA unwinding for DCA, though Nelson et al. (1989) reported the same response at 10 and 500 mg/kg in mice, raising concerns about the reliability of the assay in these studies. Chang et al. (1992) conducted both in vitro and in vivo studies to determine the ability of DCA to cause DNA damage. Primary rat (F344) hepatocytes and primary mouse hepatocytes treated with DCA for 4 hours did not in induce DNA SSBs as detected by alkaline DNA unwinding assay. No DNA strand breaks were observed in human CCRF-CEM lymphoblastoid cells in vitro exposed to DCA. Similarly, analysis of the DNA SSBs in mice killed 1 hour after a single dose of 1, 5, or 10 mM/kg DCA did not cause DNA damage. None of the F344 rats killed 4 hours after a single gavage treatment (1–10 mM/kg) produced any detectable DNA damage.

4.2.3.3. Summary

In summary, DCA has been studied using a variety but limited number of genotoxicity assays. Within the available data, DCA has been demonstrated to be mutagenic in the *S. typhimurium* assay, particularly in strain TA100, the in vitro mouse lymphoma assay and in vivo cytogenetic and gene mutation assays. DCA can cause DNA strand breaks in mouse and rat liver cells following in vivo administration by gavage.

4.2.4. CH

CH has been evaluated for its genotoxic potential using a variety of genotoxicity assays (see Tables 4-15, 4-16, and 4-17). These data are particularly important because it is known that a large flux of TCE metabolism leads to CH as an intermediate, so a comparison of their genotoxicity profiles is likely to be highly informative.

4.2.4.1. DNA Binding Studies

Limited analysis has been performed examining DNA binding potential of CH (Von Tungeln et al., 2002; Ni et al., 1995; Keller and Hd'A, 1988). Keller and Heck (1988) conducted both in vitro and in vivo experiments using $B6C3F_1$ mouse strain. The mice were pretreated with 1,500 mg/kg TCE for 10 days and then given 800 mg/kg [^{14}C] chloral. No detectable covalent binding of [^{14}C] to DNA in the liver was observed. Another study with in vivo exposures to nonradioactive CH at a concentration of 1,000 and 2,000 nmol in mice $B6C3F_1$ demonstrated an increase in malondialdehyde-derived and 8-oxo-2‘-deoxyguanosine adducts in liver DNA (Von Tungeln et al., 2002). Ni et al. (1995) observed malondialdehyde adducts in calf thymus DNA when exposed to CH and microsomes from male $B6C3F_1$ mouse liver.

Keller and Heck (1988) investigated the potential of chloral to form DNA-protein cross-links in rat liver nuclei using concentrations 25, 100, or 250 mM. No statistically significant increase in DNA-protein cross-links was observed. DNA and RNA isolated from the [^{14}C] chloral-treated nuclei did not have any detectable [^{14}C] bound. However, the proteins from choral-treated nuclei did have a concentration-related binding of [^{14}C].

Table 4-15. CH genotoxicity: bacterial, yeast, and fungal systems

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference
		With activation	Without activation	
SOS chromotest, *Escherichia coli* PQ37	10,000	–	–	Giller et al. (1995)
S. typhimurium TA100, TA1535, TA98, reverse mutation	10,000	–	–	Waskell (1978)
S. typhimurium TA100, TA1537, TA1538, TA98, reverse mutation	1,000	+	+	Haworth et al. (1983)
S. typhimurium TA100, reverse mutation	5,000 µg/plate	–	–	Leuschner and Leuschner (1991)
S. typhimurium TA100, reverse mutation	2,000 µg/plate	+	+	Ni et al. (1994)
S. typhimurium TA100, reverse mutation, liquid medium	300	+	–	Giller et al. (1995)
S. typhimurium TA100, TA104, reverse mutation	1,000 µg/plate	+	+	Beland (1999)
S. typhimurium TA104, reverse mutation	1,000 µg/plate	+	+	Ni et al. (1994)
S. typhimurium TA1535, reverse mutation	1,850	–	–	Leuschner and Leuschner (1991)
S. typhimurium TA1535, TA1537 reverse mutation	6,667	–	–	Haworth et al. (1983)
S. typhimurium TA1535, reverse mutation	10,000	–	–	Beland (1999)
S. typhimurium TA98, reverse mutation	7,500	–	–	Haworth et al. (1983)
S. typhimurium TA98, reverse mutation	10,000 µg/plate	–	+	Beland (1999)
A.nidulans, diploid strain 35X17, mitotic cross-overs	1,650	Not tested	–	Crebelli et al. (1985)
A. nidulans, diploid strain 30, mitotic cross-overs	6,600	Not tested	–	Kafer (1986)
A. nidulans, diploid strain NH, mitotic cross-overs	1,000	Not tested	–	Kappas (1989)
A. nidulans, diploid strain P1, mitotic cross-overs	990	Not tested	–	Crebelli et al. (1991)
A. nidulans, diploid strain 35X17, nondisjunctions	825	Not tested	+	Crebelli et al. (1985)
A. nidulans, diploid strain 30, aneuploidy	825	Not tested	+	Kafer (1986)
A. nidulans, haploid conidia, aneuploidy, polyploidy	1,650	Not tested	+	Kafer (1986)
A. nidulans, diploid strain NH, nondisjunctions	450	Not tested	+	Kappas (1989)

Table 4-15. CH genotoxicity: bacterial, yeast, and fungal systems (continued)

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
A. nidulans, diploid strain P1, nondisjunctions	660	Not tested	+	Crebelli et al. (1991)
A. nidulans, haploid strain 35, hyperploidy	2,640	Not tested	+	Crebelli et al. (1991)
S. cerevisiae, meiotic recombination	3,300	Not tested	Inconclusive	Sora and Agostini Carbone (1987)
S. cerevisiae, disomy in meiosis	2,500	Not tested	+	Sora and Agostini Carbone (1987)
S. cerevisiae, disomy in meiosis	3,300	Not tested	+	Sora and Agostini Carbone (1987)
S. cerevisiae, D61.M, mitotic chr. malsegregation	1,000	Not tested	+	Albertini, (1990)
Drosophila melanogaster, somatic mutation wing spot test	825		+	Zordan et al. (1994)
Drosophila melanogaster, induction of sex-linked lethal mutation	37.2 feed		Inconclusive	Beland (1999)
Drosophila melanogaster, induction of sex-linked lethal mutation	67.5 inj		–	Beland (1999)

[a]LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests; inj = injection.

+ = positive; – = negative

Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

Table 4-16. CH genotoxicity: mammalian systems—all genetic endpoints, in vitro

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference
		With activation	Without activation	
DNA-protein cross-links, rat nuclei in vitro	41,250	NT	–	Keller and Heck (1988)
DNA SSBs, rat primary hepatocytes in vitro	1,650	NT	–	Chang et al. (1992)
Gene mutation, mouse lymphoma L5178Y/TK $^{\pm}$, in vitro	1,000		(+)	Harrington-Brock et al. (1998)
SCEs, Chinese hamster ovary cells, in vitro	100	+	+	Beland (1999)
Micronucleus formation (kinetochore-positive), Chinese hamster C1 cells, in vitro	165	NT	+	Degrassi and Tanzarella (1988)
Micronucleus formation (kinetochore-negative), Chinese hamster C1 cells, in vitro	250	NT	–	Degrassi and Tanzarella (1988)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells, in vitro	400	NT	+	Parry et al., (1990)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells, in vitro	400	NT	+	Lynch and Parry (1993)
Micronucleus formation, Chinese hamster V79 cells, in vitro	316	NT	+	Seelbach et al. (1993)
Micronucleus formation, mouse lymphoma L5178Y/TK $^{\pm}$, in vitro	1,300	NT	–	Harrington-Brock et al. (1998)
Micronucleus formation, mouse lymphoma L5178Y/TK $^{\pm}$, in vitro	500	NT	+	Nesslany and Marzin (1999)
Chromosomal aberrations, Chinese Hamster CHED cells, in vitro	20	NT	+	Furnus et al. (1990)
Chromosomal aberrations, Chinese Hamster ovary cells, in vitro	1,000	+	+	Beland (1999)
Chromosomal aberrations, mouse lymphoma L5178Y/TK $^{\pm}$ cells line, in vitro	1,250	NT	(+)	Harrington-Brock et al. (1998)
Aneuploidy, Chinese hamster CHED cells, in vitro	10	NT	+	Furnus et al. (1990)
Aneuploidy, primary Chinese hamster embryonic cells, in vitro	250	NT	+	Natarajan et al. (1993)
Aneuploidy, Chinese hamster LUC2p4 cells, in vitro	250	NT	+	Warr et al. (1993)
Aneuploidy, mouse lymphoma L5178Y/TK $^{\pm}$, in vitro	1,300	NT	–	Harrington-Brock et al. (1998)
Tetraploidy and endoredupliation, Chinese hamster LUC2p4cells, in vitro	500	NT	+	Warr et al. (1993)
Cell transformation, Syrian hamster embryo cells (24-hr treatment)	350	NT	+	Gibson et al. (1995)
Cell transformation, Syrian hamster dermal cell line (24-hr treatment)	50	NT	+	Parry et al. (1996)
DNA SSBs, human lymphoblastoid cells, in vitro	1,650	NT	–	Chang et al. (1992)

Table 4-16. CH genotoxicity: mammalian systems—all genetic endpoints, in vitro (continued)

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference
		With activation	Without activation	
Gene mutation, *tk and hprt* locus, human lymphoblastoid	1,000	NT	+	Beland (1999)
SCEs, human lymphocytes, in vitro	54	NT	(+)	Gu et al. (1981a)
Micronucleus formation, human lymphocytes, in vitro	100	–	+	Van Hummelen and Kirsch-Volders (1992)
Micronucleus formation, human lymphoblastoid AHH-1 cell line, in vitro	100	NT	+	Parry et al. (1996)
Micronucleus formation, human lymphoblastoid maximum contaminant level-5 cell line, in vitro	500	NT	–	Parry et al. (1996)
Micronucleus formation (kinetochore-positive), human diploid LEO fibroblasts, in vitro	120	NT	+	Bonatti et al. (1992)
Aneuploidy (double Y induction), human lymphocytes, in vitro	250	NT	+	Vagnarelli et al. (1990)
Aneuploidy (hyperdiploidy and hypodiploidy), human lymphocytes in vitro	50	NT	+	Sbrana et al. (1993)
Polyploidy, human lymphocytes, in vitro	137	NT	+	Sbrana et al. (1993)
C-Mitosis, human lymphocytes, in vitro	75	NT	+	Sbrana et al. (1993)

[a]LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests.

+ = positive; (+) = weakly positive in an inadequate study; – = negative; NT = not tested

Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

Table 4-17. CH genotoxicity: mammalian systems—all genetic damage, in vivo

Test system/endpoint	Doses (LED or HID)[a]	Results	Reference
DNA SSBs, male Sprague-Dawley rat liver	300, oral	+	Nelson and Bull (1988)
DNA SSBs, male F344 rat liver	1,650, oral	–	Chang et al. (1992)
DNA SSBs, male B6C3F_1 mouse liver	100, oral	+	Nelson and Bull (1988)
DNA SSBs, male B6C3F_1 mouse liver	825, oral	–	Chang et al. (1992)
Micronucleus formation, male and female NMRI mice, bone-marrow erythrocytes	500, i.p.	–	Leuschner and Leuschner (1991)
Micronucleus formation, BALB/c mouse spermatids	83, i.p.	–	Russo and Levis (1992b)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes and early spermatids	83, i.p.	+	Russo and Levis (1992a)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes	200, i.p.	+	Russo et al. (1992)
Micronucleus formation, male F1 mouse bone-marrow erythrocytes	400, i.p.	–	Leopardi et al. (1993)
Micronucleus formation, C57B1 mouse spermatids	41, i.p.	+	Allen et al., 1994
Micronucleus formation, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazzini et al., (1994)
Micronucleus formation, B6C3F_1 mouse spermatids after spermatogonial stem-cell treatment	165, i.p.	+	Nutley et al. (1996)
Micronucleus formation, B6C3F_1 mouse spermatids after meiotic cell treatment	413, i.p.	–	Nutley et al. (1996)
Micronucleus formation, male F1, BALB/c mouse peripheral-blood erythrocytes	200, i.p.	–	Grawe et al. (1997)
Micronucleus formation, male B6C3F_1 mouse bone-marrow erythrocytes	500, i.p., × 3	+	Beland (1999)
Micronucleus formation, infants, peripheral lymphocytes	50, oral	+	Ikbal et al. (2004)
Chromosomal aberrations, male and female F1 mouse bone marrow cells	600, i.p.	–	Xu and Alder (1990)
Chromosomal aberrations, male and female Sprague-Dawley rat bone-marrow cells	1,000, oral	–	Leuschner and Leuschner (1991)
Chromosomal aberrations, BALB/c mouse spermatogonia treated	83, i.p.	–	Russo and Levis, (1992a)
Chromosomal aberrations, F1 mouse secondary spermatocytes	82.7, i.p.	+	Russo et al. (1984)
Chromosomal aberrations, male Swiss CD-1 mouse bone-marrow erythrocytes	400, i.p.	–	Marrazzini et al. (1994)
Chromosomal aberrations, ICR mouse oocytes	600, i.p.	–	Mailhes et al. (1993)

Table 4-17. CH genotoxicity: mammalian systems—all genetic damage, in vivo (continued)

Test system/endpoint	Doses (LED or HID)[a]	Results	Reference
Micronucleus formation, infants, peripheral lymphocytes	50, oral	+	Ikbal et al. (2004)
Polyploidy, male and female F1, mouse bone-marrow cells	600, i.p.	–	Xu and Alder (1990)
Aneuploidy F1 mouse secondary spermatocytes	200, i.p.	+	Miller and Adler (1992)
Aneuploidy, male F1 mouse secondary spermatocytes	400, i.p.	–	Leopardi et al. (1993)
Hyperploidy, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazzini et al. (1994)

[a]LED = lowest effective dose; HID = highest ineffective dose; doses are in mg/kg body weight for in vivo tests.

+ = positive; – = negative

Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

4.2.4.2. Bacterial and Fungal Systems—Gene Mutations

CH induced gene mutations in *S. typhimurium* TA100 and TA104 strains, but not in most other strains assayed. Four of six studies of CH exposure in *S. typhimurium* TA100 and two of two studies in *S. typhimurium* TA104 were positive for revertants (Beland, 1999; Giller et al., 1995; Ni et al., 1994; Haworth et al., 1983). Waskell (1978) studied the effect of CH along with TCE and its other metabolites. CH was tested at different doses (1.0–13 mg/plate) in different *S. typhimurium* strains (TA98, TA100, TA1535) for gene mutations using Ames assay. No revertant colonies were observed in strains TA98 or TA1535 both in the presence and absence of S9 mix. Similar results were obtained by Leuschner and Leuschner (1991). However, in TA100, a dose-dependent statistically significant increase in revertant colonies was obtained both in the presence and absence of S9. It should be noted that CH that was purchased from Sigma was recrystallized 1–6 times from chloroform and the authors describe this as crude CH. However, this positive result is consistent with other studies in this strain as noted above. Furthermore, Giller et al. (1995) studied CH genotoxicity in three short-term tests. Chloral-induced mutations in strain TA100 of *S. typhimurium* (fluctuation test). Similar results were obtained by Haworth et al. (1983). These are consistent with several studies of TCE, in which low, but positive responses were observed in the TA100 strain in the presence of S9 metabolic activation, even when genotoxic stabilizers were not present.

A significant increase in mitotic segregation was observed in *A. nidulans* when exposed to 5 and 10 mM CH (Crebelli et al., 1985). Studies of mitotic crossing-over in *A. nidulans* have been negative, while these same studies were positive for aneuploidy (Crebelli et al., 1991; Kappas, 1989; Käfer, 1986; Crebelli et al., 1985).

Two studies were conducted in *S. cerevisiae* to understand the chromosomal malsegregation as a result of exposure to CH (Albertini, 1990; Sora and Agostini Carbone, 1987). CH (1–25 mM) was dissolved in sporulation medium and the frequencies of various meiotic events such as recombination and disomy were analyzed. CH inhibited sporulation as a function of dose and increased diploid and disomic clones. CH was also tested for mitotic chromosome malsegregation using *S. cerevisiae* D61.M (Albertini, 1990). The tester strain was exposed to a dose range of 1–8 mg/mL. An increase in the frequency of chromosomal malsegregation was observed as a result of exposure to CH.

Limited analysis of CH mutagenicity has been performed in *Drosophila* (Beland, 1999; Zordan et al., 1994). Of these two studies, CH was positive in the somatic mutation wing spot test (Zordan et al., 1994) and equivocal in the induction of sex-linked lethal mutation when in feed but negative when exposed via injection (Beland, 1999).

4.2.4.3. Mammalian Systems

4.2.4.3.1. Gene mutations

Harrington-Brock et al. (1998) noted that CH-induced concentration related cytotoxicity in TK± mouse lymphoma cell lines without S9 activation. A nonstatistical increase in mutant frequency was observed in cells treated with CH. The mutants were primarily small colony TK mutants, indicating that most CH-induced mutants resulted from chromosomal mutations rather than point mutations. It should be noted that in most concentrations tested (350–1,600 µg/mL), cytotoxicity was observed. Percentage cell survival ranged from 96 to 4%.

4.2.4.3.2. Micronucleus

Micronuclei induction following exposure to CH is positive in most test systems in both in vitro and in vivo assays, although some negative tests also exist (Harrington-Brock et al., 1998; Allen et al., 1994; Marrazzini et al., 1994) (Ikbal et al., 2004; Beland, 1999; Nesslany and Marzin, 1999; Grawé et al., 1997; Nutley et al., 1996; Parry et al., 1996; Giller et al., 1995; Leopardi et al., 1993; Lynch and Parry, 1993; Seelbach et al., 1993; Bonatti et al., 1992; Russo and Levis, 1992b, a; Russo et al., 1992; Van Hummelen and Kirsch-Volders, 1992; Leuschner and Leuschner, 1991; Degrassi and Tanzarella, 1988). Some studies have attempted to make inferences regarding aneuploidy induction or clastogenicity as an effect of CH. Aneuploidy results from defects in chromosome segregation during mitosis and is a common cytogenetic feature of cancer cells (see Section E.3.1.5).

Giller et al. (1995) studied CH genotoxicity in three short-term tests. CH caused a significant increase in the frequency of micronucleated erythrocytes following in vivo exposure of the amphibian, *Pleurodeles waltl*, newt larvae.

CH induced aneuploidy in vitro in multiple Chinese hamster cell lines (Natarajan et al., 1993; Warr et al., 1993; Furnus et al., 1990) and human lymphocytes (Sbrana et al., 1993; Vagnarelli et al., 1990) but not in mouse lymphoma cells (Harrington-Brock et al., (1998). In vivo studies performed in various mouse strains led to increased aneuploidy in spermatocytes (Miller and Adler, 1992; Liang and Pacchierotti, 1988; Russo et al., 1984), but not oocytes (Mailhes et al., (1993)) or bone marrow cells (Leopardi et al., 1993; Xu and Adler, 1990).

The potential of CH to induce aneuploidy in mammalian germ cells has been of particular interest since Russo et al. (1984) first demonstrated that CH treatment of male mice results in significant increase in frequencies of hyperploidy in metaphase II cells. This hyperploidy was thought to have arisen from chromosomal nondisjunction in premeiotic/meiotic cell division and may be a consequence of CH interfering with spindle formation (reviewed by Russo et al. (1984)] and Liang and Brinkley (1985)]). CH also causes meiotic delay, which may be associated with aneuploidy (Miller and Adler, 1992). CH has been shown to induce micronuclei but not structural chromosomal aberrations in mouse bone-marrow cells. Micronuclei induced by nonclastogenic agents are generally believed to represent intact chromosomes that failed to

segregate into either daughter-cell nucleus at cell division (Russo et al., 1992; Xu and Adler, 1990). Furthermore, CH-induced micronuclei in mouse bone-marrow cells (Russo et al., 1992) and in cultured mammalian cells (Bonatti et al., 1992; Degrassi and Tanzarella, 1988) have shown to be predominantly kinetochore-positive in composition upon analysis with immunofluorescent methods. The presence of a kinetochore in a micronucleus is considered evidence that the micronucleus contains a whole chromosome lost at cell division (Eastmond and Tucker, 1989; Degrassi and Tanzarella, 1988; Hennig et al., 1988). Therefore, both TCE and CH appear to increase the frequency of micronuclei.

Allen et al. (1994) treated male C57B1/6J mice were given a single i.p. injection of 0, 41, 83, or 165 mg/kg CH. Spermatids were harvested at 22 hours, and 11, 13.5, and 49 days following exposure (Allen et al., 1994). Harvested spermatids were processed to identify both kinetochore-positive micronucleus (aneugen) and kinetochore-negative micronucleus (clastogen). All CH doses administered 49 days prior to cell harvest were associated with significantly increased frequencies of kinetochore-negative micronuclei in spermatids, however; dose dependence was not observed. This study is in contrast with other studies (Bonatti et al., 1992; Degrassi and Tanzarella, 1988), which demonstrated predominantly kinetochore-positive micronucleus.

The ability of CH to induce aneuploidy and polyploidy was tested in human lymphocyte cultures established from blood samples obtained from two healthy nonsmoking donors (Sbrana et al., 1993). Cells were exposed for 72 and 96 hours at doses between 50 and 250 µg/mL. No increase in percentage hyperdiploid, tetraploid, or endoreduplicated cells were observed when cells were exposed to 72 hours at any doses tested. However, at 96 hours of exposure, significant increase in hyperdiploid was observed at one dose (150 µg/mL) and was not dose dependent. Significant increase in tetraploid was observed at a dose of 137 mg/mL, but again, no dose dependence was observed.

Ikbal et al. (2004) assessed the genotoxic effects in cultured peripheral blood lymphocytes of 18 infants (age range of 31–55 days) before and after administration of a single dose of CH (50 mg/kg of body weight) for sedation before a hearing test for micronucleus frequency. A significant increase in micronuclei frequency was observed after administration of CH.

4.2.4.3.3. Chromosomal aberrations

Several studies have included chromosomal aberration analysis in both in vitro and in vivo systems exposed to CH and have had positive results in vitro—although not all studies had statistically significant increases (Harrington-Brock et al., (1998); (Beland, 1999; Furnus et al., 1990).

Analysis of CH treated mouse lymphoma cell lines for chromosomal aberrations resulted in a nonsignificant increase in chromosomal aberrations (Harrington-Brock et al., (1998).

However, it should be noted that the concentrations tested (1,250 and 1,300 µg/mL) were cytotoxic (with a cell survival of 11 and 7%, respectively). Chinese hamster embryo cells were also exposed to 0.001, 0.002, and 0.003% CH for 1.5 hours (Furnus et al., 1990). A nonstatistically significant increase in frequency of chromosomal aberrations was observed only 0.002 and 0.003% concentrations, with the increase not dose-dependent. In this study, it should be noted that the cells were only exposed for 1.5 hours to CH and cells were allowed to grow for 48 hours (two cell cycles) to obtain similar mitotic index before analyzing for chromosomal aberrations. No information on cytotoxicity was provided except that higher doses decreased the frequency of mitotic cells at the time of fixation.

In vivo chromosome aberration studies have mostly reported negative or null results (Mailhes et al., 1993; Russo and Levis, 1992b, a; Leuschner and Leuschner, 1991; Xu and Adler, 1990; Liang and Pacchierotti, 1988) with the exception of one study (Russo et al., 1984) in an F1 cross of mouse strain between C57B1/Cne × C3H/Cne.

4.2.4.3.4. SCEs

SCEs were assessed by Ikbal et al. (2004) in cultured peripheral blood lymphocytes of 18 infants (age range of 31–55 days) before and after administration of a single dose of CH (50 mg/kg of body weight) for sedation before a hearing test. The authors report a significant increase in the mean number of SCEs, from before administration (7.03 ± 0.18 SCEs/cell) and after administration (7.90 ± 0.19 SCEs/cell), with each of the 18 individuals showing an increase with treatment. Micronuclei were also significantly increased. SCEs were also assessed by Gu et al. (1981a) in human lymphocytes exposed in vitro with inconclusive results, although positive results were observed by Beland (1999) in Chinese hamster ovary cells exposed in vitro with and without an exogenous metabolic system.

4.2.4.3.5. Cell transformation

CH was positive in the two studies designed to measure cellular transformation (Parry et al., 1996; Gibson et al., 1995). Both studies exposed Syrian hamster cells (embryo and dermal) to CH and induced cellular transformation.

4.2.4.4. Summary

CH has been reported to induce micronuclei formation, aneuploidy, and mutations in multiple in vitro systems and in vivo. In vivo studies have limited results to an increased micronuclei formation mainly in mouse spermatocytes. CH was positive in some in vitro genotoxicity assays that detected point mutations, micronuclei induction, chromosomal aberrations, and/or aneuploidy. The in vivo data exhibited mixed results (Allen et al., 1994) (Leuschner and Beuscher, 1998; Nutley et al., 1996; Adler, 1993; Mailhes et al., 1993; Russo et al., 1992; Xu and Adler, 1990). Most of the positive studies showed that CH induces

aneuploidy. Based on the existing array of data, CH has the potential to be genotoxic, particularly when aneuploidy is considered in the weight of evidence for genotoxic potential. Some have suggested that CH may act through a mechanism of spindle poisoning and resulting in numerical changes in the chromosomes, but some data also suggest induction of chromosomal aberrations. These results are consistent with TCE, albeit there are more limited data on TCE for these genotoxic endpoints.

4.2.5. DCVC and DCVG

DCVC and DCVG have been studied for their genotoxic potential; however, since there is a limited number of studies to evaluate them based on each endpoint, particularly in mammalian systems, the following section has been combined to include all of the available studies for different endpoints of genotoxicity. Study details can be found in Table 4-18.

DCVC and DCVG, cysteine intermediates of TCE formed by the GST pathway, are capable of inducing point mutations as evidenced by the fact that they are positive in the Ames assay. Dekant et al. (1986c) demonstrated mutagenicity of DCVC in *S. typhimurium* strains (TA100, TA2638, and TA98) using the Ames assay in the absence of S9. The effects were decreased with the addition of a beta-lyase inhibitor aminooxyacetic acid, suggesting that bioactivation by this enzyme plays a role in genotoxicity. Vamvakas et al. (1987) tested NAcDCVC for mutagenicity following addition of rat kidney cytosol and found genotoxic activity. Furthermore, Vamvakas (1988b), in another experiment, investigated the mutagenicity of DCVG and DCVC in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and AOAA (a beta-lyase inhibitor) to inhibit genotoxicity. DCVG and DCVC both exhibited direct-acting mutagenicity, with kidney mitochondria, cytosol, or microsomes enhancing the effects for both compounds and AOAA diminishing, but not abolishing the effects. Importantly, addition of liver subcellular fractions did not enhance the mutagenicity of DCVG, consistent with in situ metabolism playing a significant role in the genotoxicity of these compounds in the kidney.

Table 4-18. TCE GSH conjugation metabolites genotoxicity

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Gene mutations (Ames test)					
S. typhimurium, TA100, 2638, 98	0.1–0.5 nmol	ND	+	DCVC was mutagenic in all three strains of *S. typhimurium* without the addition of mammalian subcellular fractions.	Dekant et al., (1986c)
S. typhimurium, TA2638	50–300 nmol	+	+	Increase in number of revertants in DCVC alone at low doses; further increase in revertants was observed in the presence of microsomal fractions. Toxicity as indicated by decreased revertants per plate were seen at higher doses.	Vamvakas et al. (1988b)
Mutation analysis					
In vitro—rat kidney epithelial cells, LOH in *Tsc* gene	10 µM	NA	–	Only 1/9 transformed cells showed LOH.	Mally et al. (2006)
In vitro—rat kidney epithelial cells, *VHL* gene (exons 1–3)	10 µM	NA	–	No mutations in *VHL* gene. Note: *VHL* is not a target gene in rodent models of chemical-induced or spontaneous renal carcinogenesis.	Mally et al. (2006)
UDS					
Porcine kidney tubular epithelial cell line (LLC-PK1)	2.5 µM–5, 10, 15, 24 hrs; 2.5–100 µM	NA	+	Dose-dependent in UDS up to 24 hrs tested at 2.5 µM. Also, there was a dose-dependent increase at lower concentrations. Higher concentrations were cytotoxic as determined by LDH release from the cells.	Vamvakas et al. (1989)
Syrian hamster embryo fibroblasts		NA	+	Increase in UDS in treatment groups.	Vamvakas et al. (1988a)

Table 4-18. TCE GSH conjugation metabolites genotoxicity (continued)

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
DNA strand breaks					
Male rabbit renal tissue (perfused kidneys and proximal tubules)	0–100 mg/kg or 10 µM to 10 mM	ND	+	Dose-dependent increase in strand breaks in both i.v. and i.p. injections (i.v. injections were done only for 10 and 20 mg/kg) were observed. Perfusion of rabbit kidney (45-min exposure) and proximal tubules (30-min exposure) experiment resulted in a dose-dependent difference in the amount of SSBs.	Jaffe et.al. (1985)
Primary kidney cells from both male rats and human	1–4 mM; 20 hrs exposure	NA	+	Statistically significant increase in all doses (1, 2, or 4 mM) both in rats and human cells.	Robbiano et al. (2004)
In vivo—male Sprague-Dawley rats exposed to TCE or DCVC—comet assay	TCE: 500–2,000 ppm, inhalation, 6 hrs/d, 5 d DCVC: 1 or 10 mg/kg, single oral dose for 16 hrs	+ (DCVC) – (TCE)	NA	No significant increase in tail length in any of the TCE exposed groups. In 1, 2 hrs exposure—1 or 10 mg to DCVC—resulted in significant increase with no dose-response, but not at 16 hrs. In 2, ND for 1 mg, significant increase at 10 mg.	Clay (2008)
Micronucleus					
Syrian hamster embryo fibroblasts		NA	–	No micronucleus formation.	Vamvakas et al. (1988a)
Primary kidney cells from both male rats and human	1–4 mM; 20 hrs exposure	NA	+	Statistically significant increase in all doses (1, 2, and 4 mM) both in rat and human cells.	Robbiano et al. (2004)
Male Sprague-Dawley rats; proximal tubule cells (in vivo)	4 mM/kg TCE exposure, single dose	NA	+	Statistically significant increase in the average frequency of micronucleated kidney cells was observed.	Robbiano et al. (1998)
Cell transformation					
Kidney tubular epithelial cell line (LLC-PK1)	1or 5 µM; 7 wks	NA	+	Induced morphological cell transformation at both concentrations tested. Furthermore, cells maintained both biochemical and morphological alterations remained stable for 30 passages.	Vamvakas et al. (1996)
Rat kidney epithelial cells (in vitro)	10 µM; 24 hrs exposure, 7 wks post incubation	NA	+	Cell transformation was higher than control; however, cell survival percentage ranged from 39 to 64%, indicating cytotoxicity.	Mally et al. (2006)

Table 4-18. TCE GSH conjugation metabolites genotoxicity (continued)

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Gene expression					
Kidney tubular epithelial cell line (LLC-PK1)	1 or 5 μM clones, 30, 60, or 90 min	NA	+	Increased c-Fos expression in 1 and 5 μM exposed clones at three different times tested.	Vamvakas et al. (1996)
Kidney tubular epithelial cell line (LLC-PK1)		NA	+	Expression of c-Fos and c-Myc increased in a time-dependent manner.	Vamvakas et al. (1993)

LDH = lactate dehydrogenase; ND = not determined; NA = not applicable

While additional data are not available on DCVG or NAcDCVC, the genotoxicity of DCVC is further supported by the predominantly positive results in other available in vitro and in vivo assays. Jaffe et al. (1985) reported DNA strand breaks due to DCVC administered in vivo, in isolated perfused kidneys, and in isolated proximal tubules of albino male rabbits. Vamvakas et al. (1989) reported dose-dependent increases in UDS in LLC-PK1 cell clones at concentrations without evidence of cytotoxicity. In addition, Vamvakas et al. (1996) reported that 7-week DCVC exposure to LLC-PK1 cell clones at noncytotoxic concentrations induces morphological and biochemical de-differentiation that persists for at least 30 passages after removal of the compound. This study also reported increased expression of the proto-oncogene c-Fos in the cells in this system. In a Syrian hamster embryo fibroblast system, DCVC did not induce micronuclei, but demonstrated a UDS response (Vamvakas et al., 1988a).

Two more recent studies are discussed in more detail. Mally et al. (2006) isolated primary rat kidney epithelial cells from *Tsc–2*$^{Ek/+}$ (Eker) rats, and reported increased transformation when exposed to 10 µM DCVC, similar to that of the genotoxic renal carcinogens *N*-methyl-*N*‘-nitro-*N*-nitrosoguanidine (Horesovsky et al., 1994). The frequency was variable but consistently higher than background. No loss-of-heterozygosity (LOH) of the *Tsc-2* gene was reported either in these DCVC transformants or in renal tumors (which were not increased in incidence) from TCE-treated Eker rats, which Mally et al. (2006) suggested support a nongenotoxic mechanism because a substantial fraction of spontaneous renal tumors in Eker rats showed LOH at this locus (Yeung et al., 1995; Kubo et al., 1994) and because LOH was exhibited both in vitro and in vivo with 2,3,4-tris(glutathion-S-yl)-hydroquinone treatment in Eker rats (Yoon et al., 2001). However, 2,3,4-tris(glutathion-S-yl)-hydroquinone is not genotoxic in standard mutagenicity assays (Yoon et al., 2001), and Kubo et al. (1994) also reported that none of renal tumors induced by the genotoxic carcinogen, *N*-ethyl-*N*-nitrosourea, showed LOH. Therefore, the lack of LOH at the *Tsc-2* locus induced by DCVC in vitro, or TCE in vivo, reported by Mally et al. (2006) is actually more similar to the response from the genotoxic carcinogen *N*-ethyl-*N*-nitrosourea than the nongenotoxic carcinogen 2,3,4-tris-(glutathion-S-yl)-hydroquinone. Therefore, these data do not substantially contradict the body of evidence on DCVC genotoxicity.

Finally, Clay (2008) evaluated the genotoxicity of DCVC in vivo using the comet assay to assess DNA breakage in the proximal tubules of rat kidneys. Rats were exposed orally to a single dose of DCVC (1 or 10 mg/kg). The animals were sacrificed either 2 or 16 hours after dosing and samples were prepared for detecting the DNA damage. DCVC (1 and 10 mg/kg) induced no significant DNA damage in rat kidney proximal tubules at the 16-hour sampling time or after 1 mg/kg DCVC at the 2-hour sampling time. While Clay et al. (2008) concluded that these data were insufficient to indicate a positive response in this assay, the study did report a statistically significant increase in percentage tail DNA 2 hours after treatment with 10 mg/kg

DCVC, despite the small number of animals at each dose (n = 5) and sampling time. Therefore, these data do not substantially contradict the body of evidence on DCVC genotoxicity.

Overall, DCVC, and to a lesser degree DCVG and NAcDCVC, have demonstrated genotoxicity based on consistent results in a number of available studies. While some recent studies (Clay, 2008; Mally et al., 2006) have reported a lack of positive responses in some in vivo measures of genotoxicity with DCVC treatment, due to a number of limitations discussed above, these studies do not substantially contradict the body of evidence on DCVC genotoxicity. It is known that these metabolites are formed in vivo following TCE exposure, specifically in the kidney, so they have the potential to contribute to the genotoxicity of TCE, especially in that tissue. Moreover, DCVC and DCVG genotoxic responses were enhanced when metabolic activation using *kidney* subcellular fractions was used (Vamvakas et al., 1988b). Finally, the lack of similar responses in in vitro genotoxicity assays with TCE, even with metabolic activation, is likely the result of the small yield (if any) of DCVC under in vitro conditions, since in vivo, DCVC is likely formed predominantly in situ in the kidney while S9 fractions are typically derived from the liver. This hypothesis could be tested in experiments in which TCE is incubated with subcellular fractions from the kidney, or from both the kidney and the liver (for enhanced GSH conjugation).

4.2.6. TCOH

Limited studies are available on the effect of TCOH on genotoxicity (see Table 4-19). TCOH is negative in the *S. typhimurium* assay using the TA100 strain (DeMarini et al., 1994; Bignami et al., 1980; Waskell, 1978). A study by Beland (1999) using *S. typhimurium* strain TA104 did not induce reverse mutations without exogenous metabolic activation; however, it did increase mutant frequency in the presence of exogenous metabolic activation at a dose >2,500 μg/plate. TCOH has not been evaluated in the other recommended screening assays. Therefore, the database is limited for the determination of TCOH genotoxicity.

Table 4-19. Genotoxicity of TCOH

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference
		With activation	Without activation	
S. typhimurium TA100, 98, reverse mutation	7,500 µg/plate	–	–	Waskell (1978)
S. typhimurium TA100, reverse mutation	0.5 µg/cm^3 vapor	–	–	DeMarini et al. (1994)
S. typhimurium TA104, reverse mutation	2,500 µg/plate	+	–	Beland (1999)
S. typhimurium TA100, 1535 reverse mutation	NA	–	–	Bignami et al. (1980)
SCEs	NA	NA	+	Gu et al. (1981b)

[a]LED = lowest effective dose; HID = highest ineffective dose.

+ = positive; – = negative; NA = doses not available, results based on the abstract

4.2.7. Synthesis and Overall Summary

TCE and its metabolites (TCA, DCA, CH, DCVC, DCVG, and TCOH) have been evaluated to varying degrees for their genotoxic activity in several in vitro systems such as bacteria, yeast, and mammalian cells, as well as in in vivo systems.

There are several challenges in interpreting the genotoxicity results obtained from TCE exposure. For example, some studies in bacteria should be interpreted with caution if conducted using technical-grade TCE since it may contain known bacterial mutagens in trace amounts as stabilizers (e.g., 1,2-epoxybutane and epichlorohydrin). Because of the volatile nature of TCE, there could be false negative results if proper precautions are not taken to limit evaporation, such as the use of a closed, sealed system. The adequacy of the enzyme-mediated activation of TCE in vitro tests is another consideration. For example, it is not clear if standard S9 fractions can adequately recapitulate the complex in vivo metabolism of TCE to reactive intermediates, which in some cases entails multiple sequential steps involving multiple enzyme systems (e.g., CYP, GST, etc.) and interorgan processing (as is described in more detail in Section 3.3). In addition, the relative potency of the metabolites in vitro may not necessarily inform their relative contribution to the overall mechanistic effects of the parent compound, TCE. Furthermore, although different assays provided data relevant to different types of genotoxic endpoints, not all effects that are relevant for carcinogenesis are encompassed. The standard battery of prokaryotic as well as mammalian genotoxicity test protocols typically specify the inclusion of significantly cytotoxic concentrations of the test compound.

With respect to potency, several TCE studies have been conducted along with numerous other chlorinated compounds and the results interpreted as a comparison of the group of compounds tested (relative potency). However, for the purposes of hazard characterization, such comparisons are not informative—particularly if they are not necessarily correlated with in vivo

carcinogenic potency. Also, differentiating the effects of TCE with respect to its potency can be influenced by many factors such as the type of cells, their differing metabolic capacities, sensitivity of the assay, need for greater concentration to show any effect, interpretation of data when the effects are marginal, and gradation of severity of effects.

Also, type of samples used, methodology used for the isolation of genetic material, and duration of exposure can particularly influence the results of several studies. This is particularly true for human epidemiological studies. For example, while some studies use tissues obtained directly from the patients, others use formalin fixed tissues sections to isolate DNA for mutation detection. Type of fixing solution, fixation time, and period of storage of the tissue blocks often affect the quality of DNA. Formic acid contained in the formalin solution or picric acid contained in Bouin's solution is known to degrade nucleic acids resulting in either low yield or poor quality of DNA. In addition, during collection of tumor tissues, contamination of neighboring normal tissue can easily occur if proper care is not exercised. This could lead to the _dilution effect' of the results (i.e., because of the presence of some normal tissue) frequency of mutations detected in the tumor tissue can be lower than expected. Due to some of these technical difficulties in obtaining proper material (DNA) for the detection of mutation, the results of these studies should be interpreted cautiously.

The following synthesis, summary, and conclusions focus on the available studies that may provide some insight into the potential genotoxicity of TCE considering the above challenges when interpreting the mutagenicity data for TCE.

Overall, evidence from a number of different analyses and a number of different laboratories using a fairly complete array of endpoints suggests that TCE, following metabolism, has the potential to be genotoxic. TCE has a limited ability to induce mutation in bacterial systems, but greater evidence of potential to bind or to induce damage in the structure of DNA or the chromosome in a number of targets. A series of carefully controlled studies evaluating TCE itself (without mutagenic stabilizers and without metabolic activation) found it to be incapable of inducing gene mutations in most standard mutation bacterial assays (Mortelmans et al., 1986; Shimada et al., 1985; Crebelli et al., 1982; Baden et al., 1979; Bartsch et al., 1979; Waskell, 1978; Henschler et al., 1977; Simmon et al., 1977). Therefore, it appears that it is unlikely that TCE is a direct-acting mutagen, though TCE has shown potential to affect DNA and chromosomal structure. TCE is also positive in some, but not all, fungal and yeast systems (Koch et al., 1988; Crebelli et al., 1985; Rossi et al., 1983; Callen et al., 1980). Data from human epidemiological studies support the possible mutagenic effect of TCE leading to *VHL* gene damage and subsequent occurrence of RCC. Association of increased *VHL* mutation frequency in TCE-exposed RCC cases has been observed (Brauch et al., 2004; Brauch et al., 1999; Brüning et al., 1997b).

TCE can lead to binding to nucleic acids and proteins (Kautiainen et al., 1997; Mazzullo et al., 1992; Bergman, 1983; Miller and Guengerich, 1983; DiRenzo et al., 1982), and such

binding appears to be due to conversion to one or more reactive metabolites. For instance, increased binding was observed in samples bioactivated with mouse and rat microsomal fractions (Mazzullo et al., 1992; Miller and Guengerich, 1983; DiRenzo et al., 1982; Banerjee and Van Duuren, 1978). DNA binding is consistent with the ability to induce DNA and chromosomal perturbations. Several studies report the induction of micronuclei in vitro and in vivo from TCE exposure (Hu et al., 2008; Robbiano et al., 2004; Wang et al., 2001; Hrelia et al., 1994; Kligerman et al., 1994). Reports of SCE induction in some studies are consistent with DNA effects, but require further study (Kligerman et al., 1994; Nagaya et al., 1989b; Gu et al., 1981a; Gu et al., 1981b; White et al., 1979).

TCA, an oxidative metabolite of TCE, exhibits little, if any genotoxic activity in vitro. TCA did not induce mutations in *S. typhimurium* strains in the absence of metabolic activation or in an alternative protocol using a closed system (Kargalioglu et al., 2002; Nelson et al., 2001; Giller et al., 1997; DeMarini et al., 1994; Rapson et al., 1980; Waskell, 1978), but a mutagenic response was induced in TA100 in the Ames fluctuation test (Giller et al., 1997). However, in vitro experiments with TCA should be interpreted with caution if steps have not been taken to neutralize pH changes caused by the compound (Mackay et al., 1995). Measures of DNA-repair responses in bacterial systems have shown induction of DNA repair reported in *S. typhimurium* but not in *E. coli*. Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations (Harrington-Brock et al., 1998). TCA was positive in some genotoxicity studies in vivo mouse, newt, and chick test systems (Giller et al., 1997; Bhunya and Jena, 1996; Birner et al., 1994; Bhunya and Behera, 1987). DNA unwinding assays have either shown TCA to be much less potent than DCA (Nelson and Bull, 1988) or negative (Styles et al., 1991; Nelson et al., 1989). Due to limitations in the genotoxicity database, the possible contribution of TCA to TCE genotoxicity is unclear.

DCA, a chloroacid metabolite of TCE, has also been studied using different types of genotoxicity assays. Although limited studies are conducted for different genetic endpoints, DCA has been demonstrated to be mutagenic in the *S. typhimurium* assays, in vitro (Kargalioglu et al., 2002; Plewa et al., 2002; DeMarini et al., 1994) in some strains, mouse lymphoma assay, (Harrington-Brock et al., 1998) in vivo cytogenetic tests (Leavitt et al., 1997; Fuscoe et al., 1996), the micronucleus induction test, the Big Blue mouse system, and other tests (Harrington-Brock et al., 1998; Leavitt et al., 1997; Fuscoe et al., 1996; DeMarini et al., 1994; Chang et al., 1992; Nelson et al., 1989; Nelson and Bull, 1988; Bignami et al., 1980). DCA can cause DNA strand breaks in mouse and rat liver cells following in vivo exposure in mice and rats (Fuscoe et al., 1996). Because of uncertainties as to the extent of DCA formed from TCE exposure, inferences as to the possible contribution from DCA genotoxicity to TCE toxicity are difficult to make.

CH is mutagenic in the standard battery of screening assays. Effects include positive results in bacterial mutation tests for point mutations and in the mouse lymphoma assay for

mutagenicity at the Tk locus (Haworth et al., 1983). In vitro tests showed that CH also induced micronuclei and aneuploidy in human peripheral blood lymphocytes and Chinese hamster pulmonary cell lines. Micronuclei were also induced in Chinese hamster embryonic fibroblasts. Several studies demonstrate that CH induces aneuploidy (loss or gain of whole chromosomes) in both mitotic and meiotic cells, including yeast (Gualandi, 1987; Sora and Agostini Carbone, 1987; Käfer, 1986; Singh and Sinha, 1979, 1976), cultured mammalian somatic cells (Degrassi and Tanzarella, 1988), and spermatocytes of mice (Liang and Pacchierotti, 1988; Russo et al., 1984). CH was negative for sex-linked recessive lethal mutations in *Drosophila* (Yoon et al., 1985). It induces SSBs in hepatic DNA of mice and rats (Nelson and Bull, 1988) and mitotic gene conversion in yeast (Bronzetti et al., 1984). Schatten and Chakrabarti (1998) showed that CH affects centrosome structure, which results in the inability to reform normal microtubule formations and causes abnormal fertilization and mitosis of sea urchin embryos. Based on the existing array of data, CH has the potential to be genotoxic, particularly when aneuploidy is considered in the weight of evidence for genotoxic potential. CH appears to act through a mechanism of spindle poisoning, resulting in numerical changes in the chromosomes. These results are consistent with TCE, albeit there are limited data on TCE for these genotoxic endpoints.

DCVC, and to a lesser degree DCVG, has demonstrated bacterial mutagenicity based on consistent results in a number of available studies (Vamvakas et al., 1988b; Vamvakas et al., 1987; Dekant et al., 1986c). DCVC has demonstrated a strong, direct-acting mutagenicity both with and without the presence of mammalian activation enzymes. It is known that these metabolites are formed in vivo following TCE exposure, so they have the potential to contribute to the genotoxicity of TCE. The lack of similar response in bacterial assays with TCE is likely the result of the small yield (if any) of DCVC under in vitro conditions, since in vivo, DCVC is likely formed predominantly in situ in the kidney (S9 fractions are typically derived from the liver). DCVC and DCVG have not been evaluated extensively in other genotoxicity assays, but the available in vitro and in vivo data are predominantly positive. For instance, several studies have reported that DCVC can induce primary DNA damage in mammalian cells in vitro and in vivo (Clay, 2008; Vamvakas et al., 1989; Jaffe et al., 1985). Long-term exposure to DCVC-induced de-differentiation of cells (Vamvakas et al., 1996). It has been shown to induce expression of the protooncogene c-Fos (Vamvakas et al., 1996) and cause cell transformation in rat kidney cells (Mally et al., 2006). In LLC-PK1 cell clones, DCVC was reported in induce UDS, but not micronuclei (Vamvakas et al., 1988a). Finally, DCVC-induced transformation in kidney epithelial cells isolated from Eker rats carrying the heterozygous *Tsc-2* mutations (Mally et al., 2006). Moreover, the lack of LOH at the *Tsc-2* locus observed in exposed cells does not constitute negative evidence of DCVC genotoxicity, as none of renal tumors induced in Eker rats by the genotoxic carcinogen *N*-ethyl-*N*-nitrosourea showed LOH (Kubo et al., 1994).

In support of the importance of metabolism, there is some concordance between effects observed from TCE and those from several metabolites. For instance, both TCE and CH have been shown to induce micronuclei in mammalian systems, but chromosomal aberrations have been more consistently observed with CH than with TCE. The role of TCA in TCE genotoxicity is less clear, as there is less concordance between the results from these two compounds. Finally, several other TCE metabolites show at least some genotoxic activity, with the strongest data from DCA, DCVG, and DCVC. While quantitatively smaller in terms of flux as compared to TCA and TCOH (for which there is almost no genotoxicity data), these metabolites may still be toxicologically important.

Thus, uncertainties with regard to the characterization of TCE genotoxicity remain, particularly because not all TCE metabolites have been sufficiently tested in the standard genotoxicity screening battery to derive a comprehensive conclusion. However, the metabolites that have been tested, particularly DCVC, have predominantly resulted in positive data, although to a lesser extent in DCVG and NAcDCVC, This supports the conclusion that these compounds are genotoxic, particularly in the kidney, where in situ metabolism produces and/or bioactivates these TCE metabolites.

4.3. CENTRAL NERVOUS SYSTEM (CNS) TOXICITY

TCE exposure results in CNS effects in both humans and animals that can result from acute, subchronic, or chronic exposure. There are studies indicating that TCE exposure results in CNS tumors and this discussion can be found in Section 4.9. The studies discussed in this section focus on the most critical neurological effects that were extracted from the neurotoxicological literature. Although there are several studies and reports that have evaluated TCE as an anesthetic, those studies were not included in this section because of the high exposure levels in comparison to the selected critical neurological effects described below. The critical neurological effects are nerve conduction changes, sensory effects, cognitive deficits, changes in psychomotor function, and changes in mood and sleep behaviors. The selection criteria that were used to determine study importance included study design and validity, pervasiveness of neurological effect, and for animal studies, the relevance of these reported outcomes in humans. More detailed information on human and animal neurological studies with TCE can be found in Appendix D.

4.3.1. Alterations in Nerve Conduction

4.3.1.1. Trigeminal Nerve Function: Human Studies

A number of human studies have been conducted that examined the effects of occupational or drinking water exposures to TCE on trigeminal nerve function (see Table 4-20). Many studies reported that humans exposed to TCE present trigeminal nerve function abnormalities as measured by blink reflex and masseter reflex test measurements (Kilburn,

2002a, b; Kilburn and Warshaw, 1993a; Feldman et al., 1992; Feldman et al., 1988). The blink and masseter reflexes are mediated primarily by the trigeminal nerve and changes in measurement suggest impairment in nerve conduction. Other studies measured the trigeminal somatosensory evoked potential (TSEP) following stimulation of the trigeminal nerve and reported statistically significantly delayed response on evoked potentials among exposed subjects compared to nonexposed individuals (Mhiri et al., 2004; Barret et al., 1987; Barret et al., 1984; Barret et al., 1982). Two studies that also measured trigeminal nerve function did not find any effect (Rasmussen et al., 1993a; El Ghawabi et al., 1973), but the methods were not provided in either study (Rasmussen et al., 1993a; El Ghawabi et al., 1973) or an appropriate control group was not included (Rasmussen et al., 1993a). These studies and results are described below and summarized in detail in Table 4-20.

Table 4-20. Summary of human trigeminal nerve and nerve conduction velocity studies

Reference[a]	Subjects	Exposure	Effect
Barret et al. (1982)	11 workers with chronic TCE exposure. Controls: 20 unexposed subjects.	Presence of TCE and TCA found through urinalysis. Atmospheric TCE concentrations and duration of exposure not reported in paper.	Following stimulation of the trigeminal nerve, significantly higher voltage stimuli was required to obtain a normal response and there was a significant increase in latency for response and decreased response amplitude.
Barret et al. (1984)	188 factory workers. No unexposed controls; lowest exposure group used as comparison.	>150 ppm; n = 54 < 150 ppm; n = 134. 7 hrs/d for 7 yrs.	Trigeminal nerve and optic nerve impairment, asthenia and dizziness were significantly increased with exposure.
Barret et al. (1987)	104 degreaser machine operators. Controls: 52 unexposed subjects Mean age 41.6 yrs.	Mean duration, 8.2 yrs, average daily exposure 7 hrs/d. Average TCOH range = 162–245 mg/g creatinine. Average TCA range = 93–131 mg/g creatinine.	Evoked trigeminal responses were measured following stimulation of the nerve and revealed increased latency to respond, amplitude or both and correlated with length of exposure ($p < 0.01$) and with age ($p < 0.05$), but not concentration.
El-Ghawabi et al. (1973)	30 money printing shop workers. Controls: 20 nonexposed males. 10 workers exposed to inks not containing TCE.	Mean TCE air concentrations ranged from 41 to 163 ppm. Exposure durations: <1 yr: n = 3 1 yr: n = 1 2 yrs: n = 2 3 yrs: n = 11 4 yrs: n = 4 ≥5 yrs: n = 9	No effect on trigeminal nerve function was noted.
Feldman et al. (1988)	21 Woburn, Massachusetts residents. 27 controls.	TCE maximum reported concentration in well water was 267 ppb; other solvents also present. Exposure duration 1−12 yrs.	Measurement of the blink reflex as mediated by the trigeminal nerve resulted in significant increases in the latency of reflex components ($p < 0.001$).

Table 4-20. Summary of human trigeminal nerve and nerve conduction velocity studies (continued)

Reference[a]	Subjects	Exposure	Effect
Feldman et al. (1992)	18 workers. 30 controls.	TCE exposure categories of —extensie," "occasional," and —chemical other than TCE." —Extensie" = chronically exposed (≥1 yr) to TCE for 5 d/wk and >50% workday. —Occpational" = chronically exposed to TCE for 1–3 d/wk and >50% workday.	The blink reflex as mediated by the trigeminal was measured. The —extensie" group revealed latencies >3 SDs above the nonexposed group mean on blink reflex components.
Kilburn and Warshaw (1993a)	160 residents living in Southwest Tucson with TCE, other solvents, and chromium in groundwater. Control: 113 histology technicians from a previous study (Kilburn and Warshaw, 1992b; Kilburn et al., 1987).	>500 ppb of TCE in well water before 1981 and 25–100 ppb afterwards. Duration ranged from 1 to 25 yrs.	Significant impairments in sway speed with eyes open and closed and blink reflex latency (R-1), which suggests trigeminal nerve impairment.
Kilburn (2002b, 2002a)	236 residents near a microchip plant in Phoenix, Arizona. Controls: 161 regional referents from Wickenburg, Arizona and 67 referents in northeastern Phoenix.	<0.2–10,000 ppb of TCE, <0.2–260,000 ppb TCA, <0.2–6,900 ppb 1,1-DCE, <0.2–1,600 ppb 1,2-DCE, <0.2–23,000 ppb perchloroethylene, <0.02–330 ppb vinyl chloride in well water. Exposure duration 2−37 yrs.	Trigeminal nerve impairment as measured by the blink reflex test; both right and left blink reflex latencies (R-1) were prolonged. Exposed group mean 14.2 + 2.1 ms (right) or 13.9 + 2.1 ms (left) vs. referent group mean of 13.4 + 2.1 ms (right) or 13.5 + 2.1 ms (left), $p = 0.0001$ (right) and 0.008 (left).
Mhiri et al. (2004)	**23 phosphate industry workers.** **Controls: 23 unexposed workers.**	Exposure ranged from 50 to 150 ppm, for 6 hrs/d for at least 2 yrs. Mean urinary TCOH and TCA levels were 79.3 ± 42 and 32.6 ± 22 mg/g creatinine.	**TSEPs were recorded. Increase in the TSEP latency was observed in 15/23 (65%) workers.**
Rasmussen et al. (1993a)	96 Danish metal degreasers. Age range: 19–68. No unexposed controls; low exposure group used as comparison.	Average exposure duration: 7.1 yrs); range of full-time degreasing: 1 mo to 36 yrs. Exposure to TCE or to CFC113: (1) Low exposure: n = 19, average full-time exposure 0.5 yr. (2) Medium exposure: n = 36, average full-time exposure 2.1 yrs. (3) High exposure: n = 41, average full-time exposure 11 yrs. TCA in high exposure group = 7.7 mg/L (maximum = 26.1 mg/L).	No statistically significant trend on trigeminal nerve function, although some individuals had abnormal function.

Table 4-20. Summary of human trigeminal nerve and nerve conduction velocity studies (continued)

Reference[a]	Subjects	Exposure	Effect
Ruijten et al. (1991)	**31 male printing workers. Mean age 44 yrs; mean duration 16 yrs. Controls: 28 unexposed; mean age 45 yrs.**	Mean cumulative exposure = 704 ppm × yr (SD 583, range: 160–2,150 ppm × yr. Mean, 17 ppm at time of study; historic TCE levels from 1976 to 1981, mean of 35 ppm. Mean duration of 16 yrs.	Measurement of trigeminal nerve function by using the blink reflex resulted in no abnormal findings. Increased latency in the masseter reflex is indicative of trigeminal nerve impairment.
Triebig et al. (1982)	24 workers (20 males, 4 females) occupationally exposed—ages 17–56. Controls: 144 individuals to establish normal nerve conduction parameters. Matched group: 24 unexposed workers (20 males, 4 females).	Exposure duration of 1–258 mo (mean 83 mo). Air exposures were between 5 and 70 ppm.	No statistically significant difference in nerve conduction velocities between the exposed and unexposed groups.
Triebig et al. (1983)	66 workers occupationally exposed. Control: 66 workers not exposed to solvents.	Subjects were exposed to a mixture of solvents, including TCE.	Exposure-response relationship observed between length of solvent exposure and statistically significant reduction in mean sensory ulnar nerve conduction velocities.

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

Integrity of the trigeminal nerve is commonly measured using blink and masseter reflexes. Five studies (Kilburn, 2002b, a; Kilburn and Warshaw, 1993a; Feldman et al., 1992; Feldman et al., 1988; Barret et al., 1984) reported a significant increase in the latency to respond to the stimuli generating the reflex. The latency increases in the blink reflex ranged from 0.4 ms (Kilburn, 2002b, a) to up to 3.44 ms (Feldman et al., 1988). The population groups in these studies were exposed by inhalation occupationally (Barret et al., 1984) and through drinking water environmentally (Kilburn, 2002b, a; Kilburn and Warshaw, 1993a; Feldman et al., 1988). Feldman et al. (1992) demonstrated persistence in the increased latency of the blink reflex response. In one subject, exposure to TCE (levels not reported by authors) occurred through a degreasing accident (high and acute exposure), and increased latency response times persisted 20 years after the accident. Another two subjects, evaluated at 9 months and 1 month following a high occupational exposure (exposure not reported by authors), also had higher blink reflex latencies with an average increase of 2.8 ms over the average response time in the control group used in the study. Although one study (Ruijten et al., 1991) did not find these increases in male printing workers exposed to TCE, this study did find a statistically significant average increase of 0.32 ms ($p < 0.05$) in the latency response time in TCE-exposed workers on the masseter reflex test, another test commonly used to measure the integrity of the trigeminal nerve.

Three studies (Mhiri et al., 2004; Barret et al., 1987; Barret et al., 1982), adopting TSEPs to measure trigeminal nerve function, found significant abnormalities in these evoked potentials. These studies were conducted on volunteers who were occupationally exposed to TCE through metal degreasing operations (Barret et al., 1987; Barret et al., 1982) or through cleaning tanks in the phosphate industry (Mhiri et al., 2004). Barret et al. (1982) reported that in 8/11 workers, an increased voltage ranging from a 25 to a 45 volt increase was needed to generate a normal TSEP and two of workers had an increased TSEP latency. Three out of 11 workers had increases in TSEP amplitudes. In a later study, Barret et al. (1987) also reported abnormal TSEPs (increased latency and/or increased amplitude) in 38% of the degreasers who were evaluated. The individuals with abnormal TSEPs were significantly older (45 vs. 40.1 years; $p < 0.05$) and were exposed to TCE longer (9.9 vs. 5.6 years; $p < 0.01$). Mhiri et al. (2004) was the only study to evaluate individual components of the TSEP and noted significant increases in latencies for all TSEP potentials (N1, P1, N2, P2, N3; $p < 0.01$) and significant decreases in TSEP amplitude (P1, $p < 0.02$; N2, $p < 0.05$). A significant positive correlation was demonstrated between exposure duration and increased TSEP latency ($p < 0.02$).

Two studies reported no statistically significant effect of TCE exposure on trigeminal nerve function (Rasmussen et al., 1993a; El Ghawabi et al., 1973). El-Ghawabi et al. (1973) conducted a study on 30 money printing shop workers occupationally exposed to TCE. Trigeminal nerve involvement was not detected, but the authors did not include the experimental methods that were used to measure trigeminal nerve involvement and did not provide any data as to how this assessment was made. Rasmussen et al. (1993a) conducted a historical cohort study on 99 metal degreasers, 70 exposed to TCE and 29 to the fluorocarbon, CFC113. It was reported that 1/21 people (5%) in the low exposure group, 2/37 (5%) in the medium exposure group, and 4/41 (10%) in the high-exposure group experienced abnormalities in trigeminal nerve sensory function, with a linear trend test p-value of 0.42. The mean urinary TCA concentration was reported for the high-exposure group only and was 7.7 mg/L (maximum concentration, 26.1 mg/L). The trigeminal nerve function findings of high-exposure group subjects were compared to that of the low-exposure group since this study did not include an unexposed or non-TCE exposed group, and decreased the sensitivity of the study.

4.3.1.2. Nerve Conduction Velocity—Human Studies

Two occupational studies assessed ulnar and median nerve function using tests of conduction latencies (Triebig et al., 1983; Triebig et al., 1982) (see Table 4-20). The ulnar nerve and median nerves are major nerves located in the arm and forearm. Triebig (1982) studied 24 healthy workers (20 males, 4 females) exposed to TCE occupationally (5–70 ppm) at three different plants and did not find statistically significant differences in ulnar or median nerve conduction velocities between exposed and unexposed subjects. This study measured exposure data, but exposures/responses were not reported by dose levels. The Triebig (1983) study is

similar in design to the previous study (Triebig et al., 1982), but with a larger number of subjects. In this study, a dose-response relationship was observed between lengths of exposure to mixed solvents that included TCE (at unknown concentration). A statistically significant reduction in nerve conduction velocities was observed for the medium- and long-term exposure groups for the sensory ulnar nerve as was a statistically significant reduction in mean nerve conduction velocity observed between exposed and control subjects.

4.3.1.3. Trigeminal Nerve Function: Laboratory Animal Studies

There is little evidence that TCE disrupts trigeminal nerve function in animal studies. Two studies demonstrated that TCE produces morphological changes in the trigeminal nerve at a dose of 2,500 mg/kg-day for 10 weeks (Barret et al., 1992; Barret et al., 1991). However, dichloroacetylene, a degradation product formed during the volatilization of TCE, was found to produce more severe morphological changes in the trigeminal nerve and at a lower dose of 17 mg/kg-day (Barret et al., 1992; Barret et al., 1991). Only one study (Albee et al., 2006) evaluated the effects of TCE on trigeminal nerve function; a subchronic inhalation exposure did not result in any significant functional changes. A summary of these studies is provided in Table 4-21.

Table 4-21. Summary of animal trigeminal nerve studies

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL[b]	Effects
Barret et al. (1991)	Direct gastric administration	Rat, Sprague-Dawley, female, 7/group	0, 2.5 g/kg, acute administration. 17 mg/kg dichloroacetylene.	LOAEL: 2.5 g/kg	Morphometric analysis was used for analyzing the trigeminal nerve. Increases in external and internal fiber diameter as well as myelin thickness were observed in the trigeminal nerve after TCE treatment.
Barret et al. (1992)	**Direct gastric administration**	**Rat, Sprague-Dawley, female, 7/group**	**0, 2.5 g/kg; one dose/d, 5 d/wk, 10 wks. 17 mg/kg dichloroacetylene**	**LOAEL: 2.5 g/kg**	**Trigeminal nerve analyzed using morphometric analysis. Increased internode length and fiber diameter in class A fibers of the trigeminal nerve observed with TCE treatment. Changes in fatty acid composition also noted.**
Albee et al. (1997)	Inhalation	Rat, F344, male, 6	0 or 300 ppm dichloro-acetylene, 2.25 hrs	LOAEL: 300 ppm dichloro-acetylene	Dichloroacetylene (TCE byproduct) exposure impaired the TSEP up to 4 d postexposure.
Albee et al. (2006)	Inhalation	Rat, F344, male and female, 10/sex/group	0, 250, 800, or 2,500 ppm	NOAEL: 2,500 ppm	No effect on TSEPs was noted at any exposure level.

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

[b]NOAEL = no-observed-adverse-effect level, LOAEL = lowest-observed-adverse-effect-level.

Barret et al. (1992; 1991) conducted two studies evaluating the effects of both TCE and dichloroacetylene on trigeminal nerve fiber diameter and internodal length as well as several markers for fiber myelination. Female Sprague-Dawley rats (n = 7/group) were dosed with 2,500 mg/kg TCE or 17 mg/kg-day dichloroacetylene by gavage for 5 days/week for 10 weeks. TCE-dosed animals only exhibited changes in the smaller Class A fibers where internode length increased marginally (<2%) and fiber diameter increased by 6%. Conversely, dichloroacetylene-treated rats exhibited significant and more robust decreases in internode length and fiber diameter in both fiber classes A (decreased 8%) and B (decreased 4%).

Albee et al. (2006) evaluated the effects of a subchronic inhalation TCE exposure in F344 rats (10/sex/group). Rats were exposed to 0, 250, 800, and 2,500 ppm TCE for 6 hours/day, 5 days/week for 13 weeks. TCE exposures were adequate to produce permanent auditory impairment even though TSEPs were unaffected. While TCE appears to be negative in disrupting the trigeminal nerve, the TCE breakdown product, dichloroacetylene, does impair trigeminal nerve function. Albee et al. (1997) showed that a single inhalation exposure of rats to 300-ppm dichloroacetylene, for 2.25 hours, disrupted trigeminal nerve evoked potentials for at least 4 days post exposure.

4.3.1.4. Discussion and Conclusions: TCE-Induced Trigeminal Nerve Impairment

Epidemiologic studies of exposure to TCE found impairment of trigeminal nerve function, assessed by the blink reflex test or the TSEP, in humans exposed occupationally by inhalation or environmentally by ingestion (see Table 4-20). Mean inhalational exposures inferred from biological monitoring or from a range of atmospheric monitoring in occupational studies was approximately 50–<150 ppm TCE exposure. Residence location is the exposure surrogate in geographical-based studies of contaminated water supplies with several solvents. Well water contaminant concentrations of TCE ranged from <0.2 to 10,000 ppb and do not provide an estimate of TCE concentrations in drinking water to studied individuals.

Two occupational studies, each including >100 subjects, reported statistically significant dose-response trends based on ambient TCE concentrations, duration of exposure, and/or urinary concentrations of the TCE metabolite TCA (Barret et al., 1987; Barret et al., 1984).

Three geographical-based studies of environmental exposures to TCE via contaminated drinking water are further suggestive of trigeminal nerve function decrements; however, these studies are more limited than occupational studies due to questions of subject selection. Both exposed subjects, who were litigants, and control subjects may not be representative of exposed (Kilburn, 2002b, a; Kilburn and Warshaw, 1993a); referents in Kilburn and Warshaw (1993a), were histology technicians and subjects in a previous study of formaldehyde and other solvent exposures and neurobehavioral effects (Kilburn and Warshaw, 1992b; Kilburn et al., 1987).

Results were mixed in a number of smaller studies. Two of these studies reported changes in

trigeminal nerve response (Mhiri et al., 2004; Barret et al., 1982), including evidence of a correlation with duration of exposure and increased latency in one study (Mhiri et al., 2004). Ruijten et al. (1991) reported no significant change in the blink reflex, but did report an increase in the latency of the masseter reflex, which also may reflect effects on the trigeminal nerve. Two other studies reported no observed effect on trigeminal nerve impairment, but the authors failed to provide assessment of trigeminal nerve function (Rasmussen et al., 1993a; El Ghawabi et al., 1973) or there was not a control (nonexposed) group included in the study (Rasmussen et al., 1993a). Therefore, because of limitations in statistical power, the possibility of exposure misclassification, and possible differences in measurement methods, these studies are not judged to provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment. Overall, the weight of evidence supports a relationship between TCE exposure and trigeminal nerve dysfunction in humans.

Impairment of trigeminal nerve function is observed in studies of laboratory animal studies. Although one subchronic animal study demonstrated no significant impairment of trigeminal nerve function following TCE exposure up to 2,500 ppm (no observed-adverse-effect level [NOAEL]) (Albee et al., 2006), morphological analysis of the nerve revealed changes in its structure (Barret et al., 1992; Barret et al., 1991). However, the dose at which an effect was observed by Barret et al. (1992; 1991) was high (2,500 mg/kg-day—lowest-observed-adverse-effect level [LOAEL]) compared to any reasonable occupational or environmental setting, although no lower doses were used. The acute or subchronic duration of these studies, as compared to the much longer exposure duration in many of the human studies, may also contribute to the apparent disparity between the epidemiologic and (limited) laboratory animal data.

The subchronic study of Barret et al. (1992) and the acute exposure study of Albee et al. (Albee et al., 1997) also demonstrated that dichloroacetylene, a (ex vivo) TCE degradation product, also induces trigeminal nerve impairment, at much lower doses than TCE. It is possible that under some conditions, co-exposure to dichloroacetylene from TCE degradation may contribute to the changes observed to be associated with TCE exposure in human studies, and this issue is discussed further below in Section 4.3.10.

Overall evidence from numerous epidemiologic studies supports a conclusion that TCE exposure induces trigeminal nerve impairment in humans. Laboratory animal studies provide limited additional support, and do not provide strong contradictory evidence. Persistence of these effects after cessation of exposure cannot be determined since exposure was ongoing in the available human and laboratory animal studies.

4.3.2. Auditory Effects

4.3.2.1. Auditory Function: Human Studies

The TCE Subregistry from the National Exposure Registry developed by the ATSDR was the subject of three studies (ATSDR, 2002; Burg and Gist, 1999; Burg et al., 1995). A fourth study (Rasmussen et al., 1993a) of degreasing workers exposed to either TCE or CFC113 also indirectly evaluated auditory function. These studies are discussed below and presented in detail in Table 4-22.

Table 4-22. Summary of human auditory function studies

Reference	Subjects	Exposure	Effect
ATSDR (2002)	116 children, under 10 yrs of age, residing near six Superfund sites. Further study of children in Burg et al. (1999; 1995). Control: 182 children.	TCE and other solvents in groundwater supplies. Exposures were modeled using tap water TCE concentrations and GIS for spatial interpolation, and LaGrange for temporal interpolation to estimate exposures from gestation to 1990 across the area of subject residences. Control = 0 ppb; low exposure group = 0–<23 ppb-yr; and high exposure group = >23 ppb-yr.	Auditory screening revealed increased incidence of abnormal middle ear function in exposed groups as indicated from acoustic reflex test. Adjusted ORs for right ear ipsilateral acoustic reflects control, OR: 1.0, low exposure group, OR: 5.1, $p < 0.05$; high exposure group, OR: 7.2, $p < 0.05$. ORs adjusted for age, sex, medical history, and other chemical contaminants. No significant decrements reported in the pure tone and typanometry screening.
Burg et al. (1995)	From an NHIS TCE subregistry of 4,281 (4,041 living and 240 deceased) residents.	Environmentally exposed to TCE and other solvents via well water in Indiana, Illinois, and Michigan.	Increase in self-reported hearing impairments for children ≤9 yrs.
Burg et al. (1999)	3,915 white registrants. Mean age 34 yrs (SD = 19.9 yrs).	Cumulative TCE exposure subgroups: <50 ppb, n = 2,867; 50–500 ppb, n = 870; 500–5,000 ppb, n = 190; >5,000 ppb, n = 35. Exposure duration subgroups: <2, 2–5, 5–10, and >10 yrs.	A statistically significant association (adjusted for age and sex) between duration of exposure and self-reported hearing impairment was found.
Rasmussen et al. (1993c)	96 Danish metal degreasers. Age range: 19–68 yrs. No unexposed controls; low exposed group is referent.	Average exposure duration: 7.1 yrs range of full-time degreasing: 1 mo to 36 yrs. Exposure to TCE or and CFC113. (1) Low exposure: n = 19, average full-time exposure 0.5 yr. (2) Medium exposure: n = 36, average full-time exposure 2.1 yrs. (3) High exposure: n = 41, average full-time exposure 11 yrs. Mean U-TCA in high exposure group = 7.7 mg/L (maximum = 26.1 mg/L).	Auditory impairments noted through several neurological tests. Significant relationship of exposure was found with acoustic-motor function ($p < 0.001$), Paced Auditory Serial Addition Test ($p < 0.001$), and Rey Auditory Verbal Learning Test ($p < 0.001$).

NHIS = National Health Interview Survey

Burg et al. (1999; 1995) reviewed the effects of TCE on 4,281 individuals (TCE Subregistry) residentially exposed to this solvent for more than 30 consecutive days. Face-to-face interviews were conducted with the TCE subregistry population and self-reported hearing loss was evaluated based on personal assessment through the interview (no clinical evaluation was conducted). TCE registrants who were ≤9 years old had a statistically significant increase in hearing impairment as reported by the subjects. The RR in this age group for hearing impairments was 2.13 (95% CI: 1.12−4.06), which decreased to 1.12 (95% CI: 0.52−2.24) for the 10−17-year-old age group and 0.32 (95% CI: 0.10−1.02) for all older age groups. A statistically significant association (when adjusted for age and sex) was found between duration of exposure (in these studies, this was length of residency) and reported hearing impairment. The ORs were 2.32 (95% CI: 1.18–4.56) for subjects exposed to TCE >2–≤5 years, 1.17 (95% CI: 0.55–2.49) for exposure >5–≤10 years, and 2.46 (95% CI: 1.30–5.02) for exposure durations >10 years.

ATSDR (2002) conducted a follow-up study to the TCE subregistry findings (Burg and Gist, 1999; Burg et al., 1995) and focused on the subregistry children located in Elkhart, Indiana, Rockford, Illinois, and Battle Creek, Michigan using clinical tests for oral motor, speech, and hearing function. Exposures were modeled using tap water TCE concentrations and geographic information system (GIS) for spatial interpolation, and LaGrange for temporal interpolation to estimate exposures from gestation to 1990 across the area of subject residences. Modeled data were used to estimate lifetime exposures (ppb-years) to TCE in residential wells. The median TCE exposure for the children was estimated from drinking water as 23 ppb/year of exposure (ranging from 0 to 702 ppb/year). Approximately 20% (17−21%, depending on ipsilateral or contralateral test reflex) of the children in the TCE subregistry and 5–7% in the control group exhibited an abnormal acoustic reflex (involuntary muscle contraction that measures movement of the stapedius muscle in the middle ear following a noise stimulus), which was statistically significant ($p = 0.003$). Abnormalities in this reflex could be an early indicator of more serious hearing impairments. No significant decrements were reported in the pure tone and typanometry screening.

Rasmussen et al. (1993c) used a psychometric test to measure potential auditory effects of TCE exposure in an occupational study. Results from 96 workers exposed to TCE and other solvents were presented in this study. Details of the exposure groups and exposure levels are provided in Table 4-22. The acoustic motor function test was used for evaluation of auditory function. Significant decrements ($p < 0.05$) in acoustic motor function performance scores (average decrement of 2.5 points on a 10-point scale) were reported for TCE exposure.

4.3.2.2. Auditory Function: Laboratory Animal Studies

The ability of TCE to permanently disrupt auditory function and produce abnormalities in inner ear histopathology has been demonstrated in several studies using a variety of test methods.

Two different laboratories have identified NOAELs following inhalation exposure for auditory function of 1,600 ppm for 12 hours/day for 13 weeks in Long-Evans rats (n = 6–10) (Rebert et al., 1991) and 1,500 ppm for 18 hours/day, 5 days/week for 3 weeks in Wistar-derived rats (n = 12) (Jaspers et al., 1993). The LOAELs identified in these and similar studies are 2,500–4,000 ppm TCE for periods of exposure ranging from 4 hours/day for 5 days to 12 hours/day for 13 weeks (e.g., Albee et al., 2006; Boyes et al., 2000; Muijser et al., 2000; Fechter et al., 1998; Crofton and Zhao, 1997; Rebert et al., 1995; Crofton et al., 1994; Rebert et al., 1993). Rebert et al. (1993) estimated acute blood TCE levels associated with permanent hearing impairment at 125 µg/mL by methods that probably underestimated blood TCE values (rats were anaesthetized using 60% carbon dioxide [CO_2]). A summary of these studies is presented in Table 4-23.

Table 4-23. Summary of animal auditory function studies

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL[a]	Effects
Rebert et al. (1991)	**Inhalation**	**Rat, Long-Evans, male, 10/group**	**Long-Evans: 0, 1,600, and 3,200 ppm; 12 hrs/d, 12 wks**	**Long-Evans: NOAEL: 1,600 ppm; LOAEL: 3,200 ppm**	**BAERs were measured. Significant decreases in BAER amplitude and an increase in latency of appearance of the initial peak (P1).**
		Rat, F344, male, 4–5/group	F344: 0, 2,000, and 3,200 ppm; 12 hrs/d, 3 wks	F344: LOAEL: 2,000 ppm	
Rebert et al. (1993)	Inhalation	Rat, Long-Evans, male, 9/group	0, 2,500, 3,000, and 3,500 ppm; 8 hrs/d, 5 d	NOAEL: 2,500 ppm; LOAEL: 3,000 ppm.	BAERs were measured 1–2 wks postexposure to assess auditory function. Significant decreases in BAERs were noted with TCE exposure.
Rebert et al. (1995)	Inhalation	Rat, Long-Evans, male, 9/group	0 and 2,800 ppm; 8 hrs/d, 5 d	LOAEL: 2,800 ppm	BAER measured 2–14 ds postexposure at a 16 kHz tone. Hearing loss ranged from 55 to 85 dB.
Crofton et al. (1994)	Inhalation	Rat, Long-Evans, male, 7–8/group	0 and 3,500 ppm TCE; 8 hrs/d, 5 d	LOAEL: 3,500 ppm	BAER measured and auditory thresholds determined 5–8 wks postexposure. Selective impairment of auditory function for mid-frequency tones (8 and 16 kHz).

Table 4-23. Summary of animal auditory function studies (continued)

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Crofton and Zhou (1997); Boyes et al. (2000)	**Inhalation**	**Rat, Long-Evans, male, 8–10/group**	**0, 800, 1,600, 2,400, and 3,200 ppm; 6 hrs/d, 5 d/wk, 13 wks**	**NOAEL: 1,600 ppm; LOAEL: 2,400 ppm**	**Auditory thresholds as measured by BAERs for the 16 kHz tone increased with TCE exposure. Measured 3–5 wks post exposure.**
		Rat, Long-Evans, male, 8–10/group	0, 1,600, 2,400, and 3,200 ppm; 6 hrs/d, 5 d	NOAEL: 2,400 ppm; LOAEL: 3,200 ppm	
		Rat, Long-Evans, male, 8–10/group	0, 800, 1,600, 2,400, and 3,200 ppm; 6 hrs/d, 5 d/wk, 4 wks	NOAEL: 2,400 ppm; LOAEL: 3,200 ppm	
		Rat, Long-Evans, male, 9–12/group	0, 4,000, 6,000, and 8,000 ppm; 6 hrs	NOAEL: 6,000 ppm; LOAEL: 8,000 ppm	
Fechter et al. (1998)	Inhalation	Rat, Long-Evans, male, 12/group	0 and 4,000 ppm; 6 hrs/d, 5 d	LOAEL: 4,000 ppm	Cochlear function measured 5–7 wks after exposure. Loss of spiral ganglion cells noted. Auditory function was significantly decreased 3 wks postexposure, as measured by compound action potentials and reflex modification.
Jaspers et al. (1993)	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 12/group	0, 1,500, and 3,000 ppm; 18 hrs/d, 5 d/wk, 3 wks	NOAEL: 1,500 ppm	Auditory function assessed repeatedly 1–5 wks postexposure for 5, 20, and 35 kHz tones; no effect at 5 or 35 kHz; decreased auditory sensitivity at 20 kHz, 3,000 ppm.
Muijser et al. (2000)	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 8	0 and 3,000 ppm; 18 hrs/d, 5 d/wk, 3 wks	LOAEL: 3,000 ppm	Auditory sensitivity decreased with TCE exposure at 4, 8, 16, and 20 kHz tones. White noise potentiated the decrease in auditory sensitivity.
Albee et al. (2006)	**Inhalation**	**Rat, F344, male and female, 10/sex/group**	**0, 250, 800, and 2,500 ppm; 6 hrs/d, 5 d/wk, 13 wks**	**NOAEL: 800 ppm; LOAEL: 2,500 ppm**	**Mild frequency specific hearing deficits; focal loss of cochlear hair cells.**

Table 4-23. Summary of animal auditory function studies (continued)

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Yamamura et al. (1983)	Inhalation	Guinea Pig, albino Hartley, male, 7–10/group	0, 6,000, 12,000, and 17,000 ppm; 4 hrs/d, 5 d	NOAEL: 17,000 ppm	No change in auditory sensitivity at any exposure level as measured by cochlear action potentials and microphonics. Study was conducted in guinea pig and species is less sensitive to auditory toxicity than rats. Studies were also not conducted in a sound-isolation chamber and effects may be impacted by background noise.

Bolded study(ies) carried forward for consideration in dose-response assessment (see Chapter 5).

BAER = brainstem auditory-evoked potential

Reflex modification was used in several studies to evaluate the auditory function in TCE-exposed animals (Boyes et al., 2000; Muijser et al., 2000; Fechter et al., 1998; Crofton and Zhao, 1997; Crofton et al., 1994; Crofton and Zhao, 1993; Jaspers et al., 1993; Yamamura et al., 1983). These studies collectively demonstrate significant decreases in auditory function at midfrequency tones (8–20 kHz tones) for TCE exposures >1,500 ppm after acute, short-term, and chronic durations. Only one study (Yamamura et al., 1983) did not demonstrate impairment in auditory function from TCE exposures as high as 17,000 ppm for 4 hours/day over 5 days. This was the only study to evaluate auditory function in guinea pigs, whereas the other studies used various strains of rats. Despite the negative finding in Yamamura et al. (1983), auditory testing was not performed in an audiometric sound attenuating chamber and extraneous noise could have influenced the outcome. It is also important to note that the guinea pig has been reported to be far less sensitive than the rat to the effects of ototoxic aromatic hydrocarbons such as toluene.

Crofton and Zhao (1997) also presented a benchmark dose (BMD) for which the calculated dose of TCE would yield a 15 dB loss in auditory threshold. This benchmark response (BMR) was selected because a 15 dB threshold shift represents a significant loss in threshold sensitivity for humans. The benchmark concentrations for a 15 dB threshold shift are 5,223 ppm for 1 day, 2,108 ppm for 5 days, 1,418 ppm for 20 days and 1,707 ppm for 65 days of exposure. While more sensitive test methods might be used and other definitions of a benchmark effect chosen with a strong rationale, these data provide useful guidance for exposure concentrations that yield hearing loss in rats.

Brainstem auditory-evoked responses (BAERs) were also measured in several studies (Albee et al., 2006; Rebert et al., 1995; Rebert et al., 1993; Rebert et al., 1991) following at exposures of 3−13 weeks. Rebert et al. (1991) measured BAERs in male Long-Evans rats

(n = 10) and F344 rats (n = 4–5) following stimulation with 4, 8, and 16 kHz sounds. The Long-Evans rats were exposed to 0, 1,600, or 3,200 ppm TCE, 12 hours/day for 12 weeks and the F344 rats were exposed to 0, 2,000, or 3,200 ppm TCE, 12 hours/day for 3 weeks. BAER amplitudes were significantly decreased at all frequencies for F344 rats exposed to 2,000 and 3,000 ppm TCE and for Long-Evans rats exposed to 3,200 ppm TCE. These data identify a LOAEL at 2,000 ppm for the F344 rats and a NOAEL at 1,600 ppm for the Long-Evans rats. In subsequent studies, Rebert et al. (1995; 1993) again demonstrated that TCE significantly decreases BAER amplitudes and also significantly increases the latency of appearance. Similar results were obtained by Albee et al. (2006) for male and female F344 rats exposed to TCE for 13 weeks. The NOAEL for this study was 800 ppm based on ototoxicity at 2,500 ppm.

Notable physiological changes were also reported in a few auditory studies. Histological data from cochleas in Long-Evans rats exposed to 4,000 ppm TCE indicated that there was a loss in spiral ganglion cells (Fechter et al., 1998). Similarly, there was an observed loss in hair cells in the upper basal turn of the cochlea in F344 rats exposed to 2,500 ppm TCE (Albee et al., 2006).

4.3.2.3. Summary and Conclusion of Auditory Effects

Human and animal studies indicated that TCE produces decrements in auditory function. In the human epidemiological studies (ATSDR, 2002; Burg and Gist, 1999; Burg et al., 1995; Rasmussen et al., 1993d), it is suggested that auditory impairments result from both an inhalation and oral TCE exposure. A LOAEL of approximately 23 ppb-years TCE (extrapolated from ≤23 ppb-years group in ATSDR (2002) from oral intake is noted for auditory effects in children. The only occupational study where auditory effects were seen reported mean urinary trichloroacetic acid (U-TCA) concentration, a nonspecific metabolite of TCE, of 7.7 mg/L for the high cumulative exposure group only (Rasmussen et al., 1993d). A NOAEL or a LOAEL for auditory changes resulting from inhalational exposure to TCE cannot be interpolated from average U-TCA concentration of subjects in the high-exposure group because of a lack of detailed information on long-term exposure levels and duration (Rasmussen et al., 1993d). Two studies (Burg and Gist, 1999; Burg et al., 1995) evaluated self-reported hearing effects in people included in the TCE subregistry comprised of people residing near Superfund sites in Indiana, Illinois, and Michigan. In Burg et al. (1995), interviews were conducted with the TCE-exposed population and it was found that children aged ≤9 years old had statistically significant hearing impairments in comparison to nonexposed children. This significant increase in hearing impairment was not observed in any other age group that was included in this epidemiological analysis. This lack of effect in other age groups may suggest association with another exposure other than drinking water; however, it may also suggest that children may be more susceptible than adults. In a follow-up analysis, Burg et al. (1999) adjusted the statistical analysis of the original data (Burg et al., 1995) for age and sex. When these adjustments were made, a

statistically significant association was reported self-reported for auditory impairment and duration of residence. These epidemiological studies provided only limited information given their use of an indirect exposure metric of residence location, no auditory testing of this studied population, and self-reporting of effects. ATSDR (2002) further tested the findings in the Burg studies (Burg and Gist, 1999; Burg et al., 1995) by contacting the children who were classified as having hearing impairments in the earlier study and conducting several follow-up auditory tests. Significant abnormalities were reported for the children in the acoustic reflex test, which suggested effects to the lower brainstem auditory pathway with the large effect measure, the OR, was reported for the high-cumulative-exposure group. Strength of analyses was its adjustment for potential confounding effects of age, sex, medical history, and other chemical contaminants in drinking water supplies. The ATSDR findings were important in that the results supported Burg et al. (1999; 1995). Rasmussen et al. (1993c) also evaluated auditory function in metal workers with inhalation exposure to either TCE or CFC113. Results from tasks, including an auditory element, suggested that these workers may have some auditory impairment. However, the tasks did not directly measure auditory function.

Animals studies strongly indicated that TCE produces deficits in hearing and provides biological context to the epidemiological study observations. Although there is a strong association between TCE and ototoxicity in the animal studies, most of the effects began to occur at higher inhalation exposures. NOAELs for ototoxicity ranged from 800 to 1,600 ppm for exposure durations of at least 12 weeks (Albee et al., 2006; Boyes et al., 2000; Crofton and Zhao, 1997; Rebert et al., 1991). Inhalation exposure to TCE was the route of administration in all of the animal studies. These studies either used reflex modification audiometry (Muijser et al., 2000; Crofton and Zhao, 1997; Crofton et al., 1994; Jaspers et al., 1993) procedures or measured BAERs (Rebert et al., 1995; Rebert et al., 1993; Rebert et al., 1991) to evaluate hearing in rats. Collectively, the animal database demonstrates that TCE produces ototoxicity at midfrequency tones (4–24 kHz), and no changes in auditory function were observed at either the low (<4 kHz) or high (>24 kHz) frequency tones. Additionally, deficits in auditory effects were found to persist for at least 7 weeks after the cessation of TCE exposure (Boyes et al., 2000; Fechter et al., 1998; Crofton and Zhao, 1997; Jaspers et al., 1993; Rebert et al., 1991). Decreased amplitude and latency were noted in the BAERs (Rebert et al., 1995; Rebert et al., 1993; Rebert et al., 1991), suggesting that TCE exposure affects central auditory processes. Decrements in auditory function following reflex modification audiometry (Muijser et al., 2000; Crofton and Zhao, 1997; Crofton et al., 1994; Jaspers et al., 1993) combined with changes observed in cochlear histopathology (Albee et al., 2006; Fechter et al., 1998) suggest that ototoxicity is occurring at the level of the cochlea and/or brainstem.

Changes in auditory function are noteworthy considering that TCE exposure is also associated with immunotoxicity and inflammatory-based diseases (discussed in Section 4.6). Autoimmune sensorineural hearing loss is a rare condition, sometimes seen with systemic

autoimmune diseases (Bovo et al., 2006; Ruckenstein, 2004). The potential role of immunotoxicity in the observed auditory impairment seen with TCE is an area that requires additional research.

4.3.3. Vestibular Function

4.3.3.1. Vestibular Function: Human Studies

The earliest reports of neurological effects resulting from TCE exposures focused on subjective vestibular system symptoms, such as headaches, dizziness, and nausea. These symptoms are subjective and self-reported. However, there is little doubt that these effects can be caused by exposures to TCE, as they have been reported extensively in the literature, resulting from occupational exposures (Liu et al., 1988; Rasmussen and Sabroe, 1986; Smith, 1970; Grandjean et al., 1955), environmental exposures (Hirsch et al., 1996), and chamber studies (Smith, 1970; Stewart et al., 1970).

Kylin et al. (1967) exposed 12 volunteers to 1,000 ppm (5,500 mg/m^3) TCE for 2 hours in a 1.5 × 2 × 2 chamber. Volunteers served as their own controls since 7 of the 12 were pretested prior to exposure and the remaining 5 were post-tested days after exposure. Subjects were tested for optokinetic nystagmus, which was recorded by electronystagmography, that is, —the potential difference produced by eye movements between electrodes placed in lateral angles between the eyes." Venous blood was also taken from the volunteers to measure blood TCE levels during the vestibular task. The authors concluded that there was an overall reduction in the limit ("fusion limit") to reach optokinetic nystagmus when individuals were exposed to TCE. Reduction of the "fusion limit" persisted for up to 2 hours after the TCE exposure was stopped and the blood TCE concentration was 0.2 mg/100 mL.

4.3.3.2. Vestibular Function: Laboratory Animal Data

The effect of TCE on vestibular function was evaluated by either: (1) promoting nystagmus (vestibular system dysfunction) and comparing the level of effort required to achieve nystagmus in the presence and absence of TCE or (2) using an elevated beam apparatus and measuring the balance. Overall, it was found that TCE disrupts vestibular function as presented below and summarized in Table 4-24.

Table 4-24. Summary of vestibular system studies

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Vestibular system studies—humans					
Kylin et al. (1967)	Inhalation	Humans, male and female, 12	1,000 ppm; 2 hrs	LOAEL: 1,000 ppm	Reduction in potential to reach nystagmus following TCE exposure.
Vestibular system studies—animals					
Tham et al. (1979)	i.v.	Rabbit, strain unknown, sex unspecified, 19	1–5 mg/kg/min	–	Positional nystagmus developed once blood levels reached 30 ppm.
Tham et al. (1984)	i.v.	Rat, Sprague-Dawley, female, 11	80 µg/kg/min	–	Excitatory effects on the vestibule-oculomotor reflex. Threshold effect at blood (TCE) of 120 ppm or 0.9 mM/L.
Niklasson et al. (1993)	Inhalation	Rat, strain unknown, male and female, 28	0, 2,700, 4,200, 6,000, or 7,200 ppm; 1 hr	LOAEL: 2,700 ppm	Increased ability to produce nystagmus.
Umezu et al. (1997)	i.p.	Mouse, ICR, male, 116	0, 250, 500, or 1,000 mg/kg, single dose and evaluated 30 mins postadministration	NOAEL: 250 mg/kg LOAEL: 500 mg/kg	Decreased equilibrium and coordination as measured by the Bridge test (staying time on an elevated balance beam).

Niklasson et al. (1993) showed acute impairment of vestibular function in male- and female-pigmented rats during acute inhalation exposure to TCE (2,700–7,200 ppm) and to trichloroethane (500–2,000 ppm). Both of these agents were able to promote nystagmus during optokinetic stimulation in a dose-related manner. While there were no tests performed to assess persistence of these effects, Tham et al. (1984; 1979) did find complete recovery of vestibular function in rabbits (n = 19) and female Sprague-Dawley rats (n = 11) within minutes of terminating a direct arterial infusion with TCE solution.

The finding that TCE can yield transient abnormalities in vestibular function is not unique. Similar impairments have also been shown for toluene, styrene, and trichloroethane (Niklasson et al., 1993) and for a broad range of aromatic hydrocarbons (Tham et al., 1984). The concentration of TCE in blood at which effects were observed for TCE (0.9 mM/L) was quite close to that observed for most of these other vestibulo-active solvents.

4.3.3.3. Summary and Conclusions for the Vestibular Function Studies

Studies of TCE exposure in both humans and animals reported abnormalities in vestibular function. Headaches, dizziness, nausea, and motor incoordination, among other subjective symptoms, are reported in occupational epidemiological studies of TCE exposure (Hirsch et al., 1996; Liu et al., 1988; Rasmussen and Sabroe, 1986; Smith, 1970; Stewart et al., 1970;

Grandjean et al., 1955). One human exposure study (Kylin et al., 1967) found that vestibular function was affected following an acute exposure to 1,000-ppm TCE (LOAEL). Individuals had a decreased threshold to reach nystagmus than when exposed to TCE than to air. Animal studies also evaluated the threshold to reach nystagmus and reported that TCE decreased the threshold to produce nystagmus in rats (LOAEL: 2,700 ppm) (Niklasson et al., 1993; Tham et al., 1984) and rabbits (Tham et al., 1984).

4.3.4. Visual Effects

4.3.4.1. Visual Effects: Human Studies

Visual impairment in humans has been demonstrated following exposures through groundwater (Reif et al., 2003; Kilburn, 2002b, a), from occupational exposure through inhalation (Rasmussen et al., 1993c; Tröster and Ruff, 1990), and from a controlled inhalation exposure study (Vernon and Ferguson, 1969). Visual functions such as color discrimination and visuospatial learning tasks are impaired in TCE-exposed individuals. Additionally, an acute exposure can impair visual depth perception. Details of the studies are provided below and summarized in Table 4-25.

Table 4-25. Summary of human visual function studies

Reference	Subjects	Exposure	Effect
Kilburn et al. (2002b, a) (2002a)	236 residents near a microchip plant in Phoenix, Arizona. Controls: 67 local referents from Phoenix, Arizona and 161 regional referents from Wickenburg, Arizona.	TCE, TCA, 1,1-DCE, 1,2-DCE, perchloroethylene, and vinyl chloride detected in well water up to 260,000 ppm; TCE concentrations in well water were 0.2–10,000 ppb. Exposure duration 2−37 yrs.	Color discrimination errors were increased among residents compared to regional referents ($p < 0.01$). No adjustment for possible confounding factors.
Reif et al. (2003)	143 residents of the Rocky Mountain Arsenal community of Denver. Referent group at lowest concentration (<5 ppb).	Exposure modeling of TCE concentrations in groundwater and in distribution system to estimate mean TCE concentration by census block of residence. High exposure group >15 ppb. Medium exposure group ≥5–≤15 ppb. Low exposure referent group <5 ppb.	Contrast sensitivity test performances (C and D) was marginally statistically significant ($p = 0.06$ and 0.07, respectively). No significant effects reported for the Benton visual retention test. Significant decrements ($p = 0.02$) were reported in the Benton visual retention test when stratified with alcohol consumption.
Rasmussen et al. (1993c)	96 Danish metal degreasers. Age range: 19–68; no unexposed controls; low exposure group was referent.	Average exposure duration: 7.1 yrs; range of full-time degreasing: 1 mo to 36 yrs. Exposure to TCE or CFC113: (1) Low exposure: n = 19, average full-time exposure 0.5 yr. (2) Medium exposure: n = 36, average full-time exposure 2.1 yrs. (3) high exposure: n = 41, average full-time exposure 11 yrs. TCA in high exposure group = 7.7 mg/L (maximum = 26.1 mg/L).	Statistically significant relationship of exposure was found with the Visual Gestalts learning and retention test (cognitive test) indicating deficits in visual performance.
Troster and Ruff (1990)	Two occupationally TCE-exposed workers. Controls: two groups of n = 30 matched controls; (all age and education matched).	Exposure concentration unknown. Exposure duration, 3–8 mo.	Both workers experienced impaired visuospatial learning.
Vernon and Ferguson (1969)	8 male volunteers age range 21–30; self controls.	0, 100, 300, and 1,000 ppm of TCE for 2 hrs.	Statistically significant effects on visual depth perception as measured by the Howard-Dolman test. NOAEL: 300 ppm; LOAEL: 1,000 ppm. No significant changes in any of the other visual test measurements.

Geographical-based studies utilized color discrimination and contrast sensitivity tests to determine the effect of TCE exposure on vision. In these studies, it was reported that TCE exposure significantly increased color discrimination errors (Kilburn, 2002b, a) or that decreased contrast sensitivity tests approached statistical significance after adjustments for several possible confounders ($p = 0.06$ or 0.07) (Reif et al., 2003). Exposure in Kilburn (2002b, a) is poorly

characterized, and for both studies, TCE is one of several contaminants in drinking water supplies; neither study provided an estimate of an individual's exposure to TCE.

Rasmussen et al. (1993c) evaluated visual function in 96 metal workers, working in degreasing at various factories and with exposure to TCE or CFC113. Visual function was tested through the visual gestalts test (visual perception) and a visual recall test. In the visual gestalts test, the number of total errors significantly increased from the low-exposure group (3.4 errors) to the high-exposure group (6.5 errors; $p = 0.01$). No significant changes were observed in the visual recall task. Troster and Ruff (1990) presented case studies conducted on two occupationally exposed workers to TCE. Both patients presented with a visual-spatial task and neither could complete the task within the number of trials allowed, suggesting visual function deficits as a measure of impaired visuospatial learning.

In a chamber exposure study (Vernon and Ferguson, 1969), eight male volunteers (ages 21–30) were exposed to 0, 100, 300, and 1,000-ppm TCE for 2 hours. Each individual was exposed to all TCE concentrations and a span of at least 3 days was given between exposures. When the individuals were exposed to 1,000-ppm TCE (5,500 mg/m^3), significant abnormalities were noted in depth perception as measured by the Howard-Dolman test ($p < 0.01$). There were no effects on the flicker fusion frequency test (threshold frequency at which the individual sees a flicker as a single beam of light) or on the form perception illusion test (volunteers presented with an illusion diagram).

4.3.4.2. Visual Effects: Laboratory Animal Data

Changes in visual function have been demonstrated in animal studies during acute (Boyes et al., 2005b; Boyes et al., 2003) and subchronic exposure (Blain et al., 1994; Rebert et al., 1991). In these studies, the effect of TCE on visual evoked responses to patterns (Boyes et al., 2005b; Boyes et al., 2003; Rebert et al., 1991) or a flash stimulus (Blain et al., 1994; Rebert et al., 1991) were evaluated. Overall, the studies demonstrated that exposure to TCE results in significant changes in the visual evoked response, which is reversible once TCE exposure is stopped. Details of the studies are provided below and are summarized in Table 4-26.

Table 4-26. Summary of animal visual system studies

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Rebert et al. (1991)	Inhalation	Rat, Long-Evans, male, 10/group	0, 1,600, and 3,200 ppm; 12 hrs/d, 12 wks	NOAEL: 1,600 ppm	Significant amplitude decreases in pattern reversal evoked potentials (N1P1 amplitude) at 6, 9, and 12 wks.
Boyes et al. (2003)	Inhalation	Rat, Long-Evans, male, 9–10/group	0 ppm, 4 hrs; 1,000 ppm, 4 hrs; 2,000 ppm, 2 hrs; 3,000 ppm, 1.3 hrs 4,000 ppm, 1 hr	LOAEL: 1,000 ppm, 4 hrs	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed visual evoked potentials. Peak brain TCE concentration correlated with dose-response.
Boyes et al. (2005a)	Inhalation	Rat, Long-Evans, male, 8–10/group	0 ppm, 4 hrs; 500 ppm, 4 hrs; 1,000 ppm, 4 hrs; 2,000 ppm, 2 hrs; 3,000 ppm, 1.3 hrs 4,000 ppm, 1 hr; 5,000 ppm, 0.8 hr	LOAEL: 500 ppm, 4 hrs	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed visual evoked potentials. Peak brain TCE concentration correlated with dose-response.
Blain et al. (1994)	**Inhalation**	**Rabbit, New Zealand albino, male, 6–8/group**	**0, 350, and 700 ppm; 4 hrs/d, 4 d/wk, 12 wks**	**LOAEL: 350 ppm**	**Significant effects noted in visual function as measured by ERG and OPs immediately after exposure. No differences in ERG or OP measurements were noted at 6 wks post-TCE exposure.**

Bolded study(ies) carried forward for consideration in dose-response assessment (see Chapter 5).

ERG = electroretinogram, OP = oscillatory potential

Boyes et al. (2005a; 2003) exposed adult, male Long-Evans rats to TCE in a head-only exposure chamber while pattern onset/offset visual evoked potentials (VEPs) were recorded. Exposure conditions were designed to provide concentration × time products of 0 ppm/hours (0 ppm for 4 hours) or 4,000 ppm/hours (see Table 4-26 for more details). VEP amplitudes were depressed by TCE exposure during the course of TCE exposure. The degree of VEP depression showed a high correlation with the estimated brain TCE concentration for all levels of atmospheric TCE exposure.

In a subchronic exposure study, Rebert et al. (1991) exposed male Long-Evans rats to 1,600 or 3,200 ppm TCE, for 12 weeks, 12 hours/day. No significant changes in flash evoked potential measurements were reported following this exposure paradigm. Decreases in pattern reversal VEPs (N1P1 amplitude) reached statistical significance following 6, 9, and 12 weeks of

exposure. The drop in response amplitude ranged from approximately 20% after 8 weeks to nearly 50% at week 14, but recovered completely within 1 week postexposure.

This transient effect of TCE on the peripheral visual system has also been reported by Blain (1994) in which New Zealand albino rabbits were exposed by inhalation to 350 and 700 ppm TCE 4 hours/day, 4 days/week for 12 weeks. Electroretinograms (ERG) and oscillatory potentials (OPs) were recorded weekly under mesopic conditions. Recordings from the 350- and 700-ppm exposed groups showed a significant increase in the amplitude of the a- and b-waves (ERG). The amplitude of the OPs was significantly decreased at 350 ppm (57%) and increased at 700 ppm (117%). These electroretinal changes returned to pre-exposure conditions within 6 weeks after the inhalation stopped.

4.3.4.3. Summary and Conclusion of Visual Effects

Changes in visual function are reported in human studies. Although central visual function was not evaluated in the human studies (such as ERGs, evoked potential measurements), clinical tests indicated deficits in color discrimination (Kilburn, 2002b, a) visual depth perception (Vernon and Ferguson, 1969), and contrast sensitivity (Reif et al., 2003). These changes in visual function were observed following both an acute exposure (Vernon and Ferguson, 1969) and residence in areas with groundwater contamination with TCE and other chemicals (Reif et al., 2003; Kilburn, 2002b, a). The exposure assessment approach of Reif et al., (2003) who adopted exposure modeling and information on water distribution patterns, is considered superior to that of Kilburn (Kilburn, 2002b, a), who used residence location as a surrogate for exposure. In the one acute inhalation study (Vernon and Ferguson, 1969), a NOAEL of 300 ppm and a LOAEL of 1,000 ppm for 2 hours was reported for visual effects. A NOAEL is not available from the drinking water studies since well water TCE concentration is a poor surrogate for an individual‘s TCE ingestion (Kilburn, 2002b, a) and there was limited statistical analysis comparing the high-exposure group to the low-exposure group (Reif et al., 2003).

Animal studies have also demonstrated changes in visual function. All of the studies evaluated central visual function by measuring changes in evoked potential response following a visual stimulus that was presented to the animal. Two acute exposure inhalation studies (Boyes et al., 2005a; Boyes et al., 2003) exposed Long-Evans rats to TCE based on a concentration × time schedule (Haber‘s law) and reported decreases in VEP amplitude. All of the exposures from these two studies resulted in decreased visual function with a LOAEL of 500 ppm for 4 hours. Another important finding that was noted is the selection of the appropriate dose-metric for visual function changes following an acute exposure. Boyes et al. (2005a; 2003) found that among other potential dose-metrics, brain TCE concentration was best correlated with changes in visual function as measured by evoked potentials under acute exposure conditions. Two subchronic exposure studies (Blain et al., 1994; Rebert et al., 1991) demonstrated visual function changes as measured by pattern reversal evoked potentials (Rebert et al., 1991) or ERGs/OPs (Blain et al., 1994). Unlike

the other three visual function studies conducted with rats, Blain et al. (1994) demonstrated these changes in rabbits. Significant changes in ERGs and OPs were noted following a 12-week exposure at 350 ppm (LOAEL) in rabbits (Blain et al., 1994), and in rats exposed to 3,200-ppm TCE for 12 weeks, there were significant decreases in pattern reversal evoked potentials, but no effect was noted in the 1,600-ppm exposure group (Rebert et al., 1991). Both subchronic studies examined visual function following an exposure-free period of either 2 weeks (Rebert et al., 1991) or 6 weeks (Blain et al., 1994) and found that visual function returned to pre-exposure levels and the changes are reversible.

4.3.5. Cognitive Function

4.3.5.1. Cognitive Effects: Human Studies

Effects of TCE on learning and memory have been evaluated in populations environmentally exposed to TCE through well water, in workers occupationally exposed through inhalation and under controlled exposure scenarios. Details of the studies are provided in Table 4-27 and discussed briefly below. In the geographical-based studies (Kilburn, 2002b, a; Kilburn and Warshaw, 1993a) cognitive function was impaired in both studies and was evaluated by testing verbal recall and digit span memory among other measures. In Arizona residents involved in a lawsuit (Kilburn and Warshaw, 1993a), significant impairments in all three cognitive measures were reported; verbal recall ($p = 0.001$), visual recall ($p = 0.03$), and digit span test ($p = 0.07$), although a question exists whether the referent group was comparable to exposed subjects and the study had a lack of consideration of possible confounding exposures in statistical analyses. Significant decreases in verbal recall ability was also reported in another environmental exposure study where 236 residents near a microchip plant with TCE concentration in well water ranging from 0.2 to 10,000 ppb (Kilburn, 2002b, a).

Table 4-27. Summary of human cognition effect studies

Reference	Subjects	Exposure	Effect
Kilburn and Warshaw (1993a)	170 residents living in Southwest Tucson with TCE, other solvents, and chromium in groundwater. Control: 68 residential referents matched to subjects from two previous studies of waste oil and oil refinery exposures.	>500 ppb of TCE in well water before 1981 and 25–100 ppb afterwards. Exposure duration ranged from 1 to 25 yrs.	Decreased performance in the digit span memory test and story recall ability.
Kilburn (2002b, a)	236 residents near a microchip plant. Controls: 67 local referents from Phoenix, Arizona and 161 regional referents from Wickenburg, Arizona.	<0.2–10,000 ppb TCE, <0.2–260,000 ppb TCA, <0.2–6,900 ppb 1,1-DCE, <0.2–1,600 1,2-DCE, <0.2–23,000 ppb perchloroethylene, <0.02–330 ppb vinyl chloride in well water. Exposure duration 2 to 37 yrs.	Cognitive effects decreased as measured by lower scores on Culture Fair 2A, vocabulary, grooved pegboard (dominant hand), trail making test, and verbal recall (i.e., memory).
Rasmussen (1993c, d)	96 Danish metal degreasers. Age range: 19–68; no external controls.	Average exposure duration: 7.1 yrs); range of full-time degreasing: 1 mo to 36 yrs. 1) Low exposure: n = 19, average full-time exposure 0.5 yr. 2) Medium exposure: n = 36, average full-time exposure 2.1 yrs. 3) High exposure: n = 41, average full-time exposure 11 yrs. TCA in high exposure group = 7.7 mg/L (maximum = 26.1 mg/L).	Cognitive impairment (psycho-organic syndrome) prevalent in exposed individuals. The incidence of this syndrome was 10.5% for low exposure, 39.5% for medium exposure, and 63.4% for high exposure. Age is a confounder. Dose-response with 9 of 15 tests; Controlling for confounds, significant relationship of exposure was found with acoustic-motor function ($p < 0.001$), Paced Auditory Serial Addition Test ($p < 0.001$), Rey Auditory Verbal-Learning Test ($p < 0.001$), vocabulary ($p < 0.001$), and visual gestalts ($p < 0.001$); significant age effects. Age is a confounder.
Troster and Ruff (1990)	Two occupationally TCE-exposed workers. Controls: two groups of n = 30 matched controls; (all age and education matched.	Exposure concentration unknown; exposure duration, 3–8 mo.	Both TCE cases exhibited significant deficits in verbal recall and visuospatial learning.
Triebig (1976)	Controlled exposure study four females, three males. Controls: four females, three males.	0, 100 ppm (550 mg/m^3), 6 hrs/d, 5 d.	There was no correlation seen between exposed and unexposed subjects for any measured psychological test results. No methods description was provided.
Triebig (1977c)	Seven men and one woman occupationally exposed with an age range from 23 to 38 yrs. No control group.	50 ppm (260 mg/m^3). Exposure duration not reported.	The psychological tests showed no statistically significant difference in the results before or after the exposure-free time period. No methods description was provided.

Table 4-27. Summary of human cognition effect studies (continued)

Reference	Subjects	Exposure	Effect
Salvini et al. (1971)	Controlled exposure study six students, male. Self used as control.	TCE concentration was 110 ppm for 4-hr intervals, twice per d. 0 ppm control exposure for all as self controls	Statistically significant results were observed for perception tests learning ($p < 0.001$) and CRT learning ($p < 0.01$).
Gamberale et al. (1976)	15 healthy men aged 20–31 yrs old. Controls: Within Subjects (15 self-controls).	0 mg/m^3, 540 mg/m^3 (97 ppm), 1,080 mg/m^3 (194 ppm), 70 min.	Repetition of the testing led to a pronounced improvement in performance as a result of the training effect; no interaction effects between exposure to TCE and training.
Stewart et al. (1970)	130 (108 males, 22 females). Controls: 63 unexposed men.	TCA metabolite levels in urine were measured: 60.8% had levels up to 20 mg/L, and 82.1% had levels up to 60 mg/L.	No significant effect on cognitive tests noted, but more effort required to perform the test in exposed group.
Chalupa (1960)	Case study—6 subjects. Average age 38.	No exposure data were reported.	80% of those with pathological EEG displayed memory loss; 30% of those with normal EEGs displayed memory loss.

EEG = electroencephalogram

Cognitive impairments are assessed in the occupational exposure and case studies (Rasmussen et al., 1993c, d; Tröster and Ruff, 1990). In metal degreasers occupationally exposed to TCE and CFC113, significant cognitive performance decreases were noted in verbal recall testing ($p = 0.03$) and verbal learning ($p = 0.04$) (Rasmussen et al., 1993d). No significant effects were found in the visual recall or digit span test for these workers. Troster and Ruff (1990) reported decrements (no statistical analysis performed) in cognitive performance as measured in verbal and visual recall tests that were conducted immediately after presentation (learning phase) and 1 hour after original presentation (retention/memory phase) for two case studies.

Several controlled (chamber) exposure studies were conducted to cognitive ability during TCE exposure and most did not find any significant decrements in the neurobehavioral measurement. Only Salvini et al. (1971) found significant decrements in cognitive function. Six males were exposed to 110 ppm (550 mg/m^3) TCE for 4-hour intervals, twice per day. Statistically significant results were observed for perception tests learning ($p < 0.001$)and choice reaction time (CRT) learning ($p < 0.01$). Triebig et al. (1977a; 1977b; 1977c; 1976) exposed seven total subjects (male and female) to 100 ppm TCE for 6 hours/day, 5 days/week and did not report any decreases in cognition, but details on the experimental procedures were not provided. Additionally, Gamberale et al. (1976) found that subjects exposed to TCE as high as 194 ppm for 70 minutes did not exhibit any impairments on a short-term memory test in comparison to an air exposure.

4.3.5.2. Cognitive Effects: Laboratory Animal Studies

Many reports have demonstrated significant differences in performance of learning tasks such as the speed to complete the task. However, there is little evidence that learning and memory function are themselves impaired by exposure. There are also limited data that suggest alterations in the hippocampus of laboratory animals exposed to TCE. Given the important role that this structure plays in memory formation, such data may be relevant to the question of whether TCE impairs memory. The studies are briefly discussed below and details are provided in Table 4-28.

Table 4-28. Summary of animal cognition effect studies

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Kjellstrand et al. (1980)	Inhalation	Gerbil, Mongolian, males and females, 12/sex/dose	0, 320 ppm; 9 mo, continuous (24 hrs/d) except 1–2 hrs/wk for cage cleaning	NOAEL: 320 ppm	No significant effect on spatial memory (radial arm maze).
Isaacson et al. (1990)	**Oral, drinking water**	**Rat, Sprague-Dawley, male weanlings, 12/dose**	**(1) 0 mg/kg-d, 8 wks. (2) 5.5 mg-d (47 mg/kg-d[b]), 4 wks + 0 mg/kg-d, 4 wks. (3) 5.5 mg/d, 4 wks (47 mg/kg-d) + 0 mg/kg-d, 2 wks + 8.5 mg/d (24 mg/kg-d), 2 wks**	**NOAEL: 5.5 mg/d, 4 wks—spatial learning** **LOAEL: 5.5 mg/d—hippocampal demyelination**	**Decreased latency to find platform in the Morris water maze (Group # 3); Hippocampal demyelination observed in all TCE-treated groups.**
Kishi et al. (1993)	Inhalation	Rats, Wistar, male, number not specified	0, 250,500, 1,000, 2,000, and 4,000 ppm, 4 hrs	LOAEL: 250 ppm	Decreased lever presses and avoidance responses in a shock avoidance task.
Umezu et al. (1997)	i.p.	Mouse, ICR, male, six exposed to all treatments (repeated exposure)	0, 125, 250, 500, and 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg	Decreased response rate in an operant response—condition avoidance task.
Oshiro et al. (2004)	Inhalation	Rat, Long-Evans, male, 24	0, 1,600, and 2,400 ppm; 6 hrs/d, 5 d/wk, 4 wks	NOAEL: 2,400 ppm	No change in reaction time in signal detection task and when challenged with amphetamine, no change in response from control.

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).
[b]mg/kg-day conversion estimated from average male Sprague-Dawley rat body weight from ages 21–49 days (118 g) for the 5.5 mg dosing period and ages 63–78 d (354 g) for the 8.5 mg dosing period.

Two studies (Umezu et al., 1997; Kulig, 1987) reported decreased performance in operant-conditioning cognitive tasks for rodents. Kishi et al. (1993) acutely exposed Wistar rats to TCE at concentrations of 250, 500, 1,000, 2,000, and 4,000 ppm for 4 hours. Rats exposed to ≥250 ppm TCE showed a significant decrease both in the total number of lever presses and in

avoidance responses compared with controls. The rats did not recover their pre-exposure performance until about 2 hours after exposure. Likewise, Umezu et al. (1997) reported a depressed rate of operant responding in male ICR strain mice (n = 6, exposed to all TCE doses, see Table 4-28) in a conditioned avoidance task that reached significance with i.p. injections of 1,000 mg/kg. Increased responding during the signaled avoidance period at lower doses (250 and 500 mg/kg) suggests an impairment in ability to inhibit responding or failure to attend to the signal.

Although cognitive impairments are noted, two additional studies indicate no change in cognition with continuous TCE exposure or improvements in cognitive tasks. No decrements in cognitive function as measured by the radial arm maze were observed in Mongolian gerbils exposed continuously by inhalation to 320 ppm TCE for 9 months (Kjellstrand et al., 1980). Improved performance was noted in a Morris swim test for weanling rats orally dosed with 5.5 mg/day for 4 weeks followed by 2 weeks of no exposure and an additional 2 weeks of 8.5 mg/day (Isaacson et al., 1990). This improved performance occurred despite a loss in hippocampal myelination.

4.3.5.3. Summary and Conclusions of Cognitive Function Studies

Human environmental and occupational exposure studies suggest impairments in cognitive function. Kilburn and Warshaw (1993a) and Kilburn (2002b, a) reported memory deficits in individuals, although a question exists whether the referent group was comparable to exposed subjects and these studies lack of consideration of possible confounding exposures in statistical analyses. Significant impairments were found in visual and verbal recall and with the digit span test. Similarly, in occupational exposure studies (Rasmussen et al., 1993c, d; Tröster and Ruff, 1990), short-term memory tests indicated that immediate memory and learning were impaired in the absence of an effect on digit span performance. In controlled exposure and/or chamber studies, two studies did not report any cognitive impairment (Gamberale et al., 1976; Stewart et al., 1970) and one study (Salvini et al., 1971) reported significant impairments in learning memory and complex choice reaction tasks. All of the controlled exposure studies were acute and/or short-term exposure studies and the sensitivity of test procedures is unknown due to the lack of methodologic information provided in the reports. Despite identified study deficiencies, these studies collectively suggest cognitive function impairment.

The animal studies measured cognitive function through spatial memory and operant responding tasks. In the two studies where spatial memory was evaluated, there was either no effect at 320 ppm TCE (Kjellstrand et al., 1980) or improved cognitive performance in weanling rats at a dose of 5.5 mg/day for 4 weeks (Isaacson et al., 1990). Improved cognitive performance was observed in weanling rats (Isaacson et al., 1990) and could be due to continuing neurodevelopment as well as compensation from other possible areas in the brain since there was a significant loss in hippocampal myelination. Significant decreases in operant responding

(avoidance/punished responding) during TCE exposure were reported in two studies (Umezu et al., 1997; Kishi et al., 1993). When TCE exposure was discontinued, operant responding return to control levels; it is unclear if the significant effects are due to decreased motor function or decreased cognitive ability.

4.3.6. Psychomotor Effects

There is considerable evidence in the literature for both animals and humans on psychomotor testing, although human and laboratory animal studies utilize very different measures of motor behavior. Generally, the human literature employs a wide variety of psychomotor tasks and assesses error rates and reaction time (RT) in the performance of the task. The laboratory animal data, by contrast, tend to include unlearned naturalistic behaviors such as locomotor activity, gait changes, and foot splay to assess neuromuscular ability.

4.3.6.1. Psychomotor Effects: Human Studies

The effects of TCE exposure on psychomotor response have been studied primarily as a change in RT with studies on motor dyscoordination resulting from TCE exposure providing subjective reporting.

4.3.6.1.1. Reaction time

Several studies have evaluated the effects of TCE on RT using simple and CRT tasks (simple reaction time [SRT] and CRT tasks). The studies are presented below and summarized in more detail in Table 4-29.

Table 4-29. Summary of human CRT studies

Reference	Subjects	Exposure	Effect
Kilburn (2002b, a)	236 residents near a microchip plant in Phoenix, Arizona. Controls: 161 regional referents from Wickenburg, Arizona. 67 referents from Phoenix, Arizona not residing near a plant.	0.2–10,000 ppb of TCE, chronic exposure.	SRT and CRT were increased in the exposed group ($p < 0.05$).
Kilburn and Warshaw (1993a)	160 residents living in Southwest Tucson with TCE and other solvents in groundwater. Control: 68 residential referents matched to subjects from two previous studies of waste oil and oil refinery exposures.	>500 ppb of TCE in well water before 1981 and 25–100 ppb afterwards. Exposure duration 1 to 25 yrs.	Mean SRT was 67 milliseconds (msec) longer than the referent group ($p < 0.0001$). CRT of the exposed subjects was between 93 and 100 msec longer in three different trials ($p < 0.0001$) compared to referents.
Reif et al. (2003)	143 residents of the Rocky Mountain Arsenal community of Denver. Referent group at lowest concentration (<5 ppb).	High exposure group >15 ppb. Medium exposure group ≥5–≤15 ppb. Low exposure referent group <5 ppb.	Significant increase in RT as measured by the SRT test ($p < 0.04$) in only among subjects who reported alcohol use (defined as having at least one drink per mo).
Kilburn and Thornton (1996)	Group A: Registered voters from Arizona and Louisiana with no exposure to TCE: n = 264, aged 18–83. Group B volunteers from California n = 29 (17 males and 12 females). Group C: exposed to TCE and other chemicals for ≥5 yrs n = 217.	No exposure or groundwater analyses reported.	Significant increase in SRT and CRT in exposed group compared to the unexposed populations.
Gamberale et al. (1976)	15 healthy men aged 20–31 yrs old. Controls: Within subjects (15 self-controls).	0, 540 mg/m^3 (97 ppm), 1,080 mg/m^3 (194 ppm), 70 min.	No change in CRT or SRT. Increase in time required to perform the RT-Addition Test (task for adding numbers) ($p < 0.05$).
Gun et al. (1978)	Four female workers from one plant exposed to TCE and four female workers from another plant exposed to TCE + nonhalogenated hydrocarbon solvent. Control: (n = 8) four unexposed female workers from each plant.	3–419 ppm, duration not specified.	TCE-only exposure increased RT in comparison to controls. In TCE + solvent group, ambient TCE was lower and mean RT shortened in Session 2, then rose subsequently to be greater than at the start.

Increases in RT were observed in environmental exposure studies by Kilburn (2002b, a), Kilburn and Warshaw (1993a), and Kilburn and Thornton (1996) as well as in an occupational exposure study by Gun et al. (1978). All populations except that of Gun et al. (1978) were exposed through groundwater contaminated as the result of environmental spills; the exposure duration was for at least 1 year and exposure levels ranged from 0.2 to 10,000 ppb for the three studies. Kilburn and Warshaw (1993a) reported that SRT significantly increased from

281 ± 55 msec to 348 ± 96 msec in individuals ($p < 0.0001$). CRT of the exposed subjects was 93 msec longer ($p < 0.0001$) than referents. Kilburn and Thornton (1996) evaluated SRT and CRT function and also found similar increases in RT. The average SRT and CRT for the combined control groups were 276 and 532 msec, respectively. These RTs increased in the TCE exposure group where the average SRT was 334 msec and CRT was 619 msec. Similarly, Kilburn (2002b, a) compared RTs between 236 TCE-exposed persons and the 161 unexposed regional controls. SRTs significantly increased from 283 ± 63 msec in controls to 334 ± 118 msec in TCE-exposed individuals ($p < 0.0001$). Similarly, CRTs also increased from 510 ± 87 to 619 ± 153 msec with exposure to TCE ($p < 0.0001$).

No effect on SRT was reported in a geographical-based study by Reif et al. (2003). SRTs were 301 msec for the lowest exposure group and 316 msec for the highest exposure group ($p = 0.42$). When the SRT data were analyzed for individuals who consumed at least on alcoholic drink per month (n = 80), a significant increase (18%, $p < 0.04$) in SRT times was observed between the lowest exposure and the highest exposure groups. In TCE-exposed individuals who did not consume alcohol (n = 55), SRTs decreased from 321 msec in the lowest exposed group to 296 msec in the highest exposed group, but this effect was not statistically significantly different. A controlled exposure (chamber study) of 15 healthy men aged 20–31 years old, were exposed to 0, 540, and 1,080 mg/m^3 TCE for 70 minutes or served as his own control, reported no statistically significant differences with the SRT or CRT tasks. However, in the RT-addition test, the level of performance varied between the different exposure conditions ($F(2.24) = 4.35$; $p < 0.05$) and between successive measurement occasions ($F(2.24) = 19.25$; $p < 0.001$).

4.3.6.1.2. Muscular dyscoordination

Three studies examined motor dyscoordination effects from TCE exposure using subjective and self-reported individual assessment. Rasmussen et al. (1993a) presented findings on muscular dyscoordination for 96 metal degreasers exposed to either TCE or CFC113. A statistically significant increasing trend of dyscoordination with TCE exposure was observed ($p = 0.01$) in multivariate regression analyses, which adjusted for the effects of age, neurological disease, arteriosclerotic disease, and alcohol abuse. Furthermore, a greater number of abnormal coordination tests were observed in the higher-exposure group compared to the low-exposure group ($p = 0.003$).

Gash et al. (2008) reported fine motor hand movement times in subjects who had filed workman compensation claims were significantly slower ($p < 0.0001$) than age-matched nonexposed controls. Exposures were based on self-reported information, and no information on the control group was presented. Troster and Ruff (1990) reported a case study conducted on two occupationally exposed workers to TCE. Mild deficits in motor speed were reported for both cases. In the first case, manual dexterity was impaired in a male exposed to TCE (unknown

concentration) for 8 months. In the second case study where a female was exposed to TCE (low concentration; exact level not specified) for 3 months, there was weakness in the quadriceps muscle as evaluated in a neurological exam and a decreased sensation to touch on one hand. Both Gash et al. (2008) and Troster and Ruff (1990) provide very limited information given their deficiencies related to lack of exposure data, self-reported information, and limited reporting of referents and statistical analysis.

4.3.6.2. Psychomotor Effects: Laboratory Animal Data

Several animal studies have demonstrated that TCE exposure produces changes in psychomotor function. At high doses (≥2,000 mg/kg), TCE causes mice to lose their righting reflex when the compound is injected i.p. (Shih et al., 2001; Umezu et al., 1997). At lower exposures (inhalation and oral), TCE produces alterations in neurobehavioral measures including locomotor activity, gait, operant responding, and reactivity. The studies are described in Sections 4.3.6.2.1–4.3.6.2.3 and summarized in Tables 4-30 and 4-31.

Table 4-30. Summary of animal psychomotor function and RT studies

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Savolainen et al. (1977)	Inhalation	Rat, Sprague-Dawley, male, 10	0 and 200 ppm; 6 hrs/d, 4 d	LOAEL: 200 ppm	Increased frequency of preening, rearing, and ambulation. Increased preening time.
Kishi et al. (1993)	Inhalation	Rats, Wistar, male, number not specified	0, 250,500, 1,000, 2,000, and 4,000 ppm, 4 hrs	LOAEL: 250 ppm	Decreased lever presses and increased responding when lever press coupled with a 10-s electric shock (decreased avoidance response).
Kulig et al. (1987)	Inhalation	Rat, Wistar, male, 8/dose	0, 500, 1,000, and 1,500 ppm; 16 hrs/d, 5 d/wk, 18 wks	NOAEL: 1,500 ppm	No change in spontaneous activity, grip strength, or hindlimb movement.
Moser et al. (1995)	**Oral**	**Rat, F344, female, 8/dose**	**0, 150, 500, 1,500, and 5,000 mg/kg, one dose**	**NOAEL: 500 mg/kg LOAEL: 1,500 mg/kg**	**Decreased motor activity; Neuro-muscular and sensorimotor impairment.**
			0, 50, 150, 500, and 1,500 mg/kg-d, 14 d	**NOAEL: 150 mg/kg-d LOAEL: 500 mg/kg-d**	**Increased rearing activity and decreased forelimb grip strength.**
Bushnell (1997)	Inhalation	Rat, Long-Evans, male, 12	0, 400, 800, 1,200, 1,600, 2,000, or 2,400 ppm, 1 hr/test d, 4 consecutive test d, 2 wks	NOAEL: 800 ppm LOAEL: 1,200 ppm	Decreased sensitivity and increased response time in the signal detection task.
Shih et al. (2001)	i.p.	Mouse, MF1, male, 6	0 and 5,000 mg/kg, acute	LOAEL: 5,000 mg/kg	Impairment of righting reflex.

Table 4-30. Summary of animal psychomotor function and RT studies (continued)

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Umezu et al. (1997)	i.p.	Mouse, ICR, male, 10/group	0, 2,000, 4,000, and 5,000 mg/kg—loss of righting reflex measure	LOAEL: 2,000 mg/kg—loss of righting reflex	Loss of righting reflex.
		Mouse, ICR, male, 6–10/group	0, 62.5, 125, 250, 500, and 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg—operant behavior NOAEL: 125 mg/kg LOAEL: 250 mg/kg—punished responding	Decreased responses (lever presses) in an operant response task for food reward. Increased responding when lever press coupled with a 20-V electric shock (punished responding).
Bushnell and Oshiro (2000)	Inhalation	Rat, Long-Evans, male, 32	0, 2,000, 2,400 and ppm; 70 min/d, 9 d	LOAEL: 2,000 ppm	Decreased performance on the signal detection task. Increased response time and decreased response rate.
Nunes et al. (2001)	**Oral**	**Rat, Sprague-Dawley, male, 10/group**	**0 and 2,000 mg/kg-d, 7 d**	**LOAEL: 2,000 mg/kg-d**	**Increased foot splay. No change in any other FOB parameter (e.g., piloerection, activity, reactivity to handling).**
Moser et al. (2003)	Oral	Rat, F344, female, 10/group	0, 40, 200, 800, and 1,200 mg/kg-d, 10 d	–	Decreased motor activity; Decreased sensitivity to tail pinch; Increased abnormality in gait; Decreased grip strength; Adverse changes in several FOB parameters.
Albee et al. (2006)	Inhalation	Rat, F344, male and female, 10/sex/group	0, 250, 800, and 2,500 ppm; 6 hrs/d, 5 d/wk, 13 wks	NOAEL: 2,500 ppm	No change in any FOB measured parameter.

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

FOB = functional observational battery

Table 4-31. Summary of animal locomotor activity studies

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Wolff and Siegmund (1978)	i.p.	Mouse, AB, male, 18	0 and 182 mg/kg, tested 30 min after injection	LOAEL: 182 mg/kg	Decreased spontaneous motor activity.
Kulig et al. (1987)	**Inhalation**	**Rat, Wistar, male, 8/dose**	**0, 500, 1,000, and 1,500 ppm; 16 hrs/d, 5 d/wk, 18 wks**	**NOAEL: 500 ppm LOAEL: 1,000 ppm**	**No change in spontaneous activity, grip strength, or hindlimb movement. Increased latency time in the 2-choice visual discrimination task (cognitive disruption and/or motor activity related effect).**
Moser et al. (1995)	Oral	Rat, F344, female, 8/dose	0, 150, 500, 1,500, and 5,000 mg/kg, one dose	NOAEL: 500 mg/kg LOAEL: 1,500 mg/kg	Decreased motor activity; neuro-muscular and sensorimotor impairment.
			0, 50, 150, 500, and 1,500 mg/kg-d, 14 d	NOAEL: 150 mg/kg-d LOAEL: 500 mg/kg-d	Increased rearing activity.
Waseem et al. (2001)	Oral	Rat, Wistar, male, 8/group	0, 350, 700, and 1,400 ppm in drinking water for 90 d	NOAEL: 1,400 ppm	No significant effect on spontaneous locomotor activity.
	Inhalation	**Rat, Wistar, male, 8/group**	**0 and 376 ppm for up to 180 d; 4 hrs/d, 5 d/wk**	**LOAEL: 376 ppm**	**Changes in locomotor activity and vary by timepoint when measured over the 180-d period.**
Moser et al. (2003)	Oral	Rat, F344, female, 10/group	0, 40, 200, 800, and 1,200 mg/kg-d, 10 d	–	Decreased motor activity; Decreased sensitivity; Increased abnormality in gait; Adverse changes in several FOB parameters.

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

4.3.6.2.1. Loss of righting reflex

Umezu et al. (1997) studied disruption of the righting reflex following acute injection (i.p.) of 2,000, 4,000, and 5,000 mg/kg TCE in male ICR mice. TCE disrupted the righting reflex at doses of 2,000 mg/kg and higher. At 2,000 mg/kg, loss of righting reflex (LORR) was observed in only 2/10 animals injected. At 4,000 mg/kg, 9/10 animals experienced LORR and 100% of the animals experienced LORR at 5,000 mg/kg.

Shih et al. (2001) reported impaired righting reflexes at exposure doses of 5,000 mg/kg (i.p.) in male MF1 mice. Mice pretreated with dimethyl sulfoxide or disulfuram (CYP2E1 inhibitor) delayed LORR in a dose related manner. By contrast, the alcohol dehydrogenase inhibitor, 4-metylpyradine, did not delay LORR that resulted from 5,000 mg/kg TCE. These

data suggest that the anesthetic properties of TCE involve its oxidation via CYP2E1 to an active metabolite.

4.3.6.2.2. Activity, sensory-motor and neuromuscular function

Changes in sensory-motor and neuromuscular activity were reported in three studies (Moser et al., 2003; Moser et al., 1995; Kishi et al., 1993). Kishi et al. (1993) exposed male Wistar rats to 250, 500, 1,000, 2,000, and 4,000 ppm TCE for 4 hours. Rats exposed to 250 ppm TCE showed a significant decrease both in the total number of lever presses and in avoidance responses at 140 minutes of exposure compared with controls. Moser et al. (1995) evaluated the effects of acute and short-term (14 day) administration of TCE in adult female F344 rats (n = 8–10/dose) on activity level, neuromuscular function, and sensorimotor function as part of a larger functional observational battery (FOB) testing. The NOAEL levels identified by the authors are 500 mg/kg (10% of the limit dose) for the acute treatment and 150 mg/kg (3% of the limit dose) for the 14-day study. In the acute study, TCE produced the most significant effects in motor activity (activity domain), gait (neuromuscular domain), and click response (sensorimotor domain). In the 14-day study, only the activity domain (rearing) and neuromuscular domain (forelimb grip strength) were significantly different ($p < 0.05$) from control animals. In a separate 10-day study (Moser et al., 2003), TCE administration significantly ($p < 0.05$) reduced motor activity, tail pinch responsiveness, reactivity to handling, hind limb grip strength and body weight. Significant increases ($p < 0.05$) in piloerection, gait scores, lethality, body weight loss, and lacrimation were also reported in comparison to controls.

There are also two negative studies that used adequate numbers of subjects in their experimental design but used lower doses than did Moser et al. (2003). Albee et al. (2006) exposed male and female F344 rats (n = 10/sex) to TCE by inhalation at exposure doses of 250, 800, and 2,500 ppm, for 6 hours/day, 5 days/week, for 13 weeks. The FOB was performed monthly, although it is not certain how much time elapsed from the end of exposure until the FOB test was conducted. No treatment-related differences in grip strength or landing foot splay were demonstrated in this study. Kulig et al. (1987) also failed to show significant effects of TCE inhalation exposure on markers of motor behavior. Wistar rats (n = 8) exposed to 500, 1,000, and 1,500 ppm, for 16 hours/day, 5 days/week, for 18 weeks failed to show changes in spontaneous activity, grip strength, or coordinated hind limb movement. Measurements were made every 3 weeks during the exposure period and occurred between 45 and 180 minutes following the previous TCE inhalation exposure.

4.3.6.2.3. Locomotor activity

The data, with regard to locomotor activity, are inconsistent. Several studies showed that TCE exposure can decrease locomotor activity including Wolff and Siegmund (1978) where AB mice (n = 18) were treated acutely with a dose of 182 mg/kg, i.p. at one of four time points

during a 24-hour day. Moser et al. (2003; 1995) reported reduced locomotor activity in female F344 rats (n = 8–10) gavaged with TCE over an acute (LOAEL = 5,000 mg/kg TCE) or subacute period (LOAEL = 500 mg/kg, but no effect at 5,000 mg/kg). In the Moser et al. (2003) study, it appears that 200 mg/kg TCE yielded a significant reduction in locomotor activity and that the degree of impairment at this dose represented a maximal effect on this measure. That is, higher doses of TCE appear to have produced equivalent or slightly less of an effect on this behavior. While this study identifies a LOAEL of 200 mg/kg TCE by gavage over a 10-day period, this is a much lower dose effect than that reported in Moser et al. (1995). Both studies (Moser et al., 2003; Moser et al., 1995) demonstrate a depression in motor activity that occurs acutely following TCE administration. Kulig et al. (1987) demonstrated that rats had increased response latency to a two choice visual discrimination following 1,000- and 1,500-ppm TCE exposures for 18 weeks. However, no significant changes in grip strength, hindlimb movement, or any other motor activity measurements were noted.

There are also a few studies (Waseem et al., 2001; Fredriksson et al., 1993) generally conducted using lower exposure doses that failed to demonstrate impairment of motor activity or ability following TCE exposure. Waseem et al. (2001) failed to demonstrate changes in locomotor activity in male Wistar rats (n = 8) dosed with TCE (350, 700, and 1,400 ppm) in drinking water for 90 days. Wistar rats (n = 8) exposed to 500, 1,000, and 1,500 ppm for 16 hours/day, 5 days/week, for 18 weeks failed to show changes in spontaneous activity. No changes in locomotor activity were observed for 17-day-old male NMRI mice that were dosed postnatally with 50 or 290 mg/kg-day from day 10 to 16 (Fredriksson et al., 1993). However, rearing activity was significantly decreased in the NMRI mice at day 60.

4.3.6.3. Summary and Conclusions for Psychomotor Effects

In human studies, psychomotor effects such as RT and muscular dyscoordination have been examined following TCE exposure. In the RT studies, statistically significant increases in CRT and SRT were reported in the Kilburn studies (2002b, a; 1996; 1993a). All of these studies were geographically based and it was suggested that the results were used for litigation and the differences between exposed and referent groups on other factors influencing reaction speed time may introduce a bias to the findings. Additionally, in these studies exposure to TCE and other chemicals occurred through drinking water for at least 1 year and TCE concentrations in well water ranged from 0.2 to 10,000 ppb. Reif et al. (2003) whose exposure assessment approach included exposure modeling of water distribution system to estimate TCE concentrations in tap water at census track of residence found that residents with drinking water containing TCE (up to >15 ppb—the highest level not specified) and other chemicals did not significantly increase CRTs or SRTs. Inhalation studies also demonstrated increased RTs. An acute exposure chamber study (Gamberale et al., 1976) tested for CRT, SRT, and RT-addition following a 70-minute exposure to TCE. A concentration-dependent significant decrease in performance was

observed with the RT-addition test and not for CRT or SRT tasks. An occupational exposure study on eight female workers exposed to TCE (Gun et al., 1978) also reported increased RT in the females exposed to TCE-only. Muscular dyscoordination for humans following TCE exposure has been reported in a few studies as a subjective observation. The studies indicated that exposure resulted in decreased motor speed and dexterity (Rasmussen et al., 1993a; Tröster and Ruff, 1990) and self-reported faster asymptomatic fine motor hand movements (Gash et al., 2008).

Animal studies evaluated psychomotor function by examining locomotor activity, operant responding, changes in gait, loss of righting reflex, and general motor behavior (see Tables 4-30 and 4-31 for references). Overall, the studies demonstrated that TCE causes loss of righting reflex at injection doses of ≥2,000 mg/kg (Shih et al., 2001; Umezu et al., 1997). Regarding general psychomotor testing, significant decreases in lever presses and avoidance were observed at inhalation exposures as low as 250 ppm for 4 hours (LOAEL; Kishi et al., 1993). Following subchronic inhalation exposures, no significant changes in psychomotor activity were noted at up to 2,500 ppm for 13 weeks (Albee et al., 2006) or at 1,500 ppm for 18 weeks (Kulig, 1987). In the oral administration studies (Moser et al., 2003; Moser et al., 1995), psychomotor effects were evaluated using an FOB. More psychomotor domains were significantly affected by TCE treatment in the acute study in comparison to the 14-day study, but a lower NOAEL (150 mg/kg-day) was reported for the 14-day study in comparison to the acute study (500 mg/kg; Moser et al., 1995). Upon closer examination of the data, a biphasic effect in one measure of the FOB (rearing) was resulting in the lower NOAEL for the 14-day study and doses that were higher and lower than the NOAEL did not produce a statistically significant increase in the number of rears. Therefore, it can be surmised that acute exposure to TCE results in significant changes in psychomotor function. However, there may be some tolerance to these psychomotor changes in increased exposure duration to TCE as evidenced by the results noted in the short-term and subchronic exposure studies.

4.3.7. Mood Effects and Sleep Disorders

4.3.7.1. Effects on Mood: Human Studies

Reports of mood disturbance (depression, anxiety) resulting from TCE exposure are numerous in the human literature. These symptoms are subjective and difficult to quantify. Studies by Gash et al. (2008), Kilburn and Warshaw (1993a), Kilburn (2002b, a), McCunney et al. (1988), Mitchell et al. (1969), Rasmussen and Sabroe (1986), and Troster and Ruff (1990) reported mood disturbances in humans. Reif et al. (2003) and Triebig et al. (1976) reported no effect on mood following TCE exposures.

4.3.7.2. Effects on Mood: Laboratory Animal Findings

It is difficult to obtain comparable data of emotionality in laboratory studies. However, Moser et al. (2003) and Albee et al. (2006) both report increases in handling reactivity among rats exposed to TCE. In the Moser study, female F344 rats received TCE by gavage for periods of 10 days at doses of 0, 40, 200, 800, and 1,200 while Albee et al. (2006) exposed F344 rats to TCE by inhalation at exposure doses of 250, 800, and 2,500 ppm for 6 hours/day, 5 days/week, for 13 weeks. These studies are summarized and described in Table 4-32.

Table 4-32. Summary of animal mood effect and sleep disorder studies

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Mood effects					
Albee et al. (2006)	**Inhalation**	Rat, F344, male and female, 10/sex/group	0, 250, 800, and 2,500 ppm; 6 hrs/d, 5 d/wk, 13 wks	NOAEL: 800 ppm	**Increased handling reactivity.**
Moser et al. (2003)	Oral	Rat, F344, female, 10/group	0, 40, 200, 800, and 1,200 mg/kg-d, 10 d	–	Decreased handling reactivity score.
Sleep disorder					
Arito et al. (1994)	**Inhalation**	Rat, Wistar, male, 5/group	0,50, 100, and 300 ppm; 8 hrs/d, 5 d/wk, 6 wks	LOAEL: 50 ppm	**Significant changes in sleep cycle as measured through EEG changes; significant decreases in wakefulness.**

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

4.3.7.3. Sleep Disturbances

Arito et al. (1994) exposed male Wistar rats to 50, 100, and 300 ppm TCE for 8 hour/day, 5 days/week, for 6 weeks and measured electroencephalographic (EEG) responses (see Table 4-32). EEG responses were used as a measure to determine the number of awake (wakefulness hours) and sleep hours. Exposure to all of the TCE levels significantly decreased amount of time spent in wakefulness during the exposure period. Some carry over was observed in the 22 hours post exposure period, with significant decreases in wakefulness seen at 100 ppm TCE. Significant changes in wakefulness-sleep elicited by the long-term exposure appeared at lower exposure levels. These data seem to identify a low dose effect of TCE and established a LOAEL of 50 ppm for sleep changes.

4.3.8. Developmental Neurotoxicity

4.3.8.1. Human Studies

In humans, CNS birth defects were observed in a few studies (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). Postnatally, observed adverse effects in humans include delayed newborn reflexes following exposure to TCE during childbirth (Beppu, 1968),

impaired learning or memory (White et al., 1997; Bernad et al., 1987, abstract); aggressive behavior (Bernad et al., 1987, abstract); hearing impairment (Burg and Gist, 1999); speech impairment (Burg and Gist, 1999; White et al., 1997); encephalopathy (White et al., 1997); impaired executive and motor function (White et al., 1997); attention deficit (White et al., 1997; Bernad et al., 1987, abstract); and autism spectrum disorder (ASD) (Windham et al., 2006). The human developmental neurotoxicity studies are discussed in more detail in Section 4.8.3.1.2.1, and summarized in Table 4-33.

Table 4-33. Summary of human developmental neurotoxicity associated with TCE exposures

Finding	Species	References
CNS defects, neural tube defects	Human	ATSDR (2001)
		Bove (1996); Bove et al. (1995)
		Lagakos et al. (1986)
Delayed newborn reflexes	Human	Beppu (1968)
Impaired learning or memory	Human	Bernad et al. (1987, abstract)
		White et al. (1997)
Aggressive behavior	Human	Bernad et al., (1987, abstract)
Hearing impairment	Human	Burg and Gist (1999)
Speech impairment	Human	Burg and Gist (1999)
		White et al. (1997)
Encephalopathy	Human	White et al. (1997)
Impaired executive function	Human	White et al. (1997)
Impaired motor function	Human	White et al. (1997)
Attention deficit	Human	White et al. (1997)
	Human	Bernad et al. (1987, abstract)
ASD	Human	Windham et al. (2006)

4.3.8.2. Animal Studies

There are a few studies demonstrating developmental neurotoxicity following TCE exposure (range of exposures) to experimental animals. These studies collectively suggest that developmental neurotoxicity result from TCE exposure; however, some types of effects such as learning and memory measures have not been evaluated. Most of the studies demonstrate either spontaneous motor activity changes (Taylor et al., 1985) or neurochemical changes such as decreased glucose uptake and changes in the specific gravity of the cortex and cerebellum (Isaacson and Taylor, 1989; Noland-Gerbec et al., 1986; Westergren et al., 1984). In addition, in most of these studies, there is no assessment of the exposure to TCE or metabolites in the pups/offspring. Details of the studies are presented below and summarized in Table 4-34.

Table 4-34. Summary of mammalian in vivo developmental neurotoxicity studies—oral exposures

Reference[a]	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL[b]	Effects
Fredriksson et al. (1993)	**Mouse, NMRI, male pups, 12 pups from 3–4 different litters/group**	**0, 50, or 290 mg/kg-d PNDs 10–16**	**LOAEL: 50 mg/kg-d**	**Rearing activity significant ↓ at both dose levels on PND 60.**
George et al. (1986)	**Rat, F334, male and female, 20 pairs/treatment group, 40 controls/sex**	**0, 0.15, 0.30, or 0.60% microencapsulated TCE in diet. Breeders exposed 1 wk premating, then for 13 wks; pregnant females throughout pregnancy (i.e., 18 wks total).**	**LOAEL: 0.15%**	**Open field testing in pups: a significant dose-related trend toward ↑ time required for male and female pups to cross the first grid in the test device.**
Isaacson and Taylor (1989)	**Rat, Sprague-Dawley, females, six dams/group**	**0, 312, or 625 mg/L (0, 4.0, or 8.1 mg/d)[c]. Dams (and pups) exposed from 14 d prior to mating until end of lactation.**	**LOAEL: 312 mg/L**	Significant ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the CA1 region of the hippocampus.
Noland-Gerbec et al. (1986)	Rat, Sprague-Dawley, females, 9–11 dams/group	0, 312 mg/L. Average total intake of dams: 825 mg TCE over 61 d. Dams (and pups) exposed from 14 d prior to mating until end of lactation.	LOAEL: 312 mg/L	Significant ↓ uptake of [^{3}H]-2-DG in whole brains and cerebella (no effect in hippocampus) of exposed pups at 7, 11, and 16 d, but returned to control levels by 21 d.
Taylor et al. (1985)	Rat, Sprague-Dawley, females, no. dams/group not reported	0, 312, 625, and 1,250 mg/L in drinking water. **Dams (and pups) exposed from 14 d prior to mating until end of lactation.**	LOAEL: 312 mg/L	**Exploratory behavior significant ↑ in 60- and 90-d-old male rats at all treatment levels.** Locomotor activity (measured through the wheel-running tasks) was higher in rats from dams exposed to 1,250 mg/L TCE.
Blossom et al. (2008)	Mouse, MRL +/+, dams and both sexes offspring, eight litters/group; three–eight pups/group	Drinking water, from GD 0 to PND 42; 0 or 0.1 mg/mL; maternal dose = 25.7 mg/kg-d; offspring PNDs 24–42 dose = 31.0 mg/kg-d.	LOAEL: 31 mg/kg-d for offspring	Righting reflex, bar holding, and negative geotaxis were not impaired. Significant association between impaired nest quality and TCE exposure. Lower GSH levels and GSH:GSSG ratios with TCE exposure.

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).
[b]LOAELs are based upon reported study findings.
[c]Dose conversions provided by study author(s).

GSSG = oxidized GSH

Taylor et al. (1985) administered TCE to female Sprague-Dawley rats in their drinking water from 14 days before breeding throughout gestation and until pups were weaned at 21 days. Measured TCE concentrations in the dams were 312−646, 625−1,102, and 1,250−1,991 mg/L in

the low-, mid-, and high-dose groups as measured from the drinking water. Pups were evaluated for exploratory activity at 28, 60, or 90 days. No significant differences were noted between control and treated pups at 28 days. At 60 days, all TCE-treated animals had significantly increased exploratory activity in comparison to age-matched controls, but only the high-dose group had increased activity at 90 days. A significant increase in spontaneous motor activity (as measured by a wheel-running task) was noted in only the high dose TCE (1,250–1,991 mg/L) group during the onset of the darkness period. This study demonstrated that both spontaneous and open field activities are significantly affected by developmental TCE exposure.

Spontaneous behavioral changes were also investigated in another study by Fredriksson et al. (1993). Male and female NMRI pups (mice) were orally administered 50 or 290 mg/kg-day for 7 days starting at PND 10. Spontaneous motor activity was investigated in male mice at ages 17 and 60 days. TCE-treated animals tested at day 17 did not demonstrate changes in any spontaneous activity measurements in comparison to control animals. Both doses of TCE (50 and 290 mg/kg-day) significantly decreased rearing in 60-day-old male mice.

Westergren et al. (1984) examined the brain specific gravity of litters from mice exposed to TCE. NMRI mice (male and female) were exposed to 150 ppm TCE (806.1 mg/m^3) for 30 days prior to mating. Exposure in males continued until the end of mating and females were exposed until the litters were born. Brains were removed from the offspring at either PNDs 1, 10, 20–22, or 29–31. At PNDs 1 and 10, significant decreases were noted in the specific gravity of the cortex. Significant decreases in the specific gravity of the cerebellum were observed at PND 10 (decrease from 1.0429 ± 0.00046 to 1.0405 ± 0.00030) and 20–22 (decrease from 1.0496 ± 0.00014 to 1.0487 ± 0.00060). Cerebellum measurements were not reported for PND 29–31 animals. Neurobehavioral assessments were not conducted in this study. Additionally, decreased brain specific gravity is suggestive of either decreased brain weight or increased brain volume (probably from edema) or a combination of the two factors and is highly suggestive of an adverse neurological effect. The effects of TCE on the cortical specific gravity were not persistent since cortices from PNDs 29–31 animals did not exhibit any significant changes. It is unclear if the effects on the cerebellum were persistent since results were not reported for the PND 29–31 animals. However, the magnitude of the change in the specific gravity of the cerebellum is decreased from PNDs 10 to 20–22, suggesting that the effect may be reversible given a longer recovery period from TCE.

The effect of TCE on glucose uptake in the brain was evaluated in rat pups exposed to TCE during gestation and through weaning. The primary source of energy utilized in the CNS is glucose. Changes in glucose uptake in the brain are a good indicator for neuronal activity modification. Noland-Grebec et al. (1986) administered 312 mg/L TCE through drinking water to female Sprague-Dawley rats from 2 weeks before breeding and up until pups reached 21 days of age. To measure glucose uptake, 2-deoxyglucose was administered intraperitoneally to male pups at either PND 7, 11, 16, or 21. Significant decreases in glucose uptake were noted in whole

brain and cerebellum at all PNDs tested. Significant decreases in glucose uptake were also observed in the hippocampus except for animals tested at PND 21. The observed decrease in glucose uptake suggests decreased neuronal activity.

Female Sprague-Dawley rats (70 days old) were administered TCE in drinking water at a level of either 4.0 or 8.1 mg/day for 14 days prior to mating and continuing up through lactation (Isaacson and Taylor, 1989). Only the male pups were evaluated in the studies. At PND 21, brains were removed from the pups, sectioned, and stained to evaluate the changes in myelin. There was a significant decrease (40% decrease) in myelinated fibers in the CA1 region of the hippocampus of the male pups. This effect appeared to be limited to the CA1 region of the hippocampus since other areas such as the optic tract, fornix, and cerebral peduncles did not have decreases in myelinated fibers.

Neurological changes were found in pups exposed to TCE in a study conducted by the National Toxicology Program (NTP) in F344 rats (George et al., 1986). TCE was administered to rats at dietary levels of 0, 0.15, 0.30, or 0.60%. No intake calculations were presented for the rat study and therefore, a dose rate is unavailable for this study. Open field testing revealed a significant ($p < 0.05$) dose-related trend toward an increase in the time required for male and female F1 weanling pups (PND 21) to cross the first grid in the testing device, suggesting an effect on the ability to react to a novel environment.

Blossom et al. (2008) treated male and female MRL +/+ mice with 0 or 0.1 mg/mL TCE in the drinking water. Treatment was initiated at the time of mating, and continued in the females (8/group) throughout gestation and lactation. Behavioral testing consisted of righting reflex on PNDs 6, 8, and 10; bar-holding ability on PNDs 15 and 17; and negative geotaxis on PNDs 15 and 17. Nest building was assessed and scored on PND 35, the ability of the mice to detect and distinguish social odors was examined with an olfactory habituation/dishabituation method at PND 29, and a resident intruder test was performed at PND 40 to evaluate social behaviors. Righting reflex, bar holding, and negative geotaxis were not impaired by treatment. There was a significant association between impaired nest quality and TCE exposure in tests of nest-building behavior; however, TCE exposure did not have an effect on the ability of the mice to detect social and nonsocial odors using habituation and dishabituation methods. Resident intruder testing identified significantly more aggressive activities (i.e., wrestling and biting) in TCE-exposed juvenile male mice as compared to controls, and the cerebellar tissue from the male TCE-treated mice had significantly lower GSH levels and GSH:oxidized GSH (GSH:GSSG) ratios, indicating increased oxidative stress and impaired thiol status, which have been previously reported to be associated with aggressive behaviors (Franco et al., 2006). Histopathological examination of the brain did not identify alterations indicative of neuronal damage or inflammation.

4.3.8.3. Summary and Conclusions for the Developmental Neurotoxicity Studies

Gestational exposure to TCE in humans has resulted in several developmental abnormalities. These changes include neuroanatomical changes such as neural tube defects (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986) and encephalopathy (White et al., 1997). Clinical neurological changes such as impaired cognition (White et al., 1997; Bernad et al., 1987), aggressive behavior (Bernad et al., 1987), and speech and hearing impairment (Burg and Gist, 1999; White et al., 1997) are also observed when TCE exposure occurs in utero.

In animal studies, anatomical and clinical developmental neurotoxicity is also observed. Following inhalation exposures of 150 ppm to mice during mating and gestation, the specific gravity of offspring brains was significantly decreased at postnatal time points through the age of weaning; this effect did not persist to 1 month of age (Westergren et al., 1984). In studies reported by Taylor et al. (1985), Isaacson and Taylor (1989), and Noland-Gerbec et al. (1986), 312 mg/L exposures in drinking water that were initiated 2 weeks prior to mating and continued to the end of lactation resulted in: (1) significant increase in exploratory behavior at PNDs 60 and 90; (2) reductions in myelination in the CA1 hippocampal region of offspring at weaning; and (3) significantly decreased uptake of 2-deoxyglucose in the rat brain at PND 21. Gestational exposures to mice (Fredriksson et al., 1993) resulted in significantly decreased rearing activity on PND 60, and dietary exposures during the course of a continuous breeding study in rats (George et al., 1986) found a significant trend toward increased time to cross the first grid in open field testing. In a study by Blossom et al. (2008), male mice exposed gestationally to TCE exhibited lower GSH levels and lower GSH:GSSG ratios, which are also observed in mice that have more aggressive behaviors (Franco et al., 2006).

4.3.9. Mechanistic Studies of TCE Neurotoxicity

4.3.9.1. Dopamine Neuron Disruption

There are very recent laboratory animal findings resulting from short-term TCE exposures that demonstrate vulnerability of dopamine neurons in the brain to this chlorinated hydrocarbon. The key limitation of these laboratory animal studies is that only one dosing regimen was included in each study. Moreover, there has been no systematic body of data to show that other chlorinated hydrocarbons such as tetrachloroethylene or aromatic solvents similarly target this cell type. Confidence in the limited data regarding dopamine neuron death and in vivo TCE exposure would be greatly enhanced by identifying a dose-response relationship. If indeed TCE can target dopamine neurons, it would be anticipated that human exposure to this agent would result in elevated rates of parkinsonism. There are no systematic studies of this potential relationship in humans, although one limited report attempted to address this possibility. Difficulties in subject recruitment into that study limit the weight that can be given to the results.

Endogenously formed chlorinated tetrahydro-beta-carbolines (TaClo) have been suggested to contribute to the development of Parkinson-like symptoms (Kochen et al., 2003; Riederer et al., 2002; Bringmann et al., 1995; Bringmann et al., 1992). TaClo can be formed endogenously from metabolites of TCE such as trichloroacetaldehyde. TaClo has been characterized as a potent neurotoxicant to the dopaminergic system. Some research groups have hypothesized that Parkinson-like symptoms resulting from TCE exposure may occur through the formation of TaClo, but not enough evidence is available to determine if this mechanism occurs.

4.3.9.1.1. Dopamine neuron disruption: human studies

There are no human studies that present evidence that TCE exposure results in dopamine neuron disruption. Nagaya et al. (1990) examined serum dopamine β-hydroxylase activity without differences observed in mean activities between control and exposed subjects. In the study, 84 male workers exposed to TCE were compared to 83 male age-matched controls. The workers had constantly used TCE in their jobs and their length of employment ranged from 0.1 to 34 years.

4.3.9.1.2. Dopamine neuron disruption: animal studies

There are limited data from mice and rats that suggest the potential for TCE to disrupt dopamine neurons in the basal ganglia (see Table 4-35). Gash et al. (2008) showed that TCE administered by gavage in F344 rats (n = 9) at an exposure level of 1,000 mg/kg-day, 5 days/week, for 6 weeks yielded degeneration of dopamine neurons in the substantia nigra and alterations in dopamine turnover as reflected in a shift in dopamine metabolite to parent compound ratios. Guehl et al. (1999) reported similar findings in OF1 mice (n = 10) that were injected i.p. with 400 mg/kg-day TCE 5 days/week for 4 weeks. Each of these studies evaluated only a single dose level of TCE, so establishing a dose-response relationship is not possible. Consequently, these data are of limited utility in risk assessment because they do not establish the potency of TCE to damage dopamine neurons. They are important, however, in identifying a potential permanent impairment that might occur following TCE exposure at relatively high exposure doses. They also identify a potential mechanism by which TCE could produce CNS injury.

Table 4-35. Summary of animal dopamine neuronal studies

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Guehl et al. (1999)	i.p. administration	Mouse, OF1, male, 10	0 and 400 mg/kg-d; 5 d/wk, 4 wks	LOAEL: 400 mg/kg-d	Significant dopaminergic neuronal death in substantia nigra.
Gash et al. (2008)	**Gavage**	**Rat, F344, male, 9/group**	**0 and 1,000 mg/kg-d; 5 d/wk, 6 wks**	**LOAEL: 1,000 mg/kg-d**	**Degeneration of dopamine-containing neurons in substantia nigra. Change in dopamine metabolism.**

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

4.3.9.1.3. Summary and conclusions of dopamine neuron studies

Only two animal studies have reported changes in dopamine neuron effects from TCE exposure (Gash et al., 2008; Guehl et al., 1999). Both studies demonstrated toxicity to dopaminergic neurons in the substantia nigra in rats or mice. LOAELs of 400 mg/kg-day (mice; Guehl et al., 1999) and 1,000 mg/kg-day (rats; Gash et al., 2008) were reported for this effect. Dopaminergic neuronal degeneration following TCE exposure has not been studied in humans. However, there were no changes in serum dopamine β-hydroxylase activity in TCE-exposed or control individuals (Nagaya et al., 1990). Loss of dopaminergic neurons in the substantia nigra also occurs in patients with Parkinson's disease and the substantia nigra is an important region in helping to control movements. As a result, loss of dopaminergic neurons in the substantia nigra may be one of the potential mechanisms involved in the clinical psychomotor effects that is observed following TCE exposure.

4.3.9.2. Neurochemical and Molecular Changes

There are limited data obtained only from laboratory animals that TCE exposure may have consequences on GABAergic (gamma-amino butyric acid [GABA]) and glutamatergic neurons (Shih et al., 2001; see Table 4-36; Briving et al., 1986). However, the data obtained are limited with respect to brain region examined, persistence of effect, and whether there might be functional consequences to these changes. The data of Briving et al. (1986) demonstrating changes in cerebellar high affinity uptake for GABA and glutamate following chronic, low-level (50 and 150 ppm) TCE exposure do not appear to be reflected in the only other brain region evaluated (hippocampus). However, glutamate levels were increased in the hippocampus. The data of Shih et al. (2001) are indirect in that it shows an altered response to GABAergic antagonist drugs in mice treated by acute injection with 250, 500, 1,000, and 2,000 mg/kg TCE. However, these data do show some dose dependency with significant findings observed with TCE exposure as low as 250 mg/kg.

Table 4-36. Summary of neurophysiological, neurochemical, and neuropathological effects with TCE exposure

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Neurophysiological studies					
Shih et al. (2001)	i.p.	Mouse, MF1, male, 6/group	0, 250 500, 1,000, or 2,000 mg/kg, 15 min; followed by tail infusion of PTZ (5 mg/mL), picrotoxin (0.8 mg/mL), bicuculline (0.06 mg/mL), strychnine (0.05 mg/mL), 4-AP (2 mg/mL), or NMDA (8 mg/mL)	–	Increased threshold for seizure appearance with TCE pretreatment for all convulsants. Effects strongest on the $GABA_A$ antagonists, PTZ, picrotoxin, and bicuculline, suggesting $GABA_A$ receptor involvement. NMDA and glycine Rc involvement also suggested.
Ohta et al. (2001)	i.p.	Mouse, ddY, male, 5/group	0, 300, or 1,000 mg/kg, sacrificed 24 hrs after injection	LOAEL: 300 mg/kg	Decreased response (long-term potentiation response) to tetanic stimulation in the hippocampus.
Neurochemical studies					
Briving et al. (1986)	Inhalation	Gerbils, Mongolian, male and female, 6/group	0, 50, or 150 ppm, continuous, 24 hrs/d, 12 mo	NOAEL: 50 ppm; LOAEL: 150 ppm for glutamate levels in hippocampus. NOAEL: 150 ppm for glutamate and GABA uptake in hippocampus. LOAEL: 50 ppm for glutamate and GABA uptake in cerebellar vermis.	Increased glutamate levels in the hippocampus. Increased glutamate and GABA uptake in the cerebellar vermis.
Subramoniam et al. (1989)	Oral	Rat, Wistar, female	0 or 1,000 mg/kg, 2 or 20 hrs. 0 or 1,000 mg/kg-d, 5 d/wk, 1 yr	–	PI and PIP decreased by 24 and 17% at 2 hrs. PIP and PIP2 increased by 22 and 38% at 20 hrs. PI, PIP, and PIP2 reduced by 52, 23, and 45% in 1 yr study.

Table 4-36. Summary of neurophysiological, neurochemical, and neuropathological effects with TCE exposure (continued)

Reference[a]	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Haglid et al. (1981)	Inhalation	Gerbil, Mongolian, male and female, 6–7/group	0, 60, or 320 ppm, 24 hrs/d, 7 d/wk, 3 mo	LOAEL: 60 ppm, brain protein changes. NOAEL: 60 ppm; LOAEL: 320 ppm, brain DNA changes	(1) Decreases in total brain soluble protein whereas increase in S100 protein. (2) Elevated DNA in cerebellar vermis and sensory motor cortex.
Neuropathological studies					
Kjellstrand et al. (1987)	**Inhalation**	Mouse, NMRI, male	0, 150, or 300 ppm, 24 hrs/d, 4 or 24 d	LOAEL: 150 ppm, 4 and 24 d	**Sciatic nerve regeneration was inhibited in both mice and rats.**
		Rat, Sprague-Dawley, female	**0, 300 ppm, 24 hrs/d, 4 or 24 d**	**NOAEL: 300 ppm, 4 d. LOAEL: 300 ppm, 24 d.**	
Isaacson and Taylor (1989)	Oral	Rat, Sprague-Dawley, females, six dams/ group	0, 312, or 625 mg/L. (0, 4.0, or 8.1 mg/d); dams (and pups) exposed from 14 d prior to mating until end of lactation	LOAEL: 312 mg/L	Significant ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the hippocampus.

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

NMDA = N-nitrosodimethylamine; PI = phosphatidyl inositol; PIP = phosphatidyl inositol-4-phosphate; PIP2 = phosphatidylinositol-4,5-bisphosphate; PTZ = pentylenetetrazole

The development and physiology of the hippocampus have also been evaluated in two different studies (Ohta et al., 2001; Isaacson and Taylor, 1989). Isaacson and Taylor (1989) found a 40% decrease in myelinated fibers from hippocampi dissected from neonatal Sprague-Dawley rats (n = 2–3) that were exposed to TCE (4 and 8.1 mg/day) in utero and during the preweaning period. Ohta et al. (2001) injected male ddY mice with 300 mg/kg TCE and found a significant reduction in response to titanic stimuli in excised hippocampal slices. Both of these studies demonstrated that there is some interaction with TCE and the hippocampal area in the brain.

Impairment of sciatic nerve regeneration was demonstrated in mice and rats exposed to TCE (Kjellstrand et al., 1987). Under heavy anesthesia, the sciatic nerve of the animals was artificially crushed to create a lesion. Prior to the lesion, some animals were pre-exposed to TCE for 20 days and then for an additional 4 days after the lesion. Another set of animals was only exposed to TCE for 4 days following the sciatic nerve lesion. For mice, regeneration of the sciatic nerve in comparison to air-exposed animals was 20 and 33% shorter in groups exposed to 150 and 300 ppm TCE for 4 days, respectively. This effect did not significantly increase in mice pre-exposed to TCE for 20 days, and the regeneration was 30% shorter in the 150-ppm group

and 22% shorter in the 300-ppm group. Comparatively, a 10% reduction in sciatic nerve regeneration length was observed in rats exposed to TCE for 20 days prior to the lesion plus the 4 days after the sciatic nerve lesion.

There are also a few in vitro studies (summarized in Table 4-37) that have demonstrated that TCE exposure alters the function of inhibitory ion channels such as $GABA_A$ and glycine receptors (Beckstead et al., 2000; Krasowski and Harrison, 2000), and serotonin receptors (Lopreato et al., 2003). Krasowski and Harrison (2000) and Beckstead et al. (2000) were able to demonstrate that human $GABA_A$ and glycine receptors could be potentiated by TCE when a receptor agonist was coapplied. Krasowski and Harrison (2000) conducted an additional experiment in order to determine if TCE was interacting with the receptor or perturbating the cellular membrane (bilipid layer). Specific amino acids on the $GABA_A$ and glycine receptors were mutated and in the presence of a receptor agonist (GABA for $GABA_A$ and glycine for glycine receptors) and in these mutated receptors, TCE-mediated potentiation was significantly decreased or abolished, suggesting that there was an interaction between TCE and these receptors. Lopreato et al. (2003) conducted a similar study with the $5HT_{3A}$ serotonin receptor and found that when TCE was coapplied with serotonin, there was a potentiation in receptor response. Additionally, TCE has been demonstrated to alter the function of voltage sensitive calcium channels (VSCCs) by inhibiting the calcium mediated-current at a holding potential of -70 mV and shifting the activation of the channels to a more hyperpolarizing potential (Shafer et al., 2005).

Table 4-37. Summary of in vitro ion channel effects with TCE exposure

Reference	Cellular system	Neuronal channel/ receptor	Concentrations	Effects
In vitro studies				
Shafer et al. (2005)	PC12 cells	VSCC	0, 500, 1,000, 1,500, or 2,000 µM	Shift of VSCC activation to a more hyperpolarizing potential. Inhibition of VSCCs at a holding potential of -70 mV.
Beckstead et al. (2000)	*Xenopus* oocytes	Human recombinant: glycine receptor α1, $GABA_A$ receptors, α1β1, α1β2γ2L	0 or 390 µM	50% potentiation of the $GABA_A$ receptors; 100% potentiation of the glycine receptor.
Lopreato et al. (2003)	*Xenopus* oocytes	Human recombinant serotonin 3A receptor	0 or 390 µM	Potentiation of serotonin receptor function.
Krasowski and Harrison (2000)	Human embryonic kidney 293 cells	Human recombinant Glycine receptor α1, $GABA_A$ receptors α2β1	Not provided	Potentiation of glycine receptor function with an EC_{50} of 0.65 ± 0.05 mM[a]. Potentiation of $GABA_A$ receptor function with an EC_{50} of 0.85 ± 0.2 mM.

[a]EC_{50} = concentration of the chemical at which 50% of the maximal effect is produced

4.3.10. Potential Mechanisms for TCE-Mediated Neurotoxicity

The mechanisms of TCE neurotoxicity have not been established despite a significant level of research on the outcomes of TCE exposure. Results from several mechanistic studies can be used to help elucidate the mechanism(s) involved in TCE-mediated neurological effects.

The disruption of the trigeminal nerve appears to be a highly idiosyncratic outcome of TCE exposure. There are limited data to suggest that it might entail a demyelination phenomenon, but similar demyelination does not appear to occur in other nerve tracts. In this regard, then, TCE is unlike a variety of hydrocarbons that have more global demyelinating action. There are some data from CNS data that focus on shifts in lipid profiles as well as data showing loss of myelinated fibers in the hippocampus. However, the changes in lipid profiles are both quite small and, also, inconsistent. And the limited data from hippocampus are not sufficient to conclude that TCE has significant demyelinating effects in this key brain region. Indeed, the bulk of the evidence from studies of learning and memory function (which would be tied to hippocampal function) suggests no clear impairments due to TCE.

Some researchers (Albee et al., 2006; Albee et al., 1997; Laureno, 1993; Barret et al., 1992; Barret et al., 1991; Laureno, 1988) have indicated that changes in trigeminal nerve function may be due to dichloroacetylene, which is formed under nonbiological conditions of high alkalinity or temperature during volatilization of TCE. In experimental settings, trigeminal nerve function (Albee et al., 1997) and trigeminal nerve morphology (Barret et al., 1992; Barret et al., 1991) were found to be more altered following a low exposure to dichloroacetylene in comparison to the higher TCE exposure. Barret et al. (1992; 1991) also demonstrated that TCE administration results in morphological changes in the trigeminal nerve. Thus, dichloroacetylene may contribute to trigeminal nerve impairment may be plausible following an inhalation exposure under conditions favoring its formation. Examples of such conditions include passing through a carbon dioxide scrubber containing alkaline materials, application to remove a wax coating from a concrete-lined stone floor, or mixture with alkaline solutions or caustic (Bingham et al., 2001; Greim et al., 1984; Saunders, 1967). However, dichloroacetylene exposures have not been identified or measured in human epidemiologic studies with TCE exposure, and thus, do not appear to be common to occupational or residential settings (Lash and Green, 1993). Moreover, changes in trigeminal nerve function have also been consistently reported in humans exposed to TCE following an oral exposure (Kilburn, 2002b, a), across many human studies of occupational and drinking water exposures under conditions with highly varying potentials for dichloroacetylene formation individuals (Feldman et al., 1988; Barret et al., 1987; Barret et al., 1984; Barret et al., 1982). As a result, the mechanism(s) for trigeminal nerve function impairment following TCE exposure is unknown (Campo et al., 2007; Mhiri et al., 2004; Kilburn, 2002a; Kilburn and Warshaw, 1993a; Ruijten et al., 1991). The varying dichloroacetylene exposure potential across these studies suggests TCE exposure, which is common to all of them, as the most likely etiologic agent for the observed effects.

The clearest consequences of TCE are permanent impairment of hearing in animal models and disruption of trigeminal nerve function in humans with animal models showing comparable changes following administration of a TCE metabolite. With regard to hearing loss, the effect of TCE has much in common with the effects of several aromatic hydrocarbons including ethylbenzene, toluene, and *p*-xylene. Many studies have attempted to determine how these solvents damage the cochlea. Of the hypotheses that have been advanced, there is little evidence to suggest oxidative stress, changes in membrane fluidity, or impairment of central efferent nerves whose endings innervate receptor cells in the cochlea. Rather, for reasons that are still uncertain, these solvents seem to preferentially target supporting cells in the cochlea whose death then alters key structural elements of the cochlea resulting ultimately in hair cell displacement and death. Recently, potential modes of action resulting in ototoxicity have been speculated to be due to blockade of neuronal nicotinic receptors present on the auditory cells (Campo et al., 2007) and potentially changes in calcium transmission (Maguin et al., 2009)ui from toluene exposure. Although these findings were reported following an acute toluene exposure, it is speculated that this mechanism may be a viable mechanism for TCE-mediated ototoxicity.

A few studies have tried to relate TCE exposure with selective impairments of dopamine neurons. Two studies (Gash et al., 2008; Guehl et al., 1999) demonstrated dopaminergic neuronal death and/or degeneration following an acute TCE administration. However, the only human TCE exposure study examining dopamine neuronal activity found no changes in serum dopamine β-hydroxylase activity in comparison to nonexposed individuals (Nagaya et al., 1990). It is thought that TaClo, which can be formed from TCE metabolites such as trichloroacetaldehyde, may be the potent neurotoxicant that selectively targets the dopaminergic system. More studies are needed to confirm the dopamine neuronal function disruption and if this disruption is mediated through TaClo.

There is good evidence that TCE and certain metabolites such as choral hydrate have CNS depressant properties and may account for some of the behavioral effects (such as vestibular effects, psychomotor activity changes, central visual changes, sleep and mood changes) that have been observed with TCE. Specifically, in vitro studies have demonstrated that TCE exposure results in changes in neuronal receptor function for the $GABA_A$, glycine, and serotonin receptors (Lopreato et al., 2003; Beckstead et al., 2000; Krasowski and Harrison, 2000). All of these inhibitory receptors that are present in the CNS are potentiated when a receptor-specific agonist and TCE are applied. These results are similar to other anesthetics and suggest that some of the behavioral functions are mediated by modifications in ion channel function. However, it is quite uncertain whether there are persistent consequences to such high dose TCE exposure. Additionally, with respect to the GABAergic system, acute administration of TCE increased the seizure threshold appearance and this effect was the strongest with convulsants that were GABA receptor antagonists (Shih et al., 2001). Therefore, this result

suggests that TCE interacts with the GABA receptor; this was also verified in vitro (Beckstead et al., 2000; Krasowski and Harrison, 2000).

TCE exposure has also been linked to decreased sensitivity to titanic stimulation in the hippocampus (Ohta et al., 2001) as well as to a significant reduction in myelin in the hippocampus in a developmental exposure study (Isaacson et al., 1990). These effects are notable since the hippocampus is highly involved in memory and learning functions. Changes in the hippocampal physiology may correlate with the cognitive changes that were reported following TCE exposure.

4.3.11. Overall Summary and Conclusions—Weight of Evidence

Both human and animal studies have associated TCE exposure with effects on several neurological domains. The strongest neurological evidence of hazard in humans is for changes in trigeminal nerve function or morphology and impairment of vestibular function. Fewer and more limited evidence exists in humans on delayed motor function and changes in auditory, visual, and cognitive function or performance. Acute and subchronic animal studies show morphological changes in the trigeminal nerve, disruption of the peripheral auditory system leading to permanent function impairments and histopathology, changes in visual evoked responses to patterns or flash stimulus, and neurochemical and molecular changes. Additional acute studies reported structural or functional changes in hippocampus, such as decreased myelination or decreased excitability of hippocampal CA1 neurons, although the relationship of these effects to overall cognitive function is not established. Some evidence exists for motor-related changes in rats/mice exposed acutely/subchronically to TCE, but these effects have not been reported consistently across all studies.

Epidemiologic evidence supports a relationship between TCE exposure and trigeminal nerve function changes, with multiple studies in different populations reporting abnormalities in trigeminal nerve function in association with TCE exposure (Mhiri et al., 2004; Kilburn, 2002b, a; Kilburn and Warshaw, 1993a; Feldman et al., 1992; Ruijten et al., 1991; Feldman et al., 1988; Barret et al., 1987; Barret et al., 1984; Barret et al., 1982). Of these, two well-conducted occupational cohort studies, each including >100 TCE-exposed workers without apparent confounding from multiple solvent exposures, additionally reported statistically significant dose-response trends based on ambient TCE concentrations, duration of exposure, and/or urinary concentrations of the TCE metabolite TCA (Barret et al., 1987; Barret et al., 1984). Limited additional support is provided by a positive relationship between prevalence of abnormal trigeminal nerve or sensory function and cumulative exposure to TCE (most subjects) or CFC113 (<25% of subjects) (Rasmussen et al., 1993a). Test for linear trend in this study was not statistically significant and may reflect exposure misclassification since some subjects included in this study did not have TCE exposure. The lack of association between TCE exposure and overall nerve function in three small studies (ulnar and medial: Triebig et al., 1983;

Triebig et al., 1982; trigeminal: El Ghawabi et al., 1973) does not provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment because of limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. Laboratory animal studies have also shown TCE-induced changes in the trigeminal nerve. Although one study reported no significant changes in TSEP in rats exposed to TCE for 13 weeks (Albee et al., 2006), there is evidence of morphological changes in the trigeminal nerve following short-term exposures in rats (Barret et al., 1992; Barret et al., 1991).

Human chamber, occupational, geographic-based/drinking water, and laboratory animal studies clearly established TCE exposure causes transient impairment of vestibular function. Subjective symptoms such as headaches, dizziness, and nausea resulting from occupational (Liu et al., 1988; Rasmussen and Sabroe, 1986; Smith, 1970; Grandjean et al., 1955), environmental (Hirsch et al., 1996), or chamber exposures (Smith, 1970; Stewart et al., 1970) have been reported extensively. A few laboratory animal studies have investigated vestibular function, either by promoting nystagmus or by evaluating balance (Umezu et al., 1997; Niklasson et al., 1993; Tham et al., 1984; Tham et al., 1979).

In addition, mood disturbances have been reported in a number of studies, although these effects also tend to be subjective and difficult to quantify (Gash et al., 2008; Kilburn, 2002b, a; Kilburn and Warshaw, 1993a; Tröster and Ruff, 1990; McCunney, 1988; Rasmussen and Sabroe, 1986; Mitchell and Parsons-Smith, 1969), and a few studies have reported no effects from TCE on mood (Reif et al., 2003; Triebig et al., 1977a; Triebig et al., 1976). Few comparable mood studies are available in laboratory animals, although both Moser et al. (2003) and Albee et al. (2006) reported increases in handling reactivity among rats exposed to TCE. Finally, a significantly increased number of sleep hours was reported by Arito et al. (1994) in rats exposed via inhalation to 50–300 ppm TCE for 8 hours/day for 6 weeks.

Four epidemiologic studies of chronic exposure to TCE observed disruption of auditory function. One large occupational cohort study showed a statistically significant difference in auditory function with cumulative exposure to TCE or CFC113 as compared to control groups after adjustment for possible confounders, as well as a positive relationship between auditory function and increasing cumulative exposure (Rasmussen et al., 1993c). Of the three studies based on populations from ATSDR's TCE Subregistry from the National Exposure Registry, more limited than Rasmussen et al. (1993c) due to inferior exposure assessment, Burg et al. (1995) and Burg and Gist (1999) reported a higher prevalence of self-reported hearing impairments. The third study reported that auditory screening revealed abnormal middle ear function in children <10-years-old, although a dose-response relationship could not be established and other tests did not reveal differences in auditory function (ATSDR, 2002). Further evidence for these effects is provided by numerous laboratory animal studies demonstrating that high-dose subacute and subchronic TCE exposures in rats disrupt the auditory system, leading to permanent functional impairments and histopathology.

Studies in humans exposed under a variety of conditions, both acutely and chronically, report impaired visual functions such as color discrimination, visuospatial learning tasks, and visual depth perception in subjects with TCE exposure. Abnormalities in visual depth perception were observed with a high acute exposure to TCE under controlled conditions (Vernon and Ferguson, 1969). Studies of lower TCE exposure concentrations also observed visuofunction effects. One occupational study (Rasmussen et al., 1993c) reported a statistically significant positive relationship between cumulative exposure to TCE or CFC113 and visual gestalts learning and retention among Danish degreasers. Two studies of populations living in a community with drinking water containing TCE and other solvents further suggested changes in visual function (Reif et al., 2003; Kilburn, 2002b, a). These studies used more direct measures of visual function as compared to Rasmussen et al. (1993c), but their exposure assessment is more limited because TCE exposure is not assigned to individual subjects (Kilburn, 2002b, a) or because there are questions regarding control selection (Kilburn, 2002b, a) and exposure to several solvents (Reif et al., 2003; Kilburn, 2002b, a).

Additional evidence of effects of TCE exposure on visual function is provided by a number of laboratory animal studies demonstrating that acute or subchronic TCE exposure causes changes in visual evoked responses to patterns or flash stimulus (Boyes et al., 2005a; Boyes et al., 2003; Blain et al., 1994). Animal studies have also reported that the degree of some effects is correlated with simultaneous brain TCE concentrations (Boyes et al., 2005a; Boyes et al., 2003) and that, after a recovery period, visual effects return to control levels (Blain et al., 1994; Rebert et al., 1991). Overall, the human and laboratory animal data together suggest that TCE exposure can cause impairment of visual function, and some animal studies suggest that some of these effects may be reversible with termination of exposure.

Studies of human subjects exposed to TCE either acutely in chamber studies or chronically in occupational settings have observed deficits in cognition. Five chamber studies reported statistically significant deficits in cognitive performance measures or outcome measures suggestive of cognitive effects (Triebig et al., 1977a; Triebig et al., 1977b; Gamberale et al., 1976; Triebig et al., 1976; Stewart et al., 1970). Danish degreasers with high cumulative exposure to TCE or CFC113 had a high risk (OR: 13.7, 95% CI: 2.0–92.0) for psychoorganic syndrome characterized by cognitive impairment, personality changes, and reduced motivation, vigilance, and initiative compared to workers with low cumulative exposure. Studies of populations living in a community with contaminated groundwater also reported cognitive impairments (Kilburn, 2002b, a; Kilburn and Warshaw, 1993a), although these studies carry less weight in the analysis because TCE exposure is not assigned to individual subjects and their methodological design is weaker.

Laboratory studies provide some additional evidence for the potential for TCE to affect cognition, though the predominant effect reported has been a change in the time needed to complete a task, rather than impairment of actual learning and memory function (Umezu et al.,

1997; Kishi et al., 1993; Kulig, 1987). In addition, in laboratory animals, it can be difficult to distinguish cognitive changes from motor-related changes. However, several studies have reported structural or functional changes in the hippocampus, such as decreased myelination (Isaacson et al., 1990; Isaacson and Taylor, 1989) or decreased excitability of hippocampal CA1 neurons (Ohta et al., 2001), although the relationship of these effects to overall cognitive function has not been established.

Two studies of TCE exposure, one chamber study of acute exposure duration and one occupational study of chronic duration, reported changes in psychomotor responses. The chamber study of Gamberale et al. (1976) reported a dose-related decrease in performance in a CRT test in healthy volunteers exposed to 100 and 200 ppm TCE for 70 minutes as compared to the same subjects without exposure. Rasmussen et al. (1993a) reported a statistically significant association with cumulative exposure to TCE or CFC113 and dyscoordination trend among Danish degreasers. Observations in a third study (Gun et al., 1978) are difficult to judge given the author's lack of statistical treatment of data. In addition, Gash et al. (2008) reported that 14/30 TCE-exposed workers exhibited significantly slower fine motor hand movements as measured through a movement analysis panel test. Studies of population living in communities with TCE and other solvents detected in groundwater supplies reported significant delays in SRTs and CRTs in individuals exposed to TCE in contaminated groundwater as compared to referent groups (Kilburn, 2002b, a; Kilburn and Thornton, 1996; Kilburn and Warshaw, 1993a). Observations in these studies are more uncertain given questions of the representativeness of the referent population, lack of exposure assessment to individual study subjects, and inability to control for possible confounders including alcohol consumption and motivation. Finally, in a presentation of two case reports, decrements in motor skills as measured by the grooved pegboard and finger tapping tests were observed (Tröster and Ruff, 1990).

Laboratory animal studies of acute or subchronic exposure to TCE observed psychomotor effects, such as loss of righting reflex (Shih et al., 2001; Umezu et al., 1997) and decrements in activity, sensory-motor function, and neuromuscular function (Moser et al., 2003; Moser et al., 1995; Kishi et al., 1993). However, two studies also noted an absence of significant changes in some measures of psychomotor function (Albee et al., 2006; Kulig, 1987). In addition, less consistent results have been reported with respect to locomotor activity in rodents. Some studies have reported increased locomotor activity after an acute i.p. dosage (Wolff and Siegmund, 1978) or decreased activity after acute- or short-term gavage dosing (Moser et al., 1995, 2003). No change in activity was observed following exposure through drinking water (Waseem et al., 2001), inhalation (Kulig, 1987), or orally during the neurodevelopment period (Fredriksson et al., 1993).

Several neurochemical and molecular changes have been reported in laboratory investigations of TCE toxicity. Kjellstrand et al. (1987) reported inhibition of sciatic nerve regeneration in mice and rats exposed continuously to 150 ppm TCE via inhalation for 24 days.

Two studies reported changes in GABAergic and glutamatergic neurons in terms of GABA or glutamate uptake (Briving et al., 1986) or response to GABAergic antagonistic drugs (Shih et al., 2001) as a result of TCE exposure, with the Briving et al. (1986) study conducted at 50 ppm for 12 months. Although the functional consequences of these changes is unclear, Tham et al. (1984; 1979) described central vestibular system impairments as a result of TCE exposure that may be related to altered GABAergic function. In addition, several in vitro studies have demonstrated that TCE exposure alters the function of inhibitory ion channels such as receptors for $GABA_A$ glycine, and serotonin (Lopreato et al., 2003; Beckstead et al., 2000; Krasowski and Harrison, 2000) or of voltage-sensitive calcium channels (Shafer et al., 2005).

4.4. KIDNEY TOXICITY AND CANCER

4.4.1. Human Studies of Kidney

4.4.1.1. Nonspecific Markers of Nephrotoxicity

Investigations of nephrotoxicity in human populations show that workers highly exposed to TCE exhibit evidence of damage to the proximal tubule (NRC, 2006). The magnitude of exposure needed to produce kidney damage is not clear. Several kidney early biological effect markers, or biomarkers, are examined in these studies, as are less sensitive clinical kidney outcomes such as glomerular filtration rate and end-stage disease. Observation of elevated excretion of urinary proteins in the four studies of TCE exposure (Bolt et al., 2004; Green et al., 2004; Brüning et al., 1999a; Brüning et al., 1999b) indicates the occurrence of a toxic insult among TCE-exposed subjects compared to unexposed controls. Two studies are of subjects with previously diagnosed kidney cancer (Bolt et al., 2004; Brüning et al., 1999a), with limited interpretation of whether effects are associated with exposure or to the disease process. Subjects in Brüning et al. (1999b) and Green et al. (2004) were disease-free. Urinary proteins are considered nonspecific markers of nephrotoxicity and include α1-microglobulin, albumin, and *N*-acetyl-β-D-glucosaminidase (NAG; Lybarger et al., 1999; Price et al., 1999; Price et al., 1996). Four studies measure α1-microglobulin with elevated excretion observed in the German studies (Bolt et al., 2004; Brüning et al., 1999a; Brüning et al., 1999b) but not Green et al. (2004). However, Green et al. (2004) found statistically significant group mean differences in NAG, another nonspecific marker of tubular toxicity, in disease-free subjects. Observations in Green et al. (2004) provide evidence of tubular damage among workers exposed to TCE at 32 ppm (mean) (range, 0.5–252 ppm). Elevated excretion of NAG as a nonspecific marker of tubular damage has also been observed with acute TCE poisoning (Carrieri et al., 2007). These and other studies relevant to evaluating TCE nephrotoxicity are discussed in more detail below.

Biological monitoring of persons who previously experienced "high" exposures to TCE (100–500 ppm) in the workplace show altered kidney function evidenced by urinary excretion of proteins suggestive of renal tubule damage. Similar results were observed in the only study available of subjects with TCE exposure at current occupational limits (NRC, 2006). Table 4-38

provides details and results from these studies. Brüning et al. (1999a) reported that a statistically significantly higher prevalence of elevated proteinuria suggestive of severe tubular damage (n = 24, 58.5%, $p < 0.01$) and an elevated excretion of α1-microglobin, another urinary biomarker of renal tubular function, were observed in 41 RCC cases with prior TCE exposure and with pending workman‘s compensation claims compared with the nonexposed renal cell cancer patients (n = 14, 28%) and to hospitalized surgical patients (n = 2, 2%). Statistical analyses did not adjust for differences in median systolic and diastolic blood pressure that appeared higher in exposed RCC cases compared to nonexposed controls. Similarly, severe tubular proteinuria is seen in 14/39 workers (35%) exposed to TCE in the electrical department, fitters shop, and through general degreasing operations of felts and sieves in a cardboard manufacturing factory compared to no subjects of 46 nonexposed males office and administrative workers from the same factory ($p < 0.01$) (Brüning et al., 1999b). Furthermore, slight tubular proteinuria was seen in 20% of exposed workers and 2% of nonexposed workers (Brüning et al., 1999b). Exposed subjects also had statistically significantly elevated levels of α1-microglobulin compared to unexposed controls. Subjects with tubular damage, as indicated by urinary protein patterns, had higher GST-alpha concentrations than nonexposed subjects ($p < 0.001$). Both sex and use of spot or 24-hour urine samples were shown to influence α1-microglobulin (Andersson et al., 2008); however, these factors are not considered to greatly influence observations given that only males were subjects and α1-microglobulin levels in spot urine sample were adjusted for creatinine concentration.

Table 4-38. Summary of human kidney toxicity studies

Subjects	Effect	Exposure	Reference
206 subjects— 104 male workers exposed to TCE; 102 male controls (source not identified)	Increased β2-microglobulin and total protein in spot urine specimen. β2-microglobulin: Exposed, 129.0 ± 113.3 mg/g creatinine. Controls, 113.6 ± 110.6 mg/g creatinine. Total protein: Exposed, 83.4 ± 113.2 mg/g creatinine. Controls, 54.0 ± 18.6 mg/g creatinine.	TCE exposure was through degreasing activities in metal parts factory or semiconductor industry. U-TTCs: Exposed, 83.4 mg/g Creatinine (range, 2–66.2 mg/g creatinine. Controls, ND. 8.4 ± 7.9 yrs mean employment duration.	Nagaya et al. (1989a)
29 metal workers	NAG in morning urine specimen, 0.17 ± 0.11 U/mmol creatinine.	Breathing zone monitoring, 3 ppm (median) and 5 ppm (mean).	Seldén et al. (1993)
191 subjects— 41 RCC cases pending cases involving compensation with TCE exposure; 50 unexposed RCC cases from the same area as TCE-exposed cases; 100 nondiseased control and hospitalized surgical patients	Increased urinary proteins patterns, α1-microglobulin, and total protein in spot urine specimen. Slight/severe tubular damage: TCE RCC cases, 93%. Nonexposed RCC cases, 46%. Surgical controls, 11%. $p < 0.01$. α1-microglobulin (mg/g creatinine): Exposed RCC cases, 24.6 ± [SD] 13.9 Unexposed RCC cases, 11.3 ± [SD] 9.8. Surgical controls, 5.5 ± [SD] 6.8.	All exposed RCC cases exposed to "high" and "very high" TCE intensity. 18 yrs mean exposure duration.	Brüning et al. (1999a)
85 male workers employed in cardboard manufacturing factory (39 TCE exposed, 46) nonexposed office and administrative controls)	Increased urinary protein patterns and excretion of proteins in spot urine specimen. Slight/severe tubular damage: TCE exposed, 67% Nonexposed, RCC cases, 9% $p < 0.001$. α1-microglobulin (mg/g creatinine): Exposed, 16.2 ± [SD] 10.3 Unexposed, 7.8 ± [SD] 6.9 $p < 0.001$. GST-alpha (µg/g creatinine): Exposed 6.0 ± [SD] 3.3 Unexposed, 2.0 ± [SD] 0.57 $p < 0.001$. No group differences in total protein or GST-pi.	"High" TCE exposure to workers in the fitters shop and electrical department. "Very high" TCE exposure to workers through general degreasing operations in carton machinery section.	Brüning et al. (1999b)

Table 4-38. Summary of human kidney toxicity studies (continued)

Subjects	Effect	Exposure	Reference
99 RCC cases and 298 hospital controls (from Brüning et al. [(2003)] and alive at the time of interview)	Increased excretion of α1-microglobulin in spot urine specimen. Proportion of subjects with α1-microglobulin <5.0 mg/L: Exposed cases, 15% Unexposed cases, 51% Exposed controls, 55% Unexposed controls, 55% $p < 0.05$, prevalence of exposed cases compared to prevalences of either exposed controls or unexposed controls. Mean α1-microglobulin: Exposed cases, 18.1 mg/L Unexposed cases, <5.0 mg/L $p < 0.05$.	All exposed RCC cases exposed to "high" and "very high" TCE intensity.	Bolt et al. (2004)
124 subjects (70 workers currently exposed to TCE and 54 hospital and administrative staff controls)	Analysis of urinary proteins in spot urine sample obtained 4 d after exposure. Increased excretion of albumin, NAG, and formate in spot urine specimen. Albumin (mg/g creatinine):[a] Exposed, 9.71 ± [SD] 11.6 Unexposed, 5.50 ± [SD] 4.27 $p < 0.05$. Total NAG (U/g creatinine): Exposed, 5.27 ± [SD] 3.78 Unexposed, 2.41 ± [SD] 1.91 $p < 0.01$. Format (mg/g creatinine): Exposed, 9.45 ± [SD] 4.78 Unexposed, 5.55 ± [SD] 3.00 $p < 0.01$. No group mean differences in GST-alpha, retinol binding protein, α1-microglobulin, β2-microglobulin, total protein, and methylmalonic acid.	Mean U-TCA of exposed workers was 64 ± [SD] 102 (Range, 1–505). Mean U-TCOH of exposed workers was 122 ± [SD] 119 (Range, 1–639). Mean TCE concentration to exposed subjects was estimated as 32 ppm (range, 0.5–252 ppm) and was estimated by applying the German occupational exposure limit (maximale arbeitsplatz konzentration, MAK) standard to U-TCA and assuming that the linear relationship holds for exposures >100 ppm. 86% of subjects with exposure to <50 ppm TCE.	Green et al. (2004)

Table 4-38. Summary of human kidney toxicity studies (continued)

Subjects	Effect	Exposure	Reference
101 cases or deaths from ESDR among male and female subjects in Hill Air Force Base aircraft maintenance worker cohort of Blair et al. (1998)	TCE exposure: Cox Proportional Hazard Analysis: Ever exposed to TCE,[b] 1.86 (1.02, 3.39). Logistic regression:[b] No chemical exposure (referent group): 1.0 <5 unit-yr, 1.73 (0.86, 3.48) 5–25 unit-yr, 1.65 (0.82, 3.35) >25 unit-yr, 1.65 (0.82, 3.35) Monotonic trend test, $p > 0.05$. Indirect low-intermittent TCE exposure, 2.47 (1.17, 5.19) Indirect peak/infrequent TCE exposure 3.55 (1.25, 10.74) Direct TCE exposure, ~~n~~ot statistically significant" but hazard ratio and CIs were not presented in paper.	Cumulative TCE exposure (intensity × duration) identified using three categories, <5 unit-yr, 5–25 unit-yr, >25 unit-yr per JEM of Stewart et al. (1991).	Radican et al. (2006)
269 cases of IgA nephropathy or membranous nephropathy glomerulonephritis followed 5 yrs (mean) for progression to ESRD	TCE exposure: Cox Proportional Hazard Analysis: Ever exposed to TCE,[b] 2.5 (0.9, 6.5) High exposure level to TCE,[b] 2.7 (0.7, 10.1).	Exposure to TCE assigned using job title and JEM; two dose surrogates, ever exposed and high exposure level.	Jacob et al. (2007)

[a]For a urine sample, 10–17 mg of albumin per g of creatinine is considered to be suspected albuminuria in males (15–25 in females) (de Jong and Brenner, 2004).
[b]Hazard ratio and 95% CI.

ESRD = end-stage renal disease; ND = not detectable

Bolt et al. (2004) measured α1-microglobulin excretion in living subjects from the RCC case-control study by Brüning et al. (2003). Some subjects in this study were highly exposed. Of the 134 with renal cell cancer, 19 reported past exposures that led to narcotic effects and 18 of the 401 controls experienced similar effects (OR: 3.71, 95% CI: 1.80–7.54) (Brüning et al., 2003). Bolt et al. (2004) found that α1-microglobulin excretion increased in exposed renal cancer patients compared with nonexposed patients controls. A lower proportion of exposed cancer patients had normal α1-microglobulin excretion, <5 mg/L, the detection level for the assay and the level considered by these investigators as associated with no clinical or subclinical tubule damage, and a higher proportion of high values, defined as ≥45 mg/L, compared to cases who did not report TCE occupational exposure and to nonexposed controls ($p < 0.05$). Exposed cases, additionally, had statistically significantly higher median concentrations of α1-micro-globulin compared to unexposed cases in creatinine-unadjusted spot urine specimens ($p < 0.05$). Reduced clearance of creatinine attributable to renal cancer does not explain the lower

percentage of normal values among exposed cases given findings of similar prevalence of normal excretion among unexposed renal cell cases and controls.

In their study of 70 current employees (58 males, 12 females) of an electronic factory with TCE exposure and 54 (50 males, 4 females) age-matched subjects drawn from hospital or administrative staff, Green et al. (2004) found that urinary excretion of albumin, total NAG and formate were increased in the exposed group compared with the unexposed group.[4] No differences between exposed and unexposed subjects were observed in other urinary proteins, including α1-microglobulin, β2-microglobulin, and GST-alpha. Green et al. (2004) stated that NAG is not an indicator of nephropathy, or kidney damage, but rather is an indicator of functional change in the kidney. Green et al. (2004) further concluded that increased urinary albumin or NAG was not related to TCE exposure; analyses to examine the exposure-response relationship found neither NAG nor albumin concentration correlated to U-TCA or employment duration (years). The National Research Council (NRC, 2006) did not consider U-TCA as sufficiently reliable to use as a quantitative measure of TCE exposure, concluding that the data reported by Green et al. (2004) were inadequate to establish exposure-response information because the relationship between U-TCA and ambient TCE intensity is highly variable and nonlinear, and conclusions about the absence of association between TCE and nephrotoxicity cannot be made based on U-TCA. Moreover, use of employment duration does not consider exposure intensity differences between subjects with the same employment duration, and bias introduced through misclassification of exposure may explain the Green et al. (2004) findings.

Seldén et al. (1993), in their study of 29 metal workers (no controls), reported a correlation between NAG and U-TCA ($r = 0.48$, $p < 0.01$) but not with other exposure metrics of recent or long-term exposure. Personal monitoring of worker breath indicated median and mean TWA TCE exposures of 3 and 5 ppm, respectively. Individual NAG concentrations were within normal reference values. Rasmussen et al. (1993b) also reported a positive relationship ($p = 0.05$) between increasing urinary NAG concentration (adjusted for creatinine clearance) and increasing duration in their study of 95 metal degreasers (no controls) exposed to either TCE (70 subjects) or CFC113 (25 subjects). Multivariate regression analyses that adjusted for age were suggestive of an association between NAG and exposure duration ($p = 0.011$). Mean urinary NAG concentration was higher among subjects with annual exposure of >30 hours/week, defined as peak exposure, compared to subjects with annual exposure of <30 hours/week (72.4 ± 44.1 compared to 45.9 ± 30.0 µg/g creatinine, $p < 0.01$).

Nagaya et al. (1989a) did not observe statistically significant group differences in urinary β2-microglobulin and total protein in spot urine specimens of male degreasers and their controls, nor were these proteins correlated with urinary total trichloro-compounds (U-TTCs). The paper

[4]Elevation of NAG in urine is a sign of proteinuria, and proteinuria is both a sign and a cause of kidney malfunction (Zandi-Nejad et al., 2004). For a urine sample, 10–17 mg of albumin per g of creatinine is considered to be suspected albuminuria in males (15–25 in females) (de Jong and Brenner, 2004).

lacks details on subject selection, whether urine collection was at the start of work week or after sufficient exposure, and presentation of *p*-values and correlation coefficients. The presentation of urinary protein concentrations stratified by broad age groups is less statistically powerful than examination of this confounder using logistic regression. Furthermore, although valid for pharmacokinetic studies, examination of renal function using U-TTC as a surrogate for TCE exposure is uncertain, as discussed above for Green et al. (2004).

4.4.1.2. End-Stage Renal Disease (ESRD)

ESRD is associated with hydrocarbon or organic solvent exposures in two studies examining this endpoint (Jacob et al., 2007; Radican et al., 2006). Table 4-38 provides details and results from Radican et al. (2006) and Jacob et al. (2007). Radican et al. (2006) assessed ESRD in a cohort of aircraft maintenance workers at Hill Air Force Base (Blair et al., 1998) with strong exposure assessment to TCE (NRC, 2006) and reported a twofold risk with overall TCE exposure and ESRD (1.86, 95% CI: 1.02, 3.39). A second study, the GN-PROGRESS retrospective cohort study, observed a twofold elevated risk for progression of glomerulonephritis to ESRD from TCE (overall exposure: 2.5, 95% CI: 0.9–6.5; high-level TCE exposure: 2.7, 95% CI: 0.7, 10.1) (Jacob et al., 2007). Statistical power was more limited in Jacob et al. (2007) because of its smaller number of exposed cases, 21 with overall exposure, compared to 56 exposed cases in Radican et al. (2006). Other occupational studies do not examine ESRD specifically, instead reporting RRs associated with deaths due to nephritis and nephrosis (Boice et al., 2006b; ATSDR, 2004a; Boice et al., 1999), all genitourinary system deaths (Ritz, 1999a; Costa et al., 1989; Garabrant et al., 1988), or providing no information on renal disease mortality in the published paper (Chang et al., 2003; Blair et al., 1998; Morgan et al., 1998).

4.4.2. Human Studies of Kidney Cancer

Cancer of the kidney and renal pelvis is the 6th leading cause of cancer in the United States with an estimated 54,390 (33,130 men and 21,260 women) newly diagnosed cases and 13,010 deaths (Jemal et al., 2008; Ries et al., 2008). Age-adjusted incidence rates based on cases diagnosed in 2001–2005 from 17 Surveillance, Epidemiology, and End Results (SEER) geographic areas are 18.3 per 100,000 for men and 9.2 per 100,000 for women. Age-adjusted mortality rates are much lower; 6.0 per 100,000 for men and 2.7 for women.

Cohort, case-control, and geographical studies have examined TCE and kidney cancer, defined either as cancer of kidney and renal pelvis in cohort and geographic-based studies or as RCC, the most common type of kidney cancer, in case-control studies. Appendix C identifies these studies‘ design and exposure assessment characteristics. Observations in these studies are presented below in Table 4-39. Rate ratios for incidence studies in Table 4-39 are, generally, larger than for mortality studies.

Table 4-39. Summary of human studies on TCE exposure and kidney cancer

Exposure group	RR (95% CI)	Number of observable events	Reference
Cohort and PMR studies—incidence			
Aerospace workers (Rocketdyne)			Zhao et al. (2005)
Any exposure to TCE	Not reported		
Low cumulative TCE score	1.00[a]	6	
Medium cumulative TCE score	1.87 (0.56, 6.20)	6	
High TCE score	4.90 (1.23, 19.6)	4	
p for trend	$p = 0.023$		
TCE, 20 yr exposure lag[b]			
Low cumulative TCE score	1.00[a]	6	
Medium cumulative TCE score	1.19 (0.22, 6.40)	7	
High TCE score	7.40 (0.47, 116)	3	
p for trend	$p = 0.120$		
All employees at electronics factory (Taiwan)			Chang et al. (2005)
Males	1.06 (0.45, 2.08)[c]	8	
Females	1.09 (0.56, 1.91)[c]	12	
Females	1.10 (0.62, 1.82)[c]	15	Sung et al. (2008)
Danish blue-collar worker with TCE exposure			Raaschou-Nielsen et al. (2003)
Any exposure, all subjects	1.2 (0.98, 1.46)	103	
Any exposure, males	1.2 (0.97, 1.48)	93	
Any exposure, females	1.2 (0.55, 2.11)	10	
Exposure lag time			
20 yrs	1.3 (0.86, 1.88)	28	
Employment duration			
<1 yr	0.8 (0.5, 1.4)	16	
1–4.9 yrs	1.2 (0.8, 1.7)	28	
≥5 yrs	1.6 (1.1, 2.3)	32	
Subcohort with higher exposure			
Any TCE exposure	1.4 (1.0, 1.8)	53	
Employment duration			
1–4.9 yrs	1.1 (0.7, 1.7)[d]	23	
≥5 yrs	1.7 (1.1, 2.4)[d]	30	

Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Biologically monitored Danish workers		1.1 (0.3, 2.8)	4	Hansen et al. (2001)
	Any TCE exposure, males	0.9 (0.2, 2.6)	3	
	Any TCE exposure, females	2.4 (0.03, 14)	1	
	Cumulative exposure (Ikeda)	Not reported		
	<17 ppm-yr			
	≥17 ppm-yr			
	Mean concentration (Ikeda)	Not reported		
	<4 ppm			
	4+ ppm			
	Employment duration	Not reported		
	<6.25 yrs			
	≥6.25			
Aircraft maintenance workers from Hill Air Force Base				Blair et al. (1998)
	TCE subcohort	Not reported		
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	1.4 (0.4, 4.7)	9	
	5–25 ppm-yr	1.3 (0.3, 4.7)	5	
	>25 ppm-yr	0.4 (0.1, 2.3	2	
	Females, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr		0	
	5–25 ppm-yr		0	
	>25 ppm-yr	3.6 (0.5, 25.6)	2	
Biologically-monitored Finnish workers				Anttila et al. (1995)
	All subjects	0.87 (0.32, 1.89)	6	
	Mean air-TCE (Ikeda extrapolation)			
	<6 ppm	Not reported		
	6+ ppm	Not reported		
Cardboard manufacturing workers in Arnsberg, Germany				Henschler et al. (1995)
	Exposed workers	7.97 (2.59, 8.59)[e]	5	
Biologically-monitored Swedish workers				Axelson et al. (1994)
	Any TCE exposure, males	1.16 (0.42, 2.52)	6	
	Any TCE exposure, females	Not reported		

Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Cardboard manufacturing workers, Atlanta area, Georgia				Sinks et al. (1992)
	All subjects	3.7 (1.4, 8.1)	6	
	All departments	∞ (3.0, ∞)[f]	5	
	Finishing department	16.6 (1.7, 453.1)[f]	3	
Cohort and PMR studies—mortality				
Computer manufacturing workers (IBM), New York				
Males		1.64 (0.45, 4.21)[g]	4	Clapp and Hoffman (2008)
Females			0	
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	2.22 (0.89, 4.57)	7	Boice et al. (2006b)
	Any exposure to TCE	Not reported		Zhao et al. (2005)
	Low cumulative TCE score	1.00[a]	7	
	Medium cumulative TCE score	1.43 (0.49, 4.16)	7	
	High TCE score	2.13 (0.50, 8.32)	3	
	p for trend	$p = 0.31$		
	TCE, 20 yr exposure lag[b]			
	Low cumulative TCE score	1.00[a]	10	
	Medium cumulative TCE score	1.69 (0.29, 9.70)	6	
	High TCE score	1.82 (0.09, 38.6)	1	
	p for trend	$p = 0.635$		
View-Master employees				ATSDR (2004a)
	Males	2.76 (0.34, 9.96)[g]	2	
	Females	6.21 (2.68, 12.23)[g]	8	
United States Uranium-processing workers (Fernald)				Ritz (1999a) (as reported in NRC, 2006)
	Any TCE exposure	Not reported		
	Light TCE exposure, 2–10 yrs duration	1.94 (0.59, 6.44)	5	
	Light TCE exposure, >10 yrs duration	0.76 (0.14, 400.0)	2	
	Mod TCE exposure, >2 yrs duration		0	

Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group	RR (95% CI)	Number of observable events	Reference
Aerospace workers (Lockheed)			Boice et al. (1999)
Routine exposure	0.99 (0.40, 2.04)	7	
Routine-Intermittent[a]	Not presented	11	
Duration of exposure			
0 yr	1.0	22	
<1 yr	0.97 (0.37, 2.50)	6	
1–4 yrs	0.19 (0.02, 1.42)	1	
≥5 yrs	0.69 (0.22, 2.12)	4	
p for trend			
Aerospace workers (Hughes)			Morgan et al. (1998)
TCE subcohort	1.32 (0.57, 2.60)	8	
Low intensity (<50 ppm)[e]	0.47 (0.01, 2.62)	1	
High intensity (>50 ppm)[e]	1.78 (0.72, 3.66)	7	
TCE subcohort (Cox analysis)			
Never exposed	1.00[a]	24	
Ever exposed	1.14 (0.51, 2.58)[h]	8	
Peak			
No/Low	1.00[a]	24	
Med/Hi	1.89 (0.85, 4.23)[h]	8	
Cumulative			
Referent	1.00[a]	24	
Low	0.31 (0.04, 2.36)[h]	1	
High	1.59 (0.68, 3.71)[h]	7	
Aircraft maintenance workers (Hill Air Force Base, Utah)			Blair et al. (1998)
TCE subcohort	1.6 (0.5, 5.1)[a]	15	
Males, cumulative exposure			
0	1.0[a]		
<5 ppm-yr	2.0 (0.5, 7.6)	8	
5–25 ppm-yr	0.4 (0.1, 4.0)	1	
>25 ppm-yr	1.2 (0.3, 4.8)	4	
Females, cumulative exposure			
0	1.0[a]		
<5 ppm-yr		0	
5–25 ppm-yr	9.8 (0.6, 157)	1	
>25 ppm-yr	3.5 (0.2, 56.4)	1	
TCE subcohort	1.18 (0.47, 2.94)[i]	18	Radican et al. (2008)
Males, cumulative exposure	1.24 (0.41, 3.71)[i]	16	
0	1.0[l]		

Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Aircraft maintenance workers (Hill Air Force Base, Utah) (continued)				Blair et al. (1998)
	<5 ppm-yr	1.87 (0.59, 5.97)[i]	10	
	5–25 ppm-yr	0.31 (0.03, 2.75)[i]	1	
	>25 ppm-yr	1.16 (0.31, 4.32)[i]	5	
	Females, cumulative exposure	0.93 (0.15, 5.76)[i]	2	
	0	1.0[a]		
	<5 ppm-yr		0	
	5–25 ppm-yr	2.86 (0.27, 29.85)[i]	1	
	>25 ppm-yr	0.97 (0.10, 9.50)[i]	1	
Cardboard manufacturing workers in Arnsberg, Germany				Henschler et al. (1995)
	TCE exposed workers	3.28 (0.40, 11.84)	2	
	Unexposed workers	- (0.00, 5.00)	0	
Deaths reported to among GE pension fund (Pittsfield, Massachusetts)		0.99 (0.30, 3.32)[f]	12	Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia				Sinks et al. (1992)
		1.4 (0.0, 7.7)	1	
U.S. Coast Guard employees				Blair et al. (1989)
	Marine inspectors	1.06 (0.22, 3.10)	3	
	Noninspectors	1.03 (0.21, 3.01)	3	
Aircraft manufacturing plant employees (Italy)				Costa et al. (1989)
	All subjects	Not reported		
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)
	All subjects	0.93 (0.48, 1.64)	12	
Case-control studies				
Population of four countries in central and eastern Europe				Moore et al. (2010)
	Any TCE exposure	1.63 (1.04, 2.54)	48	
	Any TCE exposure (High confidence exposure)	2.05 (1.13, 3.73)	29	
	Cumulative TCE exposure			
	<1.58 ppm-yr	1.19 (0.61, 2.35)	17	
	>1.58 ppm-yr	2.02 (1.14, 3.59)[j]	31	
	p for trend	$p = 0.02$		
	Average intensity			
	<0.076 ppm	1.38 (0.81, 2.35)	31	
	>0.076 ppm	2.34 (1.05, 5.21)	17	
	p for trend	$p = 0.02$		

Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group	RR (95% CI)	Number of observable events	Reference
Population of Arve Valley, France			Charbotel et al. (2009; 2007; 2006)
Any TCE exposure	1.64 (0.95, 2.84)	37	
Any TCE exposure (High confidence exposure)	1.88 (0.89, 3.98)	16	
Cumulative TCE exposure			
Referent/nonexposed	1.00[a]	49	
Low, 62.4 ppm-yr[k]	1.62 (0.75, 3.47)	12	
Medium, 253.2 ppm-yr[k]	1.15 (0.47, 2.77)	9	
High, 925 ppm-yr[k]	2.16 (1.02, 4.60)[l]	16	
Test for trend	$p = 0.04$		
Cumulative TCE exposure + peak			
Referent/nonexposed	1.00[a]	49	
Low/medium, no peaks	1.35 (0.69, 2.63)	18	
Low/medium + peaks	1.61 (0.36. 7.30)	3	
High, no peaks	1.76 (0.65, 4.73)	8	
High + peaks	2.73 (1.06, 7.07)[l]	8	
Cumulative TCE exposure, 10-yr lag			
Referent/nonexposed	1.00[a]	49	
Low/medium, no peaks	1.44 (0.69, 2.80)	19	
Low/medium + peaks	1.38 (0.32, 6.02)	3	
High, no peaks	1.50 (0.53, 4.21)	7	
High + peaks	3.15 (1.19, 8.38)	8	
TWA TCE exposure[m]			
Referent/nonexposed	1.00[a]	46	
Any TCE without cutting fluid	1.62 (0.76, 3.44)	15	
Any cutting fluid without TCE	2.39 (0.52, 11.03)	3	
<50 ppm TCE + cutting fluid	1.14 (0.49, 2,66)	12	
50 + ppm TCE + cutting fluid	2.70 (1.02, 7.17)	10	
Population of Arnsberg Region, Germany			Brüning et al. (2003)
Longest job held—TCE/PERC (CAREX)	1.80 (1.01, 3.20)	117	
Self-assessed exposure to TCE	2.47 (1.36, 4.49)	25	
Duration of self-assessed TCE exposure			
0	1.00[a]	109	
<10 yrs	3.78 (1.54, 9.28)	11	
10–20 yrs	1.80 (0.67, 4.79)	7	
>20 yrs	2.69 (0.84, 8.66)	8	

Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group	RR (95% CI)	Number of observable events	Reference
Population in five German Regions			Pesch et al., (2000b)
Any TCE exposure	Not reported		
Males	Not reported		
Females	Not reported		
TCE exposure (JTEM)			
Males			
Medium	1.3 (1.0, 1.8)	68	
High	1.1 (0.8, 1.5)	59	
Substantial	1.3 (0.8, 2.1)	22	
Females			
Medium	1.3 (0.7, 2.6)	11	
High	0.8 (0.4, 1.9)	7	
Substantial	1.8 (0.6, 5.0)	5	
Population of Minnesota			Dosemeci et al. (1999)
Ever exposed to TCE, NCI JEM			
Males	1.04 (0.6, 1.7)	33	
Females	1.96 (1.0, 4.0)	22	
Males + Females	1.30 (0.9, 1.9)	55	
Population of Arnsberg Region, Germany			Vamvakas et al. (1998)
Self-assessed exposure to TCE	10.80 (3.36, 34.75)	19	
Population of Montreal, Canada			Siemiatycki et al. (1991)
Any TCE exposure	0.8 (0.4, 2.0)[n]	4	
Substantial TCE exposure	0.8 (0.2, 2.6)[n]	2	
Geographic-based studies			
Residents in two study areas in Endicott, New York	1.90 (1.06, 3.13)	15	ATSDR (2006a) (2008b)
Residents of 13 census tracts in Redlands, California	0.80 (0.54, 1.12)[o]	54	Morgan and Cassady (2002)
Finnish residents			Vartiainen et al. (1993)
Residents of Hausjarvi	Not reported		
Residents of Huttula	Not reported		

[a]Internal referents, workers not exposed to TCE.
[b]RRs for TCE exposure after adjustment for 1st employment, SES status, age at event, and all other carcinogens, including hydrazine.
[c]Chang et al. (2005)—urinary organs combined.
[d]SIR for RCC.
[e]Henschler et al. (1995) Expected number of incident cases calculated using incidence rates from the Danish Cancer Registry.
[f]OR from nested case-control analysis.
[g]PMR.

Table 4-39. Summary of human studies on TCE exposure and kidney cancer (continued)

[h]Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex and decade (EHS, 1997).
[i]In Radican et al. (2008), kidney cancer defined as RCC (ICDA 8 code 189.0) and estimated RRs from Cox proportional hazard models were adjusted for age and sex.
[j]The OR, adjusted for age, sex, and center, for subjects with high-confidence exposure assessment with cumulative exposure, ≥1.58 ppm-yr, was 2.23 (95% CI: 1.07, 4.64) and *p*-value for trend = 0.02.
[k]Mean cumulative exposure score in Charbotel et al. (2006) (personal communication from Barbara Charbotel, University of Lyon, to Cheryl Scott, U.S. EPA, 11 April 2008).
[l]In Charbotel et al. (2006) analyses adjusted for age, sex, smoking, and BMI. The OR, adjusted for age, sex, smoking, BMI, and exposure to cutting fluids and other petroleum oils, for high cumulative TCE exposure was 1.96 (95% CI: 0.71, 5.37) and for high cumulative + peak TCE exposure was 2.63 (95% CI: 0.79, 8.83). The OR for, considering only job periods with high confidence TCE exposure assessment, adjusted for age, sex, smoking, and BMI, for high cumulative dose plus peaks was 3.80 (95% CI: 1.27. 11.40).
[m]The exposure surrogate is calculated for one occupational period only and is not the average exposure concentration over the entire employment period.
[n]90% CI.
[o]99% CI.

PERC = perchloroethylene

Additionally, a large body of evidence exists on kidney cancer risk and either job or industry titles where TCE usage has been documented. TCE has been used as a degreasing solvent in a number of jobs, task, and industries, some of which include metal, electronic, paper and printing, leather manufacturing, and aerospace/aircraft manufacturing or maintenance industries and job title of degreaser, metal workers, electrical worker, and machinist (Purdue et al., 2011; IARC, 1995b). NRC (2006) identifies characteristics for kidney cancer case-control studies that assess job title or occupation in their Table 3-8. RRs and 95% CIs reported in these studies are found in Table 4-40.

Table 4-40. Summary of case-control studies on kidney cancer and occupation or job title

Case ascertainment area/exposure group		RR (95% CI)	No. exposed cases	Reference
Swedish Cancer Registry Cases				Wilson et al. (2008)
	Machine/electronics industry	1.30 (1.08, 1.55)[a] [M]	120	
		1.75 (1.04, 2.76[a] [F]	18	
	Shop and construction metal work	1.19 (1.00, 1.40)[a] [M]	143	
	Machine assembly	1.62 (0.94, 2.59)[a] [M]		
	Metal plating work	2.70 (0.73, 6.92)[a] [M]	4	
	Shop and construction metal work	1.66 (0.71, 3.26)[a] [F]	8	
Arve Valley, France				Charbotel et al. (2006)
	Metal industry	1.02 (0.59, 1.76)	28	
	Metal workers, job title	1.00 (0.56, 1.77)	25	
	Metal industry, screw-cutting workshops	1.39 (0.75, 2.58)	22	
	Machinery, electrical and transportation equipment manufacture	1.19 (0.61, 2.33)	15	
Iowa Cancer Registry Cases				Zhang et al. (2004)
	Assemblers	2.5 (0.8, 7.6)	5	
	>10 yr employment	4.2 (1.2, 15.3)	4	
Arnsberg Region, Germany				Brüning et al. (2003)
	Iron/steel	1.15 (0.29, 4.54)	3	
	Occupations with contact to metals	1.53 (0.97, 2.43)	46	
	Longest job held	1.14 (0.66, 1.96)	24	
	Metal greasing/degreasing	5.57 (2.33, 13.32)	15	
	Degreasing agents			
	Low exposure	2.11 (0.86, 5.18)	9	
	High exposure	1.01 (0.40, 2.54)	7	
Bologna, Italy				Mattioli et al. (2002)
	Metal workers	2.21 (0.99, 5.37)	37	
	Printers	1.55 (0.17, 13.46)	7	
	Solvents	0.79 (0.31, 1.98) [M]	17	
		1.47 (0.12, 17.46) [F]	3	
Montreal, Canada				Parent et al. (2000a)
	Metal fabricating and machining industry	1.0 (0.6, 1.8)	14	
	Metal processors	1.2 (0.4, 3.4)	4	
	Printing and publishing industry	1.1 (0.4, 3.0)	4	
	Printers	3.0 (1.2, 7.5)	6	
	Aircraft mechanics	2.8 (1.0, 8.4)	4	

Table 4-40. Summary of case-control studies on kidney cancer and occupation or job title (continued)

Case ascertainment area/exposure group		RR (95% CI)	No. exposed cases	Reference
Five Regions in Germany				Pesch et al. (2000b)
	Electrical and electronic equipment assembler	3.2 (1.0, 10.3) [M]	5	
		2.7 (1.3, 5.8) [F]	11	
	Printers	3.5 (1.1, 11.2) [M]	5	
		2.1 (0.4, 11.7) [F]	2	
	Metal cleaning/degreasing, job task	1.3 (0.7, 2.3) [M]	15	
		1.5 (0.3, 7.7) [F]	2	
New Zealand Cancer Registry				Delahunt et al. (1995)
	Toolmakers and blacksmiths	1.48 (0.72, 3.03)	No information	
	Printers	0.67 (0.25, 1.83)		
Minnesota Cancer Surveillance System				Mandel et al. (1995)
	Iron or steel	1.6 (1.2, 2.2)	8	
Rhein-Neckar-Odenwald Area, Germany				Schlehofer et al. (1995)
	Metal			
	Industry	1.63 (1.07, 2.48)	71	
	Occupation	1.38 (0.89, 2.12)		
	Electronic			
	Industry	0.51 (0.26, 1.01)	14	
	Occupation	0.57 (0.25, 1.33)	9	
	Chlorinated solvents	2.52 (1.23, 5.16)	27	
	Metal and metal compounds	1.47 (0.94, 2.30)	62	
Danish Cancer Registry				Mellemgaard et al. (1994)
	Iron and steel	1.4 (0.8, 2.4) [M]	31	
		1.0 (0.1, 3.2) [F]	1	
	Solvents	1.5 (0.9, 2.4) [M]	50	
		6.4 (1.8, 23) [F]	16	
France				Aupérin et al. (1994)
	Machine fitters, assemblers, and precision instrument makers	0.7 (0.3, 1.9)	16	

Table 4-40. Summary of case-control studies on kidney cancer and occupation or job title (continued)

Case ascertainment area/exposure group		RR (95% CI)	No. exposed cases	Reference
New South Wales, Australia				McCredie and Stewart (1993)
	Iron and steel	1.18 (0.75, 1.85)[b]	52	
		2.39 (1.26, 4.52)[c]	19	
	Printing or graphics	1.18 (0.87, 2.08)[b]	29	
		0.82 (0.32, 2.11)[d]	6	
	Machinist or tool maker	1.15 (0.72, 1.86)[b]	48	
		1.83 (0.92, 3.61)[c]	16	
	Solvents	1.54 (1.11, 2.14)[b]	109	
		1.40 (0.82, 2.40)[c]	24	
Finnish Cancer Registry				Partanen et al. (1991)
	Iron and metalware work	1.87 (0.94, 3.76)	22	
	Machinists	2.33 (0.83, 6.51)	10	
	Paper and pulp; printing/publishing	2.20 (1.02, 4.72) [M]	18	
		5.95 (1.21, 29.2) [F]	7	
	Nonchlorinated solvents	3.46 (0.91, 13.2) [M]	9	
West Midlands U.K. Cancer Registry				Harrington et al. (1989)
	Organic solvents			
	Ever exposed	1.30 (0.31, 8.50)	3	
	Intermediate exposure	1.54 (0.69, 4.10)	3	
Montreal, Canada				Sharpe et al. (1989)
	Organic solvents	1.68 (0.83, 2.22)	33	
	Degreasing solvents	3.42 (0.92, 12.66)	10	
Oklahoma				Asal et al. (1988a; 1988b)
	Metal degreasing	1.7 (0.7, 3.8) [M]	19	
	Machining	1.7 (0.7, 4.3) [M]	13	
	Painter, paint manufacture	1.3 (0.7, 2.6) [M]	22	
Missouri Cancer Registry				Brownson (1988)
	Machinists	2.2 (0.5, 10.3)	3	
Danish Cancer Registry				Jensen et al. (1988)
	Iron and metal, blacksmith	1.4 (0.7, 2.9)[d]	17	
	Painter, paint manufacture	1.8 (0.7, 4.6)	10	

[a]Renal pelvis, Wilson et al. (2008).
[b]RCC, McCredie and Stewart (1993).
[c]Renal pelvis, McCredie and Stewart (1993).
[d]Renal pelvis and ureter, Jensen et al. (1988).

4.4.2.1. Studies of Job Titles and Occupations with Historical TCE Usage

Elevated risks are observed in many of the cohort or case-control studies between kidney cancer and industries or job titles with historical use of TCE (Wilson et al., 2008; Charbotel et al., 2006; Zhang et al., 2004; Brüning et al., 2003; Mattioli et al., 2002; Parent et al., 2000a;

Pesch et al., 2000b; Mandel et al., 1995; Schlehofer et al., 1995; McCredie and Stewart, 1993; Partanen et al., 1991). Overall, these studies, although indicating association with metal work exposures and kidney cancer, are insensitive for identifying a TCE hazard. The use of job title or industry as a surrogate for exposure to a chemical is subject to substantial misclassification that will attenuate rate ratios due to exposure variation and differences among individuals with the same job title. Several small case-control studies (Parent et al., 2000a; Vamvakas et al., 1998; Auperin et al., 1994; Harrington et al., 1989; Sharpe et al., 1989; Jensen et al., 1988) have insufficient statistical power to detect modest associations due to their small size and potential exposure misclassification (NRC, 2006). For these reasons, statistical variation in the risk estimate is large and observation of statistically significantly elevated risks associated with metal work in many of these studies is noteworthy. Some studies also examined broad chemical grouping such as degreasing solvents or chlorinated solvents. Observations in studies that assessed degreasing agents or chlorinated solvents reported statistically significant elevated kidney cancer risk (Brüning et al., 2003; Pesch et al., 2000b; Schlehofer et al., 1995; Mellemgaard et al., 1994; McCredie and Stewart, 1993; Harrington et al., 1989; Asal et al., 1988a; Asal et al., 1988b). Observations of association with degreasing agents, together with job title or occupations where TCE has been used historically, provide a signal and suggest an etiologic agent common to degreasing activities.

4.4.2.2. Cohort and Case-Controls Studies of TCE Exposure

Cohort and case-controls studies that include JEMs for assigning TCE exposure potential to individual study subjects show associations with kidney cancer, specifically RCC, and TCE exposure. Support for this conclusion derives from findings of increased risks in cohort studies (Zhao et al., 2005; Raaschou-Nielsen et al., 2003; Henschler et al., 1995) and in case-control studies from the Arnsberg region of Germany (Brüning et al., 2003; Pesch et al., 2000b; Vamvakas et al., 1998), the Arve Valley region in France (Charbotel et al., 2009; Charbotel et al., 2006), the United States (Dosemeci et al., 1999; Sinks et al., 1992), and the four central and eastern Europe countries of Czech Republic, Poland, Romania, and Russia (Moore et al., 2010).

A consideration of a study‘s statistical power and exposure assessment approach is necessary to interpret observations in Table 4-39. Most cohort studies are underpowered to detect a doubling of kidney cancer risks including the essentially null studies by Greenland et al. (1994), Axelson et al. (1994 [incidence]), Anttila et al. (1995 [incidence]), Blair et al. (1998 [incidence and mortality]), Morgan et al. (1998), Boice et al. (1999), and Hansen et al. (2001). Only the exposure duration-response analysis of Raaschou-Nielsen et al. (2003) had over 80% statistical power to detect a doubling of kidney cancer risk (NRC, 2006), and they observed a statistically significant association between kidney cancer and ≥5-year employment duration. Rate ratios estimated in the mortality cohort studies of kidney cancer (e.g., Boice et al., 2006b; Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998; Axelson et al., 1994; Greenland et al., 1994;

Sinks et al., 1992; Garabrant et al., 1988) are likely underestimated to some extent because their reliance on death certificates and increased potential of nondifferential misclassification of outcome in these studies, although the magnitude is difficult to predict (NRC, 2006). Cohort or PMR studies with more uncertain exposure assessment approaches, e.g., studies of all subjects working at a factory (Clapp and Hoffman, 2008; Sung et al., 2007; Chang et al., 2005; ATSDR, 2004a; Chang et al., 2003; Costa et al., 1989; Garabrant et al., 1988), do not show association but are quite limited given their lack of attribution of higher or lower exposure potentials; risks are likely diluted due to their inclusion of no or low exposed subjects.

Two studies were carried out in geographic areas with a high frequency and a high degree of TCE exposure and were designed with a priori hypotheses to test for the effects of TCE exposure on renal cell cancer risk (Charbotel et al., 2009; Charbotel et al., 2006; Brüning et al., 2003) and a third study carried out in four central and eastern European countries with high RCC rates unexplained by established risk factors (Moore et al., 2010; Ferlay et al., 2008). For these reasons, their observations have important bearing to the epidemiologic evidence evaluation. These studies found a twofold elevated risk with any TCE exposure after adjustment for several possible confounding factors including smoking (2.47, 95% CI: 1.36, 4.49) for self-assessed exposure to TCE (Brüning et al., 2003); any confidence job with high cumulative TCE exposure, 925 ppm-years (2.16, 95% CI: 1.02, 4.60) with a positive and statistically significant trend test, $p = 0.04$, high confidence jobs with high cumulative TCE exposure (3.34, 95% CI: 1.27, 8.74) (Charbotel et al., 2006); high confidence assessment of high TCE cumulative exposure ≥1.58 ppm-years (2.23, 95% CI: 1.07, 4.64) with a positive and statistically significant trend test, $p = 0.02$ (Moore et al., 2010). Furthermore, RCC risk in Charbotel et al. (2005) increased to over threefold (95% CI: 1.19, 8.38) in statistical analyses, which considered a 10-year exposure lag period. An exposure lag period is often adopted in analysis of cancer epidemiology to reduce exposure measurement biases (Salvan et al., 1995). Most exposed cases in this study were exposed to TCE below any current occupational standard (26 of 37 cases [70%]) had held a job with a highest TWA (<50 ppm) (Charbotel et al., 2009). A subsequent analysis of Charbotel et al. (2009) using an exposure surrogate defined as the highest TWA for any job held, an inferior surrogate given that TCE exposures in other jobs were not considered, reported an almost threefold elevated risk (2.80, 95% CI: 1.12, 7.03) adjusted for age, sex, body mass index (BMI), and smoking with exposure to TCE in any job to ≥50-ppm TWA (Charbotel et al., 2009). Considering all jobs, Moore et al. (2010) reported a risk of 2.34 (95% CI: 1.05, 5.21) for average TCE intensity (>0.76 ppm), an exposure metric similar to a TWA exposure category. Zhao et al. (2005) compared 2,689 TCE-exposed workers at a California aerospace company to nonexposed workers from the same company as the internal referent population, and found a monotonic increase in incidence of kidney cancer by increasing cumulative TCE exposure. In addition, a fivefold increased incidence was associated with high cumulative TCE exposure. This relationship for high cumulative TCE exposure, lagged 20 years, was accentuated with

adjustment for other occupational exposures (RR = 7.40, 95% CI: 0.47, 116), although the CIs were increased. An increased CI with adjustments is not unusual in occupational studies, as exposure is usually highly correlated with them, so that adjustments often inflate SE without removing any bias (NRC, 2006). Observed risks were lower for kidney cancer mortality and because of reliance on cause of death on death certificates are likely underestimated because of nondifferential misclassification of outcome (Percy et al., 1981). Boice et al. (2006b), another study of 1,111 workers with potential TCE exposure at this company and which overlaps with Zhao et al. (2005), found a twofold increase in kidney cancer mortality (standardized mortality ratio [SMR] = 2.22, 95% CI: 0.89, 4.57). This study examined mortality in a cohort whose definition date differs slightly from Zhao et al. (2005), working between 1948 and 1999 with vital status as of 1999 (Boice et al., 2006b) compared to working between 1950 and 1993 with follow-up for mortality as of 2001 (Zhao et al., 2005), and used a qualitative approach for TCE exposure assessment. Boice et al. (2006b) is a study of fewer subjects identified with potential TCE exposure, of fewer kidney cancer deaths [7 deaths; 10 incident cases, 10 deaths in Zhao et al. (2005)], of subjects with more recent exposures, and with a inferior exposure assessment approach compared to Zhao et al. (2005); a finding of a twofold mortality increase (95% CI: 0.89, 4.57) is noteworthy given the insensitivities.

Zhao et al. (2005), Charbotel et al. (2006), and Moore et al. (2010), furthermore, are three of the few studies to conduct a detailed assessment of exposure that allowed for the development of a JEM that provided rank-ordered levels of exposure to TCE and other chemicals. NRC (2006) discussed the inclusion of rank-ordered exposure levels is a strength increasing precision and accuracy of exposure information compared to more inferior exposure assessment approaches in some other studies such as duration of exposure or a grouping of all exposed subjects.

The finding in Raaschou-Nielsen et al. (2003) of an elevated RCC risk with longer employment duration is noteworthy given this study's use of a relatively insensitive exposure assessment approach. One strength of this study is the presentation of incidence ratios for a subcohort of higher exposed subjects, those with at least a 1-year duration of employment and first employment before 1980, as a sensitivity analysis for assessing the effect of possible exposure misclassification bias. RCC risk was higher in this subcohort compared to the larger cohort and indicated some potential for misclassification bias in the grouped analysis. For both the cohort and subcohort analyses, risk appeared to increase with increasing employment duration, although formal statistical tests for trend are not presented in the published paper.

4.4.2.2.1. Discussion of controversies on studies in the Arnsberg region of Germany

Two previous studies of workers in this region, a case-control study of Vamvakas et al. (1998) and Henschler et al. (1995), a study prompted by a kidney cancer case cluster, observed strong associations between kidney cancer and TCE exposure. A fuller discussion of the studies

from the Arnsberg region and their contribution to the overall weight of evidence on cancer hazard is warranted in this evaluation given the considerable controversy (Cherrie et al., 2001; Mandel, 2001; Green and Lash, 1999; McLaughlin and Blot, 1997; Bloemen and Tomenson, 1995; Swaen, 1995) surrounding Henschler et al. (1995) and Vamvakas et al. (1998).

Criticisms of Henschler et al. (1995) and Vamvakas et al. (1998) relate, in part, to possible selection biases that would lead to inflating observed associations and limited inferences of risk to the target population. Specifically, these include: (1) the inclusion of kidney cancer cases first identified from a cluster and the omission of subjects lost to follow-up from Henschler et al. (1995); (2) use of a Danish population as referent, which may introduce bias due to differences in coding cause of death and background cancer rate differences (Henschler et al., 1995); (3) follow-up of some subjects outside the stated follow-up period (Henschler et al., 1995); (4) differences between hospitals in the identification of cases and controls in Vamvakas et al. (1998); (5) lack of temporality between case and control interviews (Vamvakas et al., 1998); (6) lack of blinded interviews (Vamvakas et al., 1998); (7) age differences in Vamvakas et al. (1998) cases and controls that may lead to a different TCE exposure potential; (8) inherent deficiencies in Vamvakas et al. (1998) as reflected by its inability to identify other known kidney cancer risk factors; and (9) exposure uncertainty, particularly unclear intensity of TCE exposure. Overall, NRC (2006) noted that some of the points above may have contributed to an underestimation of the true exposure distribution of the target population (points 5, 6, and 7), other points would underestimate risk (points 3), and that these effects could not have explained the entire excess risk observed in these studies (points 1, 2, and 4). The NRC (2006) furthermore disagreed with the exposure uncertainty criticism (point 9), and concluded TCE exposures, although of unknown intensity, were substantial and, clearly showed graded differences on several scales in Vamvakas et al. (1998) consistent with this study's semiquantitative exposure assessment.

Brüning et al. (2003) was carried out in a broader region in southern Germany, which included the Arnsberg region and a different set of cases and control identified from a later time period than Vamvakas et al. (1998). The TCE exposure range in this study was similar to that in Vamvakas et al. (1998), although at a lower exposure prevalence because of the larger and more heterogeneous ascertainment area for cases and controls. For "ever exposed" to TCE, Brüning et al. (2003) observed a risk ratio of 2.47 (95% CI: 1.36, 4.49) and a fourfold increase in risk (95% CI: 1.80, 7.54) among subjects with any occurrence of narcotic symptom and a sixfold increase in risk (95% CI: 1.46, 23.99) for subjects who had daily occurrences of narcotic symptoms; risks which are lower than observed in Vamvakas et al. (1998). The lower rate ratio in Brüning et al. (2003) might indicate bias in the Vamvakas et al. (1998) study or statistical variation between studies related to the broader base population included in Brüning et al. (2003).

Observational studies such as epidemiologic studies are subject to biases and confounding, which can be minimized but never completely eliminated through a study's design and statistical analysis methods. While Brüning et al. (2003) overcome many of the deficiencies of Henschler et al. (1995) and Vamvakas et al. (1998), nonetheless, possible biases and measurement errors could be introduced through their use of prevalent cases and residual noncases, use of controls from surgical and geriatric clinics, nonblinding of interviewers, a 2-year difference between cases and controls in median age, use, or proxy or next-of-kin interviews, and self-reported occupational history.

The impact of any one of the above points could either inflate or depress observed associations. Biases related to a longer period for case compared to control ascertainment could go in either direction. Next-of-kin interviewers for deceased cases, all controls being alive at the time of interview, would be expected to underestimate risk if exposures were not fully reported and thus, misclassified. On the other hand, the control subjects who were enrolled when the interviews were conducted might not represent the true exposure distribution of the target population through time and would lead to overestimate of risk. Selection of controls from clinics is not expected to greatly influence observed associations since these clinics specialized in the type of care they provided (NRC, 2006). Brüning et al. (2003) is not the only kidney case-control study where interviewers were not blinded; in fact, only the study of Charbotel et al. (2006) included blinding of interviewers. Blinding of interviewers is preferred to reduce possible bias. The Brüning et al. (2003) study's use of frequency matching using 5-year age groupings is common in epidemiologic studies and any biases introduced by age difference between cases and controls is expected to be minimal because the median age difference was 3 years.

Despite these issues, the three studies of the Arnsberg region, with very high apparent exposure and different base populations showed a significant elevation of risk and all have bearing on kidney cancer hazard evaluations. The emphasis provided by each study for identifying a kidney cancer hazard depends on its strengths and weaknesses. Brüning et al. (2003) overcomes many of the deficiencies in Henschler et al. (1995) and Vamvakas et al. (1998). The finding of a statistically significantly approximately threefold elevated OR with occupational TCE exposure in Brüning et al. (2003) strengthens the signal previously reported by Henschler et al. (1995) and Vamvakas et al. (1998). A previous study of cardboard workers in the United States (Sinks et al., 1992), a study like Henschler et al. (1995), which was prompted by a reported cancer cluster, had observed association with kidney cancer incidence, particularly with work in the finishing department where TCE use was documented. Henschler et al. (1995), Vamvakas et al. (1998), and Sinks et al. (1992) are less likely to provide a precise estimate of the magnitude of the association given greater uncertainty in these studies compared to Brüning et al. (2003). For this reason, Brüning et al. (2003) is preferred for meta-analysis treatment since it is considered to better reflect risk in the target population than the two other studies. Another

study (Charbotel et al., 2006) of similar exposure conditions of a different base population and of different case and control ascertainment methods as the Arnsberg region studies has become available since the Arnsberg studies. This study shows a statistically significant elevation of risk and high cumulative TCE exposure in addition to a positive trend with rank-order exposure levels. Charbotel et al. (2006) added evidence to observations from earlier studies on high TCE exposures in Southern Germany and suggested that peak exposure may add to risk associated with cumulative TCE exposure.

4.4.2.3. Examination of Possible Confounding Factors

Examination of potential confounding factors is an important consideration in the evaluation of observations in the epidemiologic studies on TCE and kidney cancer. A known risk factor for kidney cancer is cigarette smoking. Obesity, diabetes, hypertension and antihypertensive medications, and analgesics are linked to kidney cancer, but causality has not been established (McLaughlin et al., 2006; Moore et al., 2005). On the other hand, fruit and vegetable consumption is considered protective of kidney cancer risk (McLaughlin et al., 2006). Studies by Asal et al. (1988a; 1988b), Partanen et al. (1991), McCredie and Stewart (1993), Aupérin et al. (1994), Chow et al. (1994), Mellemgaard et al. (1994), Mandel et al. (1995), Vamvakas et al. (1998), Dosemeci et al. (1999), Pesch et al. (2000b), Brüning et al. (2003), and Charbotel et al. (2006) controlled for smoking, and all studies except Pesch et al. (2000b) controlled for BMI. Moore et al. (2010) examined, but did not find, smoking or BMI as potential confounders because statistical examination of cigarette smoking and BMI altered risk estimates for the association between TCE exposure and kidney cancer by <10%. Vamvakas et al. (1998) and Dosemeci et al. (1999) controlled for hypertension and/or diuretic intake in the statistical analysis. Because it is unlikely that exposure to TCE is associated with smoking, BMI, hypertension, or diuretic intake, these possible confounders do not significantly affect the estimates of risk (NRC, 2006).

Direct examination of possible confounders is less common in cohort studies than in case-control studies where information is obtained from study subjects or their proxies. Use of internal controls, such as for Zhao et al. (2005), in general, minimizes effects of potential confounding due to smoking or socioeconomic (SES) status since exposed and referent subjects are drawn from the same target population. Information on possible confounding due to BMI (obesity) and to diabetes is lacking in cohort studies; however, any uncertainties are likely small given the generally healthy nature of an employed population and its favorable access to medical care.

The effect of smoking as a possible confounder may be assessed indirectly through: (1) examination of risk ratios for other smoking-related sites; (2) examination of the expected contribution by smoking to cancer risks; and (3) examination of lung cancer in nine TCE cohort studies in which there is a high likelihood of TCE exposure in individual study subjects (and

which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review using meta-analysis methods). Some information on smoking-related lung and kidney cancer risks may be obtained from IARC (2004a) for indirectly evaluating the expected magnitude by smoking on kidney cancer risks in TCE cohort studies. Five cohort studies of cigarette smoking reported risk estimates for both lung and kidney cancers with an observed ratio of lung:kidney cancer risks of 3.5–10.6 for active smokers, who will have higher smoking-related risks than former smokers (see Table 4-41). The nine cohort studies (Radican et al., 2008; Zhao et al., 2005; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994) present lung cancer risks and reported risks for overall TCE exposure ranging from 0.69 (95% CI: 0.31, 1.30) by Axelson et al. (1994) to 1.4 (95% CI: 1.32, 1.52) by Raaschou-Nielsen et al. (2003) (see Table 4-81). Smoking was more prevalent in the Raaschou-Nielsen et al. (2003) cohort than the background population as suggested by the elevated risks for lung and other smoking-related sites. If smoking fully contributes to the observed 40% excess lung cancer risk in this study and based on observations in the five smoking cohorts, the expected contribution by smoking to RCC risk is estimated as 1–6% and far smaller than the 20 and 40% excess in RCC risk in the cohort and subcohort. The use of internal referents who are unexposed subjects drawn from the occupational settings as TCE exposed subjects in three studies reduces any confounding related to smoking as referents (Radican et al., 2008; Zhao et al., 2005; Morgan et al., 1998). In the other cohort studies lacking direct adjustment for smoking and internal referents, difference in cigarette smoking between exposed and referent subjects is not sufficient to fully explain observed excess kidney cancer risks associated with TCE, particularly high TCE exposure. Lung cancer risk estimates are lower than or equal to kidney cancer risk estimates and inconsistent with observations in the five smoking cohorts (Hansen et al., 2001; Boice et al., 1999; Axelson et al., 1994).

Table 4-41. Summary of lung and kidney cancer risks in active smokers

Cohort	RR		Ratio lung; kidney	Reference
	Lung	Kidney		
MRFIT (USA) 1975–1985, men	6.7	1.9	3.5	Kuller et al. (1991)
British Doctor's Study (United Kingdom) 1957–1991, men	14.9[a]	1.4[a]	10.6	Doll et al. (1994)
U.S. Veterans Study (United States) 1954–1980, men	11.6	1.5	7.7	McLaughlin et al. (1995)
Swedish Census Study (Sweden) 1963–1989, women	4.7	1.1	4.3	Nordlund et al. (1999, 1997)
Cancer Prevention Study II (United States) 1982–1986, women	12.4	1.4	9.1	Garfinkel and Stellman (1988); Heath et al. (1997)

[a]Relative mortality rate compared to nonsmokers.

Source: from IARC (2004a)

Meta-analysis methods were adopted, additionally, as a tool for examining risk estimates from the nine cohort studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review reporting lung cancer to assess the presence of potential systematic error related to confounding from smoking. Significant heterogeneity was observed across the nine studies of overall exposure (I^2 = 90%) and for six of the nine studies with highest exposure groups (I^2 = 80%). Although the appropriateness of conducting any meta-analysis without attempting to explain the heterogeneity is arguable, the summary estimate from the primary random effects meta-analysis of the nine studies was 0.96 (95% CI: 0.76, 1.21) for overall TCE exposure, and 0.96 (95% CI: 0.72, 1.27) for the highest group exposure reported by six studies. These observations suggest potential confounding by smoking of kidney cancer summary risk estimates can be reasonably excluded in cohort studies of TCE exposure.

Mineral oils such as cutting fluids or hydrazine common to some job titles with potential TCE exposures (such as machinists, metal workers, and test stand mechanics) were included as covariates in statistical analyses of Zhao et al. (2005), Boice et al. (2006b) and Charbotel et al. (2009; 2006) or evaluated as a single exposure for cases and controls in Moore et al. (Karami et al., 2011; 2010). A TCE effect on kidney cancer incidence was still evident, although effect estimates were often imprecise due to lowered statistical power (Charbotel et al., 2009; Charbotel et al., 2006; Zhao et al., 2005). Observed associations were similar in analyses including chemical co-exposures in both Zhao et al. (2005) and Charbotel et al. (2009; 2006) compared to chemical co-exposure unadjusted risks. The association or OR between high TCE score and kidney cancer incidence in Zhao et al. (2005) was 7.71 (95% CI: 0.65, 91.4) after

adjustment for other carcinogens including hydrazine and cutting oils, compared to analyses unadjusted for chemical co-exposures (4.90, 95% CI: 1.23, 19.6).

In Charbotel et al. (2006), exposure to TCE was strongly associated with exposure to cutting fluids and petroleum oils (22 of the 37 TCE-exposed cases were exposed to both). Statistical modeling of all factors significant at 10% threshold showed the OR for cutting fluids to be almost equal to one, whereas the OR for the highest level of TCE exposure was close to two (Charbotel et al., 2006). Moreover, when exposure to cutting oils was divided into three levels, a decrease in OR with level of exposure was found. In conditional logistic regression adjusted for cutting oil exposure, the OR for RCC and TCE was similar to ORs unadjusted for cutting fluid exposures (high cumulative TCE exposure: 1.96 [95% CI: 0.71–5.37] compared to 2.16 [95% CI: 1.02–4.60]; high cumulative and peak: 2.63 [95% CI: 0.79–8.83] compared to 2.73 [95% CI: 1.06–7.07] (Charbotel et al., 2006). Charbotel et al. (2009) further examined TCE exposure defined as the highest TWA in any job held, inferior to cumulative exposure given its lack of consideration of TCE exposure potential in other jobs, either as exposure to TCE alone, cutting fluids alone, or to both after adjusting for smoking, BMI, age, sex, and exposure to other oils (TCE alone: 1.62 [95% CI: 0.75, 3.44]); cutting fluids alone: 2.39 (95% CI: 0.52, 11.03); TCE >50-ppm TWA + cutting fluids: 2.70 (95% CI: 1.02, 7.17). There were few cases exposed to cutting fluids alone (n = 3) or to TCE alone (n = 15), all of whom had TCE exposure (in the highest exposed job held) of <35 ppm TWA, and the subgroup analyses were of limited statistical power. A finding of higher risk for both cutting oil and TCE exposure ≥50 ppm compared to cutting oil alone supports a TCE effect for kidney cancer. Adjustment for cutting oil exposures, furthermore, did not greatly affect the magnitude of TCE effect measures in the many analyses presented by Charbotel et al. (2009; 2006) suggesting cutting fluid exposure as not greatly confounding TCE effect measures. Two other kidney case-control studies of TCE exposure examined the effect of cutting oil as a single occupational exposure on kidney cancer risk (Karami et al., 2011; Brüning et al., 2003). Although Brüning et al. (2003) reported an OR of 2.11 (95% CI: 0.66, 6.70) for self-reported cutting oil exposure and kidney cancer, cutting oil exposure did not appear highly correlated with TCE exposure as only 5 cases reported exposure to cutting oils compared to 25 cases reporting TCE exposure. Karami et al. (2011), who examined mineral oil or cutting fluid exposure among cases and controls in Moore et al. (2010), reported an OR of 0.8 (95% CI: 0.6, 1.1) and 1.1 (95% CI: 0.8, 1.4), for cutting oil mists or other mineral oil mists respectively, and provides evidence that the reported association with TCE exposure in Moore et al. (2010) is not likely confounded by cutting or mineral oil exposures. Moreover, cutting oils and mineral oils have not been associated with kidney cancer in other cohort or case-control studies (Mirer, 2010; NIOSH, 1998), which provide additional support for potential confounding by cutting oils as of minimal concern.

Boice et al. (2006b) was unable to directly examine hydrazine exposure on TCE effect measures because of a lack of model convergence in statistical analyses. Three of seven TCE-exposed kidney cancer cases were identified with hydrazine exposure of ≤1.5 years and the absence of exposure to the other four cases suggested confounding related to hydrazine was unlikely to greatly modify observed association between TCE and kidney cancer.

4.4.2.4. Susceptible Populations—Kidney Cancer and TCE Exposure

Two studies of kidney cancer cases from the Arnsberg region in Germany and the study of kidney cancer cases from three Central and Eastern European countries have examined the influence of polymorphisms of the GST metabolic pathway on RCC risk and TCE exposure (Moore et al., 2010; Wiesenhütter et al., 2007; Brüning et al., 1997a). In their study of 45 TCE-exposed male and female RCC cases pending legal compensation and 48 unmatched male TCE-exposed controls, Brüning et al. (1997a) observed a higher prevalence of exposed cases homozygous and heterozygous for GSTM1 positive, 60%, than the prevalence for this genotype among exposed controls, 35%. The frequency of GSTM1 positive was lower among this control series than the frequency found in other European population studies, 50% (Brüning et al., 1997a). The prevalence of the GSTT1 positive genotype was 93% among exposed cases and 77% among exposed controls. The prevalence of GSTT1 positive genotype in the European population is 75% (Brüning et al., 1997a).

Wiesenhütter et al. (2007) compares the frequency of genetic polymorphism among subjects from the renal cancer case-control study of Brüning et al. (2003) and to the frequencies of genetic polymorphisms in the areas of Dormund and Lutherstadt Wittenberg, Germany. Wiesenhütter et al. (2007) identified the genetic frequencies of GSTM1 and GSTT1 phenotypes for 98 of the original 134 cases (73%) and 324 of the 401 controls (81%). The prevalence of GSTM1 positive genotype was 48% among all RCC cases, 40% among TCE-exposed cases, and 52% among all controls. The prevalence of GSTT1 positive genotypes was 81% among all cases and 81% among all controls. The prevalence of GSTT1 positive genotypes reported in this paper for all TCE-exposed cases was 20%. Wiesenhütter et al. (2007) noted background frequencies in the German population in the expanded control group were 50% for GSTM1 positive and 81% for GSTT1 positive genotypes. The observations are limited as the paper is sparsely reported and numbers of exposed (n = 4) and unexposed (n = 15) GSTT1 positive cases do not sum to the 79 cases with the GSTT1 positive genotype identified in the table‘s first row.

Moore et al. (2010) presents associations between TCE exposure and RCC risk stratified by GSTT genotype and for single nucleotide polymorphisms (SNPs) of the renal cysteine conjugate β-lyase gene. Genotyping was available for 925 of the 1,097 cases and 1,192 of the 1,476 controls. The percentage of cases and controls genotyped did not significantly differ among TCE-exposed and unexposed subjects nor was the active GSTT1 genotype association with kidney cancer risk (0.94, 95% CI: 0.75, 1.19). However, adopting statistical analysis

examining TCE exposure and kidney cancer that stratified on GSTT1 polymorphism as null (deleted allele) or active (≥1 intact allele), Moore et al. (2010) reported significant associations for GSTT1 active genotype and no association was suggested for subjects with GSTT1 null genotype. The risk estimate for the association for TCE exposure and kidney cancer among subjects with an active GSTT1 genotype was 1.88 (95% CI: 1.06, 3.33), with higher risk estimates for long exposure duration, cumulative exposure, and average exposure intensity (≥13.5 years, 2.13 [95% CI: 1.04, 4.39]; ≥1.58 ppm-years, 2.59 [95% CI: 1.25, 5.35]; ≥0.076 ppm, 2.77 [95% CI: 1.01, 7.58]) and a positive trend with increasing exposure duration, cumulative exposure or average intensity categories ($p \leq 0.03$) (Moore et al., 2010). The associations between TCE exposure and kidney cancer was stronger for subjects with a functionally active GSTT1 than those for all subjects (both genotypes combined) (see Table 4-39). Moore et al. (2010) tested but did not find statistical interaction between GSTT1 genotype and TCE exposure ($p \geq 0.17$). Moore et al. (2010) also examined the effect of polymorphisms of the cysteine conjugate β-lyase gene on TCE risk and reported interaction between TCE exposure and four minor alleles (SNPs rs2293968, rs2280841, rs2259043, and rs941960) ($p < 0.05$). Associations with TCE exposure and kidney cancer were threefold higher compared to unexposed subjects with these SNPs.

Observations in Brüning et al. (1997a) and Wiesenhütter et al. (2007) must be interpreted cautiously. Few details were provided in these studies on selection criteria and not all subjects from the Brüning et al. (2003) case-control study were included. For GSTM1 positive, the higher prevalence among exposed cases in Brüning et al. (1997a) compared Wiesenhütter et al. (2007) and the lower prevalence among controls compared to background frequency in the European population may reflect possible selection biases. On the other hand, the broader base population included in Brüning et al. (2003) may explain the observed lower frequency of GSTM1 positive cases in Wiesenhütter et al. (2007). Moreover, Wiesenhütter et al. (2007) does not report genotype frequencies for controls by exposure status and this information is essential to an examination of whether RCC risk and TCE exposure may be modified by polymorphism status. The statistical analyses in both studies was a simple comparison of exposure prevalence between cases and controls and did not include analyses that stratified on exposure status. An examination of exposure prevalence is limited as Moore et al. (2010), too, reported TCE exposure prevalence as similar between exposed cases and controls. Associations between TCE exposure and kidney cancer for GSTT1 active genotype, however, were reported in stratified analyses. The more rigorous study design and statistical methods in Moore et al. (2010) affords more weight to their reported observations than for Brüning et al. (1997a) and Wiesenhütter et al. (2007). Moore et al. (2010) provides evidence of greater susceptibility to TCE exposure and kidney cancer among subjects with a functionally active GSTT polymorphism, particularly among those with certain alleles in single nucleotide polymorphisms of the cysteine conjugation β-lyase gene region.

Of the three larger (in terms of number of cases) studies that did provide results separately by sex, Dosemeci et al. (1999) suggested that there may be a sex difference for TCE exposure and RCC (OR: 1.04, [95% CI: 0.6, 1.7]) in males and 1.96 (95% CI: 1.0, 4.0 in females), while Raaschou-Nielsen et al. (2003) report the same standardized incidence ratio (SIR = 1.2) for both sexes and crude ORs calculated from data from the Pesch et al. (2000b) study (provided in a personal communication from Beate Pesch, Forschungsinstitut für Arbeitsmedizin, to Cheryl Scott, EPA, 21 February 2008) are 1.28 for males and 1.23 for females. Whether the Dosemeci et al. (1999) observations are due to susceptibility differences or to exposure differences between males and females cannot be evaluated. Blair et al. (1998) and Hansen et al. (2001) also present some results by sex, but these two studies have too few cases to be informative about a sex difference for kidney cancer.

4.4.2.5. Meta-Analysis for Kidney Cancer

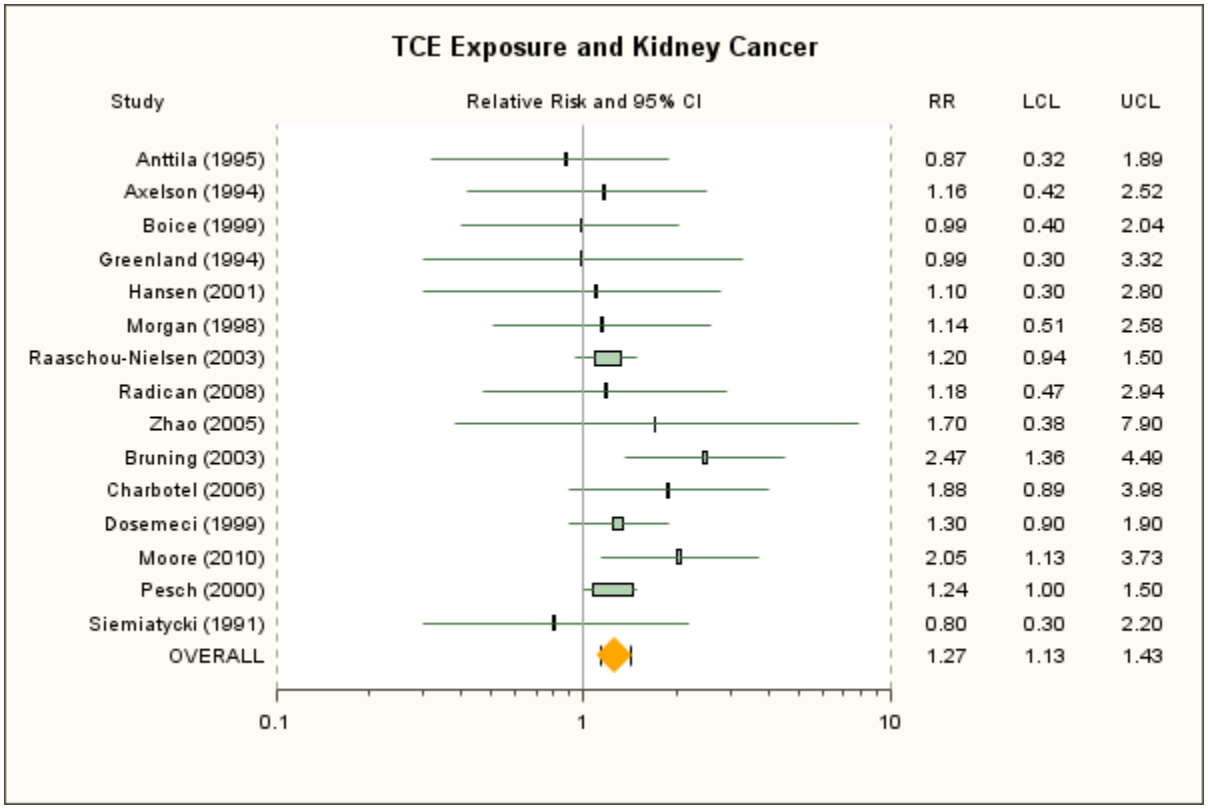
Meta-analysis (detailed methodology in Appendix C) was adopted as a tool for examining the body of epidemiologic evidence on kidney cancer and TCE exposure and to identify possible sources of heterogeneity. The meta-analyses of the overall effect of TCE exposure on kidney cancer suggest a small, statistically significant increase in risk that was stronger in a meta-analysis of the highest exposure group. There was no observable heterogeneity for any of the meta-analyses of the 15 studies and no indication of publication bias. Thus, these findings of increased risks of kidney cancer associated with TCE exposure are robust.

The meta-analysis of kidney cancer examines 15 cohort and case-control studies identified through a systematic review and evaluation of the epidemiologic literature on TCE exposure (Moore et al., 2010; Charbotel et al., 2006; Zhao et al., 2005; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Pesch et al., 2000b; Boice et al., 1999; Dosemeci et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991). Details of the systematic review and meta-analysis of the TCE studies are fully discussed in Appendix B and C.

The summary relative risk (RRm) estimate from the primary random effects meta-analysis of the 15 studies was 1.27 (95% CI: 1.13, 1.43). The analysis was dominated by two (contributing almost 70% of the weight) or three (almost 80% of the weight) large studies (Raaschou-Nielsen et al., 2003; Pesch et al., 2000b; Dosemeci et al., 1999). Figure 4-1 arrays individual studies by their weight. No single study was overly influential; removal of individual studies resulted in RRm estimates that were all statistically significant ($p < 0.005$) and ranged from 1.24 (with the removal of Brüning et al., (2003)) to 1.30 (with the removal of Raaschou-Nielsen et al., (2003)). Similarly, the overall RRm estimate was not highly sensitive to alternate RR estimate selections nor was publication bias apparent. There was no apparent heterogeneity across the 15 studies, i.e., the random effects model and the fixed effect model gave the same

results (p_{hetero} = 0.67; I = 0%). Nonetheless, subgroup analyses were done examining the cohort and case-control studies separately with the random effects model; the resulting RRm estimates were 1.16 (95% CI: 0.96, 1.40) for the cohort studies and 1.48 (1.15, 1.91) for the case-control studies. There was no heterogeneity in the cohort subgroup (p = 0.998; I^2 = 0%). There was heterogeneity in the case-control subgroup, but it was not statistically significant (p = 0.14) and the I^2 value of 41% suggests that the extent of the heterogeneity in this subgroup was low-to-moderate.

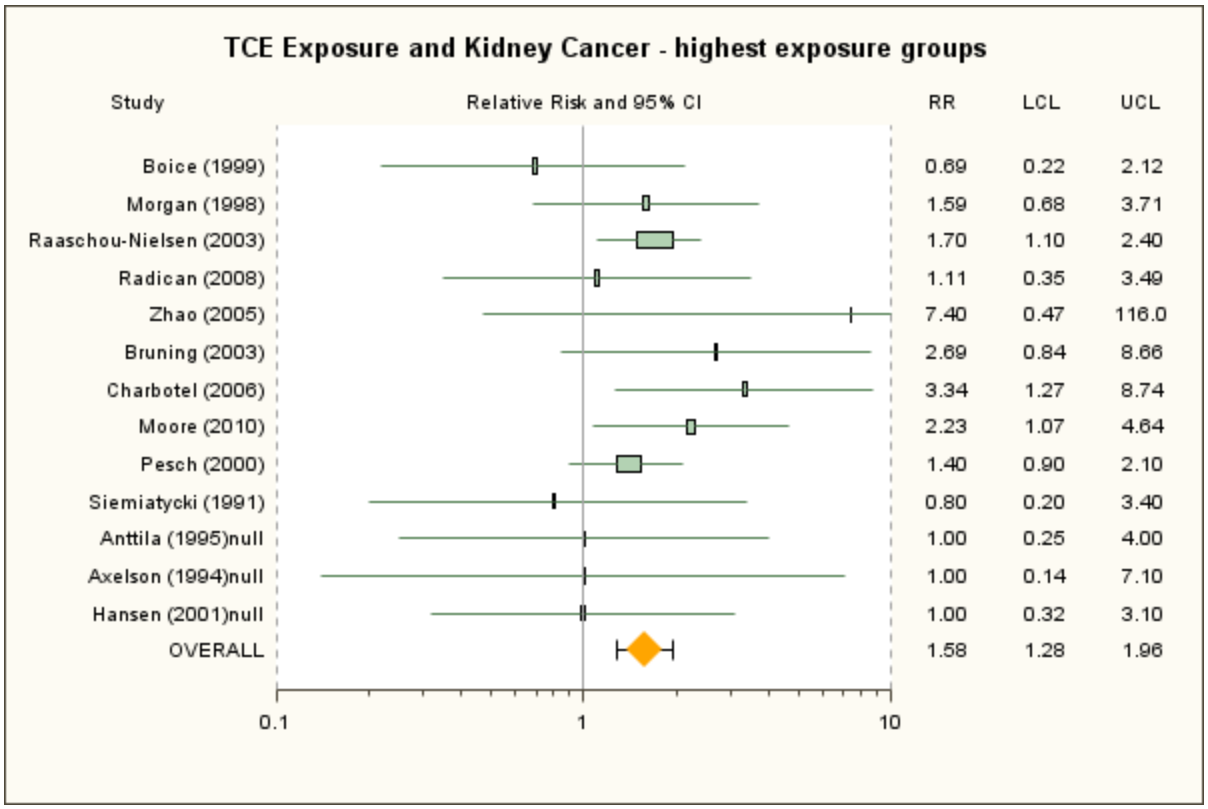
Ten studies reported risks for higher exposure groups (Moore et al., 2010; Charbotel et al., 2006; Zhao et al., 2005; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Pesch et al., 2000b; Boice et al., 1999; Dosemeci et al., 1999; Morgan et al., 1998; Siemiatycki, 1991). Different exposure metrics were used in the various studies, and the purpose of combining results across the different highest exposure groups was not to estimate an RRm associated with some level of exposure. Instead, the focus on the highest exposure category was meant to result in an estimate less affected by exposure misclassification. In other words, it is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any vs. none) exposure comparison. Thus, if TCE exposure increases the risk of kidney cancer, the effects should be more apparent in the highest exposure groups.



Random effects model; fixed effect model same. The summary estimate is in the bottom row, represented by the diamond. Symbol sizes reflect relative weights of the studies.

Figure 4-1. Meta-analysis of kidney cancer and overall TCE exposure.

The RRm estimate from the random effects meta-analysis of the studies with results presented for higher exposure groups was 1.64 (95% CI: 1.31, 2.04), higher than the RRm from the overall kidney cancer meta-analysis. As with the overall analyses, the meta-analyses of the highest-exposure groups were dominated by Pesch et al. (2000b) and Raaschou-Nielsen et al. (2003), which provided about 60% of the weight. Axelson et al. (1994), Anttila et al. (1995), and Hansen et al. (2001) do not report risk ratios for kidney cancer by higher exposure and a sensitivity analysis was carried out to address reporting bias. The RRm estimate from the primary random effects meta-analysis with null RR estimates (i.e., RR = 1.0) included for Axelson et al. (1994), Anttila et al. (1995), and Hansen et al. (2001) to address reporting bias associated with ever exposed was 1.58 (95% CI: 1.28, 1.96). Figure 4-2 arrays individual studies by their weight. The inclusion of these three additional studies contributed <7% of the total weight. No single study was overly influential; removal of individual studies resulted in RRm estimates that were all statistically significant ($p < 0.005$) and that ranged from 1.52 [with the removal of Raaschou-Nielsen et al., (2003)] to 1.64 [with the removal of Pesch et al. (2000b)]. Similarly, the RRm estimate was not highly sensitive to alternate RR estimate selections (all with $p < 0.0005$) and other than a negligible amount of heterogeneity observed in the sensitivity analysis with the Pesch JEM alternate ($I^2 = 0.64\%$), there was no observable heterogeneity across the studies for any of the meta-analyses conducted with the highest-exposure groups, including those in which RR values for Anttila, Axelson, and Hansen were assumed ($I^2 = 0\%$), For Pesch, the job-task exposure matrix (JTEM) approach is preferred because it seemed to be a more comprehensive and discriminating approach, taking actual job tasks into account, rather than just larger job categories. No subgroup analyses (e.g., cohort vs. case-control studies) were done with the highest exposure group results.



With assumed null RR estimates for Antilla, Axelson, and Hansen (see Appendix C text). Random effects model; fixed effect model same. The summary estimate is in the bottom row, represented by the diamond. Symbol sizes reflect relative weights of the studies.

Figure 4-2. Meta-analysis of kidney cancer and TCE exposure—highest exposure groups.

NRC (2006) deliberations on TCE commented on two prominent evaluations of the then-current TCE epidemiologic literature using meta-analysis techniques, Wartenberg et al. (2000) and Kelsh et al. (2005), submitted by Exponent-Health Sciences to NRC during their deliberations and who updated their analysis by including subsequently published studies of Boice et al. (2006b) and Charbotel et al. (2006) but not Radican et al. (2008), and presented summary relative risk (RRm) estimates for cohort and (Kelsh et al., 2005)case-control studies, separately, and combined (Kelsh et al., 2010). Wartenberg et al. (2000) reported an RRm of 1.7 (95% CI: 1.1, 2.7) for kidney cancer incidence in the TCE subcohorts (Blair et al., 1998; Anttila et al., 1995; Henschler et al., 1995; Axelson et al., 1994). For kidney cancer mortality in TCE subcohorts (Boice et al., 1999; Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998; Henschler et al., 1995), Wartenberg et al. (2000) reported an RRm of 1.2 (95% CI: 0.8, 1.7). Kelsh et al. (2010) examined a slightly different grouping of cohort studies as did Wartenberg et al. (2000), presenting an RRm estimate for kidney cancer incidence and mortality combined. The RRm for kidney cancer in Group I cohort studies (Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994) was 1.34 (95% CI: 1.07–1.67) with no evidence of heterogeneity and, in Group II cohort studies, studies lacking documented TCE exposure (Chang et al., 2003; Henschler et al., 1995; Sinks et al., 1992; Seldén and Ahlborg, 1991; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988), was 1.58 (95% CI: 0.75, 3.32) with evidence of heterogeneity. Removing both Henschler et al. (1995) and Sinks et al. (1992), considered by Kelsh et al. (2010) as outliers, eliminated observed heterogeneity and the summary risk estimate was 0.88 (95% CI: 0.8, 1.33). Kelsh et al. (2010), also, presented separately a RRm for renal cancer case-control studies and TCE. For case-control studies (Charbotel et al., 2005; Brüning et al., 2003; Pesch et al., 2000b; Dosemeci et al., 1999; Vamvakas et al., 1998; Greenland et al., 1994; Siemiatycki, 1991), the RRm for RCC was 1.57 (95% CI: 1.06, 2.30) with evidence of heterogeneity, and 1.33 (95% CI: 1.02, 1.73) with no evidence of heterogeneity in a sensitivity analysis removing Vamvakas et al. (1998), a study Kelsh et al. (2010) considered as an outlier. Last, Kelsh et al. (2010) presented three RRm estimates for renal cell cancer Groups I and II cohort and case-control studies combined: 1.30 (95% CI: 1.04, 1.61) with evidence of heterogeneity and included 23 studies with kidney cancer risk estimates for all subjects, those with documented TCE exposure and those unexposed to TCE, and Ritz (1999a) in Group I studies; 1.42 (95% CI 1.13, 1.77) with evidence of heterogeneity and included 23 studies, with TCE subcohort kidney cancer risk estimates replacing the total cohort estimate for Group I studies; and 1.24 (95% CI; 1.06, 1.45) with no evidence of heterogeneity and included 20 studies, counting TCE subcohort kidney cancer risk estimates in Group I studies and removing the three studies Kelsh et al. (2010) considered as outliers.

The present analysis was conducted according to NRC (2006) suggestions for transparency, systematic review criteria, and examination of both cohort and case-control

studies. EPA's meta-analysis has several advantages to previous ones of TCE exposure and cancer. The selection criteria adopted in this meta-analysis were intended to identify informative studies for the evaluation of TCE exposure and cancer, studies with reduced systematic errors. Neither Henschler et al. (1995) nor Vamvakas et al. (1998), two studies with incomplete cohort identification or potential selection bias of study controls, met our inclusion criteria, and their inclusion in other meta-analysis may have contributed to the observed heterogeneity in kidney cancer RRm (Kelsh et al., 2010). Studies with background or low TCE exposure potential also did not meet another selection criterion as our analysis focused on TCE exposure potential inferred to each subject by reference to industrial hygiene records, individual biomarkers, JEMs, water distribution models, or questionnaire responses that likely had fewer biases associated with exposure misclassification, although this bias would not have been completely minimized. Inclusion of studies of lower exposure potential in meta-analyses can have important implications for identifying a cancer hazard (Vlaanderen et al., 2011; Zhang et al., 2009; Steinmaus et al., 2008). The present analysis includes the recently published studies of Charbotel et al. (2006), Moore et al. (2010), and updated mortality of the Blair et al. (1998) cohort by Radican et al. (2008). As discussed above, the summary estimate from the primary random effects meta-analysis of the 15 studies was 1.27 (95% CI: 1.13, 1.43). Additionally, EPA examined kidney cancer risk for higher exposure group. The RRm estimate from the random effects meta-analysis of the studies with results presented for higher exposure groups was 1.64 (95% CI: 1.31, 2.04), higher than the RRm from the overall kidney cancer meta-analysis, and 1.58 (95% CI: 1.28, 1.96) in the meta-analysis with null RR estimates (i.e., RR = 1.0) to address possible reporting bias for three studies.

4.4.3. Human Studies of Somatic Mutation of *VHL* Gene

Studies have been conducted to identify mutations in the *VHL* gene in RCC patients, with and without TCE exposures (Wells et al., 2009; Toma et al., 2008; Charbotel et al., 2007; Furge et al., 2007; Brauch et al., 1999; Schraml et al., 1999; Kenck et al., 1996). Inactivation of the *VHL* gene through mutations, LOH, and imprinting has been observed in about 70% of sporadic renal clear cell carcinomas, the most common RCC subtype (Kenck et al., 1996). Other genes or pathways, including c-Myc activation and VEGF, have also been examined as to their role in various RCC subtypes (Toma et al., 2008; Furge et al., 2007). Furge et al. (2007) reported that there are molecularly distinct forms of RCC and possibly molecular differences between clear-cell RCC subtypes. This study was performed using tissues obtained from paraffin blocks. These results are supported by a more recent study that examined the genetic abnormalities of clear cell RCC using frozen tissues from 22 clear cell-RCC patients and paired normal tissues (Toma et al., 2008). This study found that 20 (91%) of the 22 cases had LOH on chromosome 3p (harboring the *VHL* gene). Alterations in copy number were also found on chromosome 9 (32% of cases), chromosome arm 14q (36% of cases), chromosome arm 5q (45% of cases), and

chromosome 7 (32% of cases), suggesting roles for multiple genetic changes in RCC, and is also supported by genomes-wide single-nucleotide polymorphism analysis (Toma et al., 2008).

Several papers link mutation of the *VHL* gene in RCC patients to TCE exposure. These reports are based on comparisons of *VHL* mutation frequencies in TCE-exposed cases from RCC case-control studies or from comparison to background mutation rates among RCC case series (see Table 4-42). Brüning et al. (1997b) first reported a high somatic mutation frequency (100%) in a series of 23 RCC cases with medium to high intensity TCE exposure as determined by an abnormal single stand conformation polymorphism (SSCP) pattern, with most variations found in exon two. Only four samples were sequenced at the time of publication and showed mutations in exon one, two and three (see Table 4-42). Some of the cases in this study were from the case-control study of Vamvakas et al. (1998) (see Section 4.4.3 and Appendix B).

Brauch et al. (2004; 1999) analyzed renal cancer cell tissues for mutations of the *VHL* gene and reported increased occurrence of mutations in patients exposed to high concentrations of TCE. In the first study (Brauch et al., 1999), an employer‘s liability or worker‘s compensation registry was used to identify 44 RCC cases, 18 of whom were also included in Brüning et al. (1997b). Brauch et al. (1999) found multiple mutations in 42% of the exposed patients who experienced any mutation and 57% showed loss of heterozygosity. A hot spot mutation of cytosine to thymine at nucleotide 454 (C454T) was found in 39% of samples that had a *VHL* mutation and was not found in renal cell cancers from nonexposed patients or in lymphocyte DNA from either exposed or nonexposed cases or controls. As discussed above, little information was given on how subjects were selected and whether there was blinding of from the RCC case-control study of Vamvakas et al. (1998). Brauch et al. (2004) compared age at diagnosis and histopathologic parameters of tumors as well as somatic mutation characteristics in the *VHL* tumor suppressor gene between the TCE-exposed and non-TCE-exposed RCC patient groups (TCE-exposed from their previous 1999 publication to the non TCE-exposed cases newly sequenced in this study). RCC did not differ with respect to histopathologic characteristics in either patient group. Comparing results from TCE-exposed and nonexposed patients revealed clear differences with respect to: (1) frequency of somatic *VHL* mutations; (2) incidence of C454T transition; and (3) incidence of multiple mutations. The C454T hot spot mutation at codon 81 was exclusively detected in tumors from TCE-exposed patients, as were multiple mutations. Also, the incidence of *VHL* mutations in the TCE-exposed group was at least twofold higher than in the nonexposed group. Overall, these finding support the view that the effect of TCE is not limited to clonal expansion of cells mutated spontaneously or by some other agent.

Table 4-42. Summary of human studies on somatic mutations of the *VHL* gene[a]

TCE exposure status	Brüning et al. (1997b)	Brauch et al. (1999)		Schraml et al. (1999)		Brauch et al. (2004)		Charbotel et al. (2007)	
	Exposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed
Number of subjects/ number with mutations (%)	23/23 (100%)	44/33 (75%)	73/42 (58%)	9/3 (33%)	113/38 (34%)	17/14 (82%)	21/2 (10%)	25/2 (9%)	23/2 (8%)
RCC subtype	Unknown	Unknown		Clear cell 9 (75%) Papillary 2 (18%) Oncocytomas 1 (8%)	Unknown	Clear cell 37 (%) Oncocytic adenoma 1 (%) Bilateral metachronous 1 (%)		Clear cell 51 (75%) Papillary 10 (10–15%) Chromophobe 4 (5%) Oncocytomas 4 (5%)	
Tissue type analyzed	Paraffin	Paraffin, fresh (lymphocyte)		Paraffin		Paraffin		Paraffin, frozen tissues, Bouin's fixative	
Assay	SSCP[b], sequencing[b]	SSCP, sequencing, restriction enzyme digestion		CGH, sequencing		Sequencing		Sequencing	
Number of mutations	23	50	42	4	50	24	2	2	2
Type of mutation Missense Nonmissense[c]	 1 3	 27 23	 NA NA	 1 3	 Unknown Unknown	 17 7	 2 0	 1 1	 1 1

[a]Adapted from NRC (2006) with addition of Schraml et al. (1999) and Charbotel et al. (2007).
[b]By SSCP. Four (4) sequences confirmed by comparative genomic hybridization.
[c]Includes insertions, frameshifts, and deletions.

CGH = comparative genomic hybridization

Brauch et al. (2004) were not able to analyze all RCCs from the Vamvakas study (Vamvakas et al., 1998), in part because samples were no longer available. Using the data described by Brauch et al. (2004) (*VHL* mutation found in 15 exposed and 2 nonexposed individuals, and *VHL* mutation not found in 2 exposed and 19 unexposed individuals), the calculated OR is 71.3. The lower bound of the OR including the excluded RCCs is derived from the assumption that all 20 cases that were excluded were exposed but did not have mutations in *VHL* (*VHL* mutations were found in 15 exposed and 2 unexposed individuals and *VHL* was not found in 22 exposed and 18 unexposed individuals), leading to an OR of 6.5 that remains statistically significant.

Charbotel et al. (2007) examines somatic mutations in the three *VHL* coding exons in RCC cases from their case-control study (Charbotel et al., 2006). Of the 87 RCCs in the case-control study, tissue specimens were available for 69 cases (79%) of which 48 were clear cell-RCC. *VHL* sequencing was carried out for only the clear cell-RCC cases, 66% of the 73 clear cell-RCC cases in Charbotel et al. (2006). Of the 48 clear cell-RCC cases available for *VHL* sequencing, 15 subjects were identified with TCE exposure (31%), an exposure prevalence lower than 43% observed in the case-control study. Partial to full sequencing of the *VHL* gene was carried out using polymerase chain reaction (PCR) amplification and *VHL* mutation pattern recognition software of Béroud et al. (1998). Full sequencing of the *VHL* gene was possible for only 26 RCC cases (36% of all RCC cases). Single point mutations were identified in four cases (8% prevalence): two unexposed cases, a G > C mutation in exon 2 splice site and a G > A in exon 1; one case identified with low/medium exposure, T > C mutation in exon 2, and, one case identified with high TCE exposure, T > C in exon 3. It should be noted that the two cases with T > C mutations were smokers unlike the cases with G > A or G > C mutations. The prevalence of somatic *VHL* mutation in this study is quite low compared to that observed in other RCC case series from this region; around 50% (Gallou et al., 2001; Bailly et al., 1995). To address possible bias from misclassification of TCE exposure, Charbotel et al. (2006) examined renal cancer risk for jobs associated with a high level of confidence for TCE exposure. As would be expected if bias was a result of misclassification, they observed a stronger association between higher confidence TCE exposure and RCC, suggesting that some degree of misclassification bias is associated with their broader exposure assessment approach. Charbotel et al. (2007) do not present findings on *VHL* mutations for those subjects with higher level of confidence TCE exposure assignment.

Schraml et al. (1999) did not observe statistically significant differences in DNA sequence or mutation type in a series of 12 RCCs from subjects exposed to solvents including varying TCE intensity and a parallel series of 113 clear cell carcinomas from non-TCE exposed patients. Only nine of the RCC were clear cell-RCC and were sequenced for mutations. *VHL* mutations were observed in clear cell tumors only; 4 mutations in 3 TCE-exposed subjects compared to 50 mutations in tumors of 38 nonexposed cases. Details as to exposure conditions

are limited to a statement that subjects had been exposed to high doses of solvents, potential for mixed solvent exposures, and that exposure included a range of TCE concentrations. Limitations of this study include having a wider range of TCE exposure intensities as compared to the studies described above (Brauch et al., 1999; Brüning et al., 1997b), which focused on patients exposed to higher levels of TCE, and the limited number of TCE-exposed subjects analyzed, being the smallest of all available studies on RCC, TCE, and *VHL* mutation. For these reasons, Schraml et al. (1999) is quite limited for examining the question of *VHL* mutations and TCE exposure.

Szymańska et al. (2010) examined somatic mutations in three *VHL* coding exons in 359 RCC cases, 334 with clear-cell carcinomas, from the case-control study of Moore et al. (2010) as part of a pilot examination of mutation in three other genes, TP53, EGFR, and KRAS. The prevalence of *VHL* mutations was high in the RCC series, 72% of the tumors carried at least one function mutation, Although occupational exposures were not examined and data were not presented, Szymańska et al. (2010) reported that *VHL* mutations were not associated with TCE exposure.

A number of additional methodological issues need to be considered in interpreting these studies. Isolation of DNA for mutation detection has been performed using various tissue preparations, including frozen tissues, formalin fixed tissues and tissue sections fixed in Bouin's solution. Ideally, studies would be performed using fresh or freshly frozen tissue samples to limit technical issues with the DNA extraction. When derived from other sources, the quality and quantity of the DNA isolated can vary, as the formic acid contained in the formalin solution, fixation time and period of storage of the tissue blocks often affect the quality of DNA. Picric acid contained in Bouin's solution is also known to degrade nucleic acids resulting in either low yield or poor quality of DNA. In addition, during collection of tumor tissues, contamination of neighboring normal tissue can easily occur if proper care is not exercised. This could lead to the _dilution effect' of the results—i.e., because of the presence of some normal tissue, frequency of mutations detected in the tumor tissue can be lower than expected. These technical difficulties are discussed in these papers, and should be considered when interpreting the results. Additionally, selection bias is possible given tissue specimens were not available for all RCC cases in Vamvakas et al. (1998) or in Charbotel et al. (2006). Some uncertainty associated with misclassification bias is possible given the lack of TCE exposure information to individual subjects in Schraml et al. (1999) and in Charbotel et al. (2007) from their use of broader exposure assessment approach compared to that associated with the higher confident exposure assignment approach. A recent study by Nickerson et al. (2008) addresses many of these concerns by utilizing more sensitive methods to look at both the genetic and epigenetic issues related to *VHL* inactivation. This study was performed on DNA from frozen tissue samples and used a more sensitive technique for analysis for mutations (endonuclease scanning) as well as analyzing for methylation changes that may lead to inactivation of the *VHL* gene. This method of analysis was validated on tissue samples with known mutations. Of the 205 clear cell-RCC

samples analyzed, 169 showed mutations in the *VHL* gene (82.4%). Of those 36 without mutation, 11 were hypermethylated in the promoter region, which will also lead to inactivation of the *VHL* gene. Therefore, this study showed inactivating alterations in the *VHL* gene (either by mutation or hypermethylation) in 91% tumor samples analyzed.

The limited animal studies examining the role of *VHL* mutation following exposure to chemicals including TCE are described below in Section 4.4.6.1.1. Conclusions as to the role of *VHL* mutation in TCE-induced kidney cancer, taking into account both human and experimental data, are presented below in Section 4.4.7.

4.4.4. Kidney Noncancer Toxicity in Laboratory Animals

Acute, subchronic, and chronic exposures to TCE cause toxicity to the renal tubules in rats and mice of both sexes, via both inhalation (see Table 4-43) and oral (see Table 4-44) exposures. Nephrotoxicity from acute exposures to TCE has only been reported at relatively high doses, although histopathological changes have not been investigated in these experiments. Information about specific location of lesions is presented where available. TCE exposure for 13-weeks (corn oil gavage) led to increased nephrotoxicity but no significant increases in preneoplastic or neoplastic lesions as compared to controls (Mally et al., 2006). Chronic nephropathy was also observed in both sexes of Osborne-Mendel rats following exposure to TCE (549 and 1,097 mg/kg-day, 78 week). Chakrabarti and Tuchweber (1988) found that TCE administered to male F344 rats by i.p. injection (723–2,890 mg/kg) or by inhalation (1,000–2,000 ppm for 6 hours) produced elevated urinary NAG, GGT, glucose excretion, blood urea nitrogen (BUN), and high molecular weight protein excretion, characteristic signs of proximal tubular, and possibly glomerular injury, as soon as 24 hours postexposure. In the i.p. injection experiments, inflammation was observed, although some inflammation is expected due to the route of exposure, and nephrotoxicity effects were only statistically significantly elevated at the highest dose (2,890 mg/kg). In the inhalation experiments, the majority of the effects were statistically significant at both 1,000 and 2,000 ppm. Similarly, at these exposures, renal cortical slice uptake of *p*-aminohippurate was inhibited, indicating reduced proximal tubular function. Cojocel et al. (1989) found similar effects in mice administered TCE by i.p. injection (120–1,000 mg/kg) at 6 hours postexposure, such as the dose-dependent increase in plasma BUN concentrations and decrease in *p*-aminohippurate accumulation in renal cortical slices. In addition, malondialdehyde (MDA) and ethane production were increased, indicating lipid peroxidation.

Table 4-43. Inhalation studies of kidney noncancer toxicity in laboratory animals

Reference[a]	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Kidney effect(s) discussed in Section 4.4.4
Chakrabarti and Tuchweber (1988)	F344 rats (M)	Inhalation	0–20,00 ppm, 6 hrs	6/group	Increased signs of proximal tubular damage.
Green et al. (1998)	F344 rats (M)	Inhalation	0, 250, and 500 ppm, 6 hrs/d for 1, 7, 15, 21, 28 d	3–5/group	Increased formic acid excretion; plasma and urinary markers of nephrotoxicity unchanged.
Kjellstrand et al. (1983a)	**NMRI mice (M and F)**	**Inhalation**	**0–3.600 ppm, variable time periods of 1–24 hrs/d, for 30 or 120 d**	**10–20/group**	**Increased kidney weight.**
Maltoni et al. (1986)	**Sprague-Dawley rats, (M and F) B6C3F$_1$ mice (M and F)**	**Inhalation**	**0, 100, 300, and 600 ppm, 7 hrs/d, 5 d/wk, 104 wks exposure, observed for lifespan**	**116–141/group**	**Meganucleocytosis in male rats (Details in Table 4-49).**
Mensing et al. (2002)	Long-Evans rats (M)	Inhalation	0–500 ppm, 6 hrs/5 d/wk, 6 mo	5/group	Increased signs of nephrotoxicity.
Woolhiser et al. (2006)	**Sprague-Dawley rats (F)**	**Inhalation**	**0, 100, 300, and 1,000 ppm, 6 hrs/d, 5 d/wk, 4 wks**	**16/group**	**Increased kidney weight.**

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

Table 4-44. Oral and i.p. studies of kidney noncancer toxicity in laboratory animals

Reference[a]	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Kidney effect(s) discussed in Section 4.4.4
Chakrabarti and Tuchweber (1988)	F344 rats (M)	i.p. injection	0–2,890 mg/kg-d	6/group	Increased signs of proximal tubular damage.
Cojocel et al. (1989)	NMRI mice (M)	i.p. injection (sesame oil)	0–1,000 mg/kg	4/group	Increased signs of nephrotoxicity.
Green et al. (1997a)	F344 rats (M) $B6C3F_1$ mice (M)	Gavage (corn oil)	0, 500, and 2,000 mg/kg-d, 1 or 10 d	5 or 10/group	Increases in biochemical markers of kidney damage.
Green et al. (2003)	F344 rats (M)	Drinking water	0–54.3 mg/kg-d, 52 wks	60/group	Increased kidney weights and tubular degeneration.
Mally et al. (2006)	Eker rat (M)	Gavage (corn oil)	0–1,000 mg/kg-d body weight, 5 d/wk, 13 wks	10/group	Increased nephrotoxicity.
Maltoni et al. (1986)	Sprague-Dawley rats (M and F)	Gavage (olive oil)	0, 50, and 250 mg/kg-d 4–5 d/wk, 52 wks	30/group	Megakaryocytosis in male rats (details in Table 4.47).
NCI (1976)	**Osborne-Mendel rats (M and F) $B6C3F_1$ mice (M and F)**	**Gavage (corn oil)**	**0–2,339 mg/kg-d, variable doses, 5 d/wk, 78 wks**	**50/group**	**Toxic nephrosis in all exposed animals (details in Table 4.46).**
NTP (1988)	**ACI, August, Marshall, and Osborne-Mendel rats (M and F)**	**Gavage (corn oil)**	**0, 500, and 1,000 mg/kg-d, 5 d/wk, 103 wks**	**50/group**	**Cytomegaly and toxic nephropathy observed in all exposed rats (details in Table 4-48).**
NTP (1990)	**F344 rats (M and F) $B6C3F_1$ mice (M and F)**	**Gavage (corn oil)**	**Rats: 0–2,000 mg/kg-d, Mice: 0–6,000 mg/kg-d, 5d/wk, 13 wks**	**10/group**	**Cytomegaly and karyomegaly of renal tubular epithelium in mice and rats (details in Table 4-45).**
Peden-Adams et al. (2008)	MRL mice (M and F)	Drinking water	0; 1,400; and 14,000 ppb; lifetime	6/group	Increased kidney weight in male mice.

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

Kidney weight increases have been observed following inhalation exposure to TCE in both mice (Kjellstrand et al. (1983a) and rats (Woolhiser et al., 2006) and following lifetime drinking water exposure in a genetically-prone murine model (Peden-Adams et al., 2008). Kjellstrand et al. (1983a) demonstrated an increase in kidney weights in both male (20% compared to control) and female (10% compared to control) mice following intermittent and continuous TCE whole-body inhalation exposure (up to 120 days). This increase was significant in males as low as 75 ppm exposure and in females starting at 150 ppm. The latter inhalation study, an unpublished report by Woolhiser et al. (2006), was designed to examine immunotoxicity of TCE but also contains information regarding kidney weight increases in female Sprague-Dawley rats exposed to 0-, 100-, 300-, and 1,000-ppm TCE for 6 hours/day, 5 days/week, for 4 weeks. Relative kidney weights were significantly elevated (17.4% relative to controls) at 1,000 ppm. However, the small number of animals and the variation in initial animal weight limit the ability of this study to determine statistically significant increases. The Peden-Adams et al. (2008) study was designed to assess the effects of TCE exposure in a genetically-prone murine lupus model. Although the study did not demonstrate an increase in the development of autoimmune disease markers (for details, see Section 4.6.2), changes in body weight and organ weights in males were observed. Following lifetime exposure to TCE (14.000 ppb) in drinking water, males exhibited a decreasing trend in body mass of 12% from controls (female body weights not altered). Spleen, thymic, and kidney mass in females were not altered following exposure to TCE, while an 18% increase in kidney mass was observed in the high-dose treatment group (14.000 ppb) in males.

Similarly, overt signs of subchronic nephrotoxicity, such as changes in blood or urinary biomarkers, are also primarily a high-dose phenomenon, although histopathological changes are evident at lower exposures. Green et al. (1997a) reported administration of 2,000 mg/kg-day TCE by corn oil gavage for 42 days in F344 rats caused increases of around twofold of control results in urinary markers of nephrotoxicity such as urine volume and protein (both 1.8 ×), NAG (1.6 ×), glucose (2.2 ×) and alkaline phosphatase (ALP; 2.0 ×), similar to the results of the acute study of Chakrabarti and Tuchweber (1988), above. No morphological changes were observed in kidneys from any animals (Green et al., 1997a). At lower dose levels, Green et al. (1998) reported that plasma and urinary markers of nephrotoxicity were unchanged. In particular, after 1–28 day exposures to 250 or 500 ppm TCE for 6 hours/day, there were no statistically significant differences in plasma levels of BUN or in urinary levels of creatinine, protein, ALP, NAG, or GGT. However, increased urinary excretion of formic acid, accompanied by changes in urinary pH and increased ammonia, was found at these exposures. Interestingly, at the same exposure level of 500 ppm (6 hours/day, 5 days/week, for 6 months), Mensing et al. (2002) reported elevated excretion of low molecular weight proteins and NAG, biomarkers of nephrotoxicity, but after the longer exposure duration of 6 months.

Numerous studies have reported histological changes from TCE exposure for subchronic and chronic durations (Mensing et al., 2002; NTP, 1990; Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986). As summarized in Table 4-45, in 13-week studies in F344 rats and B6C3F$_1$ mice, NTP (1990) reported relatively mild cytomegaly and karyomegaly of the renal tubular epithelial cells at the doses 1,000–6,000 mg/kg-day (at the other doses, tissues were not examined). The NTP report noted that "these renal effects were so minimal that they were diagnosed only during a reevaluation of the tissues... prompted by the production of definite renal toxicity in the 2-year study." In the 6-month, 500-ppm inhalation exposure experiments of Mensing et al. (2002), some histological changes were noted in the glomeruli and tubuli of exposed rats, but they provided no detailed descriptions beyond the statement that "perivascular, interstitial infections and glomerulonephritis could well be detected in kidneys of exposed rats."

Table 4-45. Summary of renal toxicity and tumor findings in gavage studies of TCE by NTP (1990)[a]

Sex	Dose (mg/kg)[b]	Cytomegaly and karyomegaly incidence (severity[c])	Adenoma (overall; terminal)	Adenocarcinoma (overall; terminal)
1/d, 5 d/wk, 13-wk study, F344/N rats				
Male	0, 125, 250, 500, 100	Tissues not evaluated	None reported	
	2,000	8/9 (minimal/mild)		
Female	0, 62.5, 125, 250, 500	Tissues not evaluated		
	1,000	5/10 (equivocal/minimal)		
1/d, 5 d/wk, 13-wk study, B6C3F$_1$ mice				
Male	0, 375, 750, 1,500	Tissues not evaluated	None reported	
	3,000	7/10[d] (mild/moderate)		
	6,000	–[e]		
Female	0, 375, 750, 1,500	Tissues not evaluated		
	3,000	9/10 (mild/moderate)		
	6,000	1/10 (mild/moderate)		
1/d, 5 d/wk, 103-wk study, F344/N rats				
Male	0	0% (0)	0/48; 0/33	0/48; 0/33
	500	98% (2.8)	2/49; 0/20	0/49; 0/20
	1,000	98% (3.1)	0/49; 0/16	3/49; 3/16[f]
Female	0	0% (0)	0/50; 0/37	0/50; 0/37
	500	100% (1.9)	0/49; 0/33	0/49; 0/33
	1,000	100% (2.7)	0/48; 0/26	1/48; 1/26
1/d, 5 d/wk, 103-wk study, B6C3F$_1$ mice				
Male	0	0% (0)	1/49; 1/33	0/49; 0/33
	1,000	90% (1.5)	0/50; 0/16	1/50; 0/16
Female	0	0% (0)	0/48; 0/32	0/48; 0/32
	1,000	98% (1.8)	0/49; 0/23	0/49; 0/23

[a]Study carried forward for consideration in dose-response assessment (see Chapter 5).
[b]Corn oil vehicle.
[c]Numerical scores reflect the average grade of the lesion in each group (1, slight; 2, moderate; 3, well marked; and 4, severe).
[d]Observed in four mice that died after 7–13 weeks and in three that survived the study.
[e]All mice died during the first week.
[f]$p = 0.028$.

After 1–2 years of chronic TCE exposure by gavage (NTP, 1990, 1988; NCI, 1976) or inhalation (Maltoni et al., 1988; Maltoni et al., 1986) (see Tables 4-45 to 4-49), both the incidence and severity of these effects increases, with mice and rats exhibiting lesions in the tubular epithelial cells of the inner renal cortex that are characterized by cytomegaly, karyomegaly, and toxic nephrosis. As with the studies at shorter duration, these chronic studies reported cytomegaly and karyomegaly of tubular cells. NTP (1990) specified the area of damage as the pars recta, located in the corticomedullary region. It is important to note that these effects are distinct from the chronic nephropathy and inflammation observed in control mice and rats (Lash et al., 2000b; Maltoni et al., 1988; Maltoni et al., 1986; NCI, 1976).

Table 4-46. Summary of renal toxicity and tumor findings in gavage studies of TCE by NCI (1976)[a]

Sex	Dose (mg/kg)[b]	Toxic nephrosis (overall; terminal)	Adenoma or adenocarcinoma (overall; terminal)[c]
1/d, 5 d/wk, 2-yr study, Osborne-Mendel rats			
Males	0	0/20; 0/2	0/20; 0/2
	549	46/50; 7/7	1/50;[d] 0/7
	1,097	46/50; 3/3	0/50; 0/3
Females	0	0/20; 0/8	0/20; 0/8
	549	39/48; 12/12	0/48; 0/12
	1,097	48/50; 13/13	0/50; 0/13
1/d, 5 d/wk, 2-yr study, B6C3F$_1$ mice			
Males	0	0/20; 0/8	0/20; 0/8
	1,169	48/50; 35/35	0/50; 0/35
	2,339	45/50; 20/20	1/50;[e] 1/20
Females	0	0/20; 0/17	0/20; 0/17
	869	46/50; 40/40	0/50; 0/40
	1,739	46/47;[f] 39/39	0/47; 0/39

[a]Study carried forward for consideration in dose-response assessment (see Chapter 5).
[b]Treatment period was 48 weeks for rats, 66 weeks for mice. Doses were changed several times during the study based on monitoring of body weight changes and survival. Dose listed here is the TWA dose over the days on which animals received a dose.
[c]A few malignant mixed tumors and hamartomas of the kidney were observed in control and low-dose male rats, but are not counted here.
[d]Tubular adenocarcinoma.
[e]Tubular adenoma.
[f]One mouse was reported with "nephrosis," but not "nephrosis toxic," and so was not counted here.

Table 4-47. Summary of renal toxicity findings in gavage studies of TCE by Maltoni et al. (1988; 1986)

Sex	Dose (mg/kg)[a]	Megalonucleocytosis[b] (overall; corrected[c])
1/d, 4–5 d/wk, 52-wk exposure, observed for lifespan, Sprague-Dawley rats		
Males	0	0/20; 0/22
	50	0/30; 0/24
	250	14/30; 14/21
Females	0	0/30; 0/30
	50	0/30; 0/29
	250	0/30; 0/26

[a]Olive oil vehicle.
[b]Renal tubuli megalonucleocytosis is the same as cytomegaly and karyomegaly of renal tubuli cells (Maltoni et al., 1988; Maltoni et al., 1986).
[c]Denominator for "corrected" incidences is the number of animals alive at the time of the first kidney lesion in this experiment (39 weeks).

Table 4-48. Summary of renal toxicity and tumor incidence in gavage studies of TCE by NTP (1988)[a]

Sex	Dose (mg/kg)[b]	Cytomegaly	Toxic nephropathy	Adenoma (overall; terminal)	Adenocarcinoma (overall; terminal)
1/d, 5 d/wk, 2-yr study, ACI rats					
Male	0	0/50	0/50	0/50; 0/38	0/50; 0/38
	500	40/49	18/49	0/49; 0/19	1/49; 0/19
	1,000	48/49	18/49	0/49; 0/11	0/49; 0/11
Female	0	0/48	0/48	0/48; 0/34	0/48; 0/34
	500	43/47	21/47	2/47; 1/20	1/47; 1/20
	1,000	42/43	19/43	0/43; 0/19	1/43; 0/19
1/d, 5 d/wk, 2-yr study, August rats					
Male	0	0/50	0/50	0/50; 0/21	0/50; 0/21
	500	46/50	10/50	1/50; 0/13	1/50; 1/13
	1,000	46/49	31/49	1/49; 1/16	0/49; 0/16
Female	0	0/49	0/49	1/49; 1/23	0/49; 0/23
	500	46/48	8/48	2/48; 1/26	2/48; 2/26
	1,000	50/50	29/50	0/50; 0/25	0/50; 0/25
1/d, 5 d/wk, 2-yr study, Marshall rats					
Male	0	0/49	0/49	0/49; 0/26	0/49; 0/26
	500	48/50	18/50	1/50; 0/12	0/50; 0/12
	1,000	47/47	23/47	0/47; 0/6	1/47; 0/6
Female	0	0/50	0/50	1/50; 0/30	0/50; 0/30
	500	46/48	30/48	1/48; 1/12	1/48; 0/12
	1,000	43/44	30/44	0/44; 0/10	1/44; 1/10
1/d, 5 d/wk, 2-yr study, Osborne-Mendel rats					
Male	0	0/50	0/50	0/50; 0/22	0/50; 0/22
	500	48/50	39/50	6/50; 5/17	0/50; 0/17
	1,000	49/50	35/50	1/50; 1/15	1/50; 0/15
Female	0	0/50	0/50	0/50; 0/20	0/50; 0/20
	500	48/50	30/50	0/50; 0/11	0/50; 0/11
	1,000	49/49	39/49	1/49; 0/7	0/49; 0/7

[a]Study carried forward for consideration in dose-response assessment (see Chapter 5).
[b]Corn oil vehicle.

Table 4-49. Summary of renal toxicity and tumor findings in inhalation studies of TCE by Maltoni et al. (1988; 1986)[a]

Sex	Concentration (ppm)	Meganucleocytosis[b] (overall; corrected)	Adenoma (overall; corrected)	Adenocarcinoma (overall; corrected)
7 hrs/d, 5 d/wk, 2-yr exposure, observed for lifespan, Sprague-Dawley rats[c]				
Male	0	0/135; 0/122	0/135; 0/122	0/135; 0/122
	100	0/130; 0/121	1/130; 1/121	0/130; 0/121
	300	22/130; 22/116	0/130; 0/116	0/130; 0/116
	600	101/130; 101/124	1/130; 1/124	4/130; 4/124
Female	0	0/145; 0/141	0/145; 0/141	0/145; 0/141
	100	0/130; 0/128	1/130; 1/128	0/130; 0/128
	300	0/130; 0/127	0/130; 0/127	0/130; 0/127
	600	0/130; 0/127	0/130; 0/127	1/130; 1/127
7 hrs/d, 5 d/wk, 78-wk exposure, observed for lifespan, $B6C3F_1$ mice[d]				
Male	0	0/90	0/90	0/90
	100	0/90	0/90	1/90
	300	0/90	0/90	0/90
	600	0/90	0/90	0/90
Female	0	0/90	0/90	1/90
	100	0/90	0/90	0/90
	300	0/90	0/90	0/90
	600	0/90	0/90	0/90

[a]Study carried forward for consideration in dose-response assessment (see Chapter 5); three inhalation experiments in this study found no renal megalonucleocytosis, adenomas, or adenocarcinomas: BT302 (8-week exposure to 0, 100, or 600 ppm in Sprague-Dawley rats); BT303 (8-week exposure to 0, 100, or 600 ppm in Swiss mice); and BT305 (78-week exposure to 0, 100, 300, or 600 ppm in Swiss mice).
[b]Renal tubuli meganucleocytosis is the same as cytomegaly and karyomegaly of renal tubuli cells (Maltoni et al., 1988; Maltoni et al., 1986).
[c]Combined incidences from experiments BT304 and BT304bis. Corrected incidences reflect number of rats alive at 47 weeks, when the first renal tubular megalonucleocytosis in these experiments appeared.
[d]Female incidences are from experiment BT306, while male incidences are from experiment BT306bis, which was added to the study because of high, early mortality due to aggressiveness and fighting in males in experiment BT306. Corrected incidences not show, because only the renal adenocarcinomas appeared at 107 weeks in the male and 136 weeks in the female, when the most of the mice were already deceased.

These effects of TCE on the kidney appear to be progressive. Maltoni et al. (1988; 1986) noted that the incidence and degree of renal toxicity increased with increased exposure time and increased time from the start of treatment. As mentioned above, signs of toxicity were present in the 13 week study (NTP, 1988), and NTP (1990) noted cytomegaly at 26 weeks. NTP (1990) noted that as —exposure time increased, affected tubular cells continued to enlarge and additional tubules and tubular cells were affected," with toxicity extending to the cortical area as kidneys became more extensively damaged. NTP (1990, 1988) noted additional lesions that increased in frequency and severity with longer exposure, such as dilation of tubules and loss of tubular cells lining the basement membrane (—striped appearance" (NTP, 1988) or flattening of these cells

(NTP, 1990)). NTP (1990) also commented on the intratubular material and noted that the tubules were empty or "contained wisps of eosinophilic material."

With gavage exposure, these lesions were present in both mice and rats of both sexes, but were on average more severe in rats than in mice, and in male rats than in female rats (NTP, 1990). Thus, it appears that male rats are most sensitive to these effects, followed by female rats and then mice. This is consistent with the experiments of Maltoni et al. (1988; 1986), which only reported these effects in male rats. The limited response in female rats or mice of either sex in these experiments may be related to dose or strain. The lowest chronic gavage doses in the National Cancer Institute (NCI, 1976) and NTP (1990, 1988) F344 rat experiments was 500 mg/kg-day, and in all of these cases, at least 80% (and frequently 100%) of the animals showed cytomegaly or related toxicity. By comparison, the highest gavage dose in the Maltoni et al. (1988; 1986) experiments (250 mg/kg-day) showed lower incidences of renal cytomegaly and karyomegaly in male Sprague-Dawley rats (47 and 67%, overall and corrected incidences) and none in female rats. The $B6C3F_1$ mouse strain was used in the NCI (1976), NTP (1990), and Maltoni et al. (1988; 1986) studies (see Tables 4-45–4-49). While the two gavage studies (NTP, 1990; NCI, 1976) were consistent, reporting at least 90% incidence of cytomegaly and karyomegaly at all studied doses, whether dose accounts for the lack of kidney effects in Maltoni et al. (1988; 1986) requires comparing inhalation and gavage dosing. Such comparisons depend substantially on the internal dose-metric, so conclusions as to whether dose can explain differences across studies cannot be addressed without dose-response analysis using PBPK modeling. Some minor differences were found in the multistrain NTP study (1988), but the high rate of response makes distinguishing among them difficult. Soffritti (personal communication with JC Caldwell, February 14, 2006) did note that the colony from which the rats in Maltoni et al. (1988; 1986) experiments were derived had historically low incidences of chronic progressive nephropathy and renal cancer.

4.4.5. Kidney Cancer in Laboratory Animals

4.4.5.1. Inhalation Studies of TCE

A limited number of inhalation studies examined the carcinogenicity of TCE, with no statistically-significantly increases in kidney tumor incidence reported in mice or hamsters (Maltoni et al., 1988; 1986; Fukuda et al., 1983; Henschler et al., 1980). The cancer bioassay by Maltoni et al. (1988; 1986) reported no statistically significant increase in kidney tumors in mice or hamsters, but renal adenocarcinomas were found in male (4/130) and female (1/130) rats at the high dose (600 ppm) after 2 years of exposure and observation at natural death. In males, these tumors seemed to have originated in the tubular cells, and were reported to have never been observed in over 50,000 Sprague-Dawley rats (untreated, vehicle-treated, or treated with different chemicals) examined in previous experiments in the same laboratory (Maltoni et al., 1986). The renal adenocarcinoma in the female rat was cortical and reported to be similar to that

seen infrequently in historical controls. This study also demonstrated the appearance of increased cytokaryomegaly or megalonucleocytosis in the tubular cells, a lesion that was significantly and dose-dependently increased in male rats only (see Table 4-49). Maltoni et al. (1986) noted that some considerations supported either the hypothesis that these were precursor lesions of renal adenocarcinomas cancer or the hypothesis that these are not precursors but rather the morphological expression of TCE-induced regressive changes. The inhalation studies by Fukuda et al. (1983) in Sprague-Dawley rats and female ICR mice reported one clear cell carcinoma in rats exposed to the highest concentration (450 ppm) but saw no increase in kidney tumors in mice. This result was not statistically significant (see Table 4-50) and no details are given about the specific location of the tumors. One negative study (Henschler et al., 1980) tested NMRI mice, Wistar rats, and Syrian hamsters of both sexes (60 animals per strain), and observed no significant increase in renal tubule tumors any of the species tested. Benign adenomas were observed in male mice and rats, a single adenocarcinoma was reported in male rats at the highest dose, and no renal adenocarcinomas were reported in females of either species (see Table 4-50). RCCs appear to be very rare in Wistar rats, with historical control rates reported to be about 0.4% in males and 0.2% in females (Poteracki and Walsh, 1998), so these data are very limited in power to detect small increases in their incidence.

Table 4-50. Summary of renal tumor findings in inhalation studies of TCE by Henschler et al. (1980)[a] and Fukuda et al. (1983)[b]

Sex	Concentration (ppm)	Adenomas	Adenocarcinomas
6 hrs/d, 5 d/wk, 18-mo exposure, 30-mo observation, Han:NMRI mice (Henschler et al., 1980)			
Males	0	4/30	1/30
	100	1/29	0/30
	500	1/29	0/30
Females	0	0/29	0/29
	100	0/30	0/30
	500	0/28	0/28
6 hrs/d, 5 d/wk, 18-mo exposure, 36-mo observation, Han:WIST rats (Henschler et al., 1980)			
Males	0	2/29	0/29
	100	1/30	0/30
	500	2/30	1/30
Females	0	0/28	0/28
	100	0/30	0/30
	500	1/30	0/30
7 hrs/d, 5 d/wk, 2-yr study, Crj:CD (Sprague-Dawley) rats (Fukuda et al., 1983)			
Females	0	0/50	0/50
	50	0/50	0/50
	150	0/47	0/47
	450	0/51	1/50

[a]Henschler et al. (1980) observed no renal tumors in control or exposed Syrian hamsters.
[b]Fukuda et al. (1983) observed no renal tumors in control or exposed Crj:CD-1 (ICR) mice.

4.4.5.2. Gavage and Drinking Water Studies of TCE

Several chronic gavage studies exposing multiple strains of rats and mice to 0–3,000 mg/kg TCE for at least 52 weeks have been conducted (see Tables 4-45 to 4-48, 4-51) (Maltoni et al., 1988; Maltoni et al., 1986, NCI, 1976; NTP, 1988, 1990; Henschler et al., 1984; Van Duuren et al., 1979). Van Duuren et al. (1979) examined TCE and 14 other halogenated compounds for carcinogenicity in both sexes of Swiss mice. While no excess tumors were observed, the dose rate (0.5 mg once per week, or an average dose rate of approximately 2.4 mg/kg-day for a 30 g mouse) is about 400-fold lower than that in the other gavage studies. Inadequate design and reporting of this study limit the ability to use the results as an indicator of TCE carcinogenicity. In the NCI (1976) study, the results for Osborne-Mendel rats were considered by the authors to be inconclusive due to significant early mortality. Two male rats demonstrated kidney lesions (dilated renal pelvis and dark red renal medulla), but in rats of both sexes, no increase was seen in primary tumor induction over that observed in controls. While both sexes of $B6C3F_1$ mice showed a compound-related increase in nephropathy, no increase in tumors over controls was observed. The NCI study (1976) used technical-grade TCE that contained two known carcinogenic compounds as stabilizers (epichlorohydrin and 1,2-epoxybutane). However, a subsequent study by Henschler et al. (1984) in mice reported no significant differences in systemic tumorigenesis between pure, industrial, and stabilized TCE, suggesting that concentrations of these stabilizers are too low to be the cause of tumors. A later gavage study by NTP (1988), using TCE stabilized with diisopropylamine, observed an increased incidence of renal tumors in all four strains of rats (ACI, August, Marshall, and Osborne-Mendel). All animals exposed for up to 2 years (rats and mice) had non-neoplastic kidney lesions (tubular cell cytomegaly), even if they did not later develop kidney cancer (see Table 4-48). This study was also considered inadequate by the authors because of chemically induced toxicity, reduced survival, and incomplete documentation of experimental data. The final NTP study (1990) in male and female F344 rats and $B6C3F_1$ mice used epichlorohydrin-free TCE. Only in the highest-dose group (1,000 mg/kg) of male F344 rats was renal carcinoma statistically significant increased. The results for detecting a carcinogenic response in rats were considered by the authors to be equivocal because both groups receiving TCE showed significantly reduced survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the high-dose group) of death by gavage error. However, historical control incidences at NTP of kidney tumors in F344 rats is very low,[5] lending biological significance to their occurrence in this study, despite the study's limitations. Cytomegaly and karyomegaly were also increased, particularly in male rats. The toxic nephropathy (specific location in kidney

[5]NTP (1990) reported a historical control incidence of 0.4% in males. The NTP web site reports historical control rates of renal carcinomas for rats dosed via corn oil gavage on the NIH-07 diet (used before 1995, when the TCE studies were conducted) to be 0.5% (2/400) for males and 0% (0/400) for females (http://ntp-server.niehs.nih.gov/ntp/research/database_searches/historical_controls/path/r_gavco.txt). In addition, the two occurences in males came from the same study, with all other studies reporting 0/50 carcinomas.

not stated) observed in both rats and mice and contributed to the poor survival rate (see Table 4-45). As discussed previously, this toxic nephropathy was clearly distinguishable from the spontaneous chronic progression nephropathy commonly observed in aged rats.

Table 4-51. Summary of renal tumor findings in gavage studies of TCE by Henschler et al. (1984)[a] and Van Duuren et al. (1979)[b]

Sex (TCE dose)	Control or TCE exposed (stabilizers if present)	Adenomas	Adenocarcinomas
5 d/wk, 18-mo exposure, 24-mo observation, Swiss mice (Henschler et al., 1984)			
Males (2.4g/kg body weight)	Control (none)	1/50	1/50
	TCE (triethanolamine)	1/50	1/50
	TCE (industrial)	0/50	0/50
	TCE (epichlorohydrin (0.8%))	0/50	0/50
	TCE (1,2-epoxybutane (0.8%))	2/50	2/50
	TCE (both epichlorohydrin (0.25%) and 1,2-epoxybutane (0.25%))	0/50	0/50
Females (1.8 g/kg body weight)	Control (none)	0/50	1/50
	TCE (triethanolamine)	4/50	0/50
	TCE (industrial)	0/50	0/50
	TCE (epichlorohydrin (0.8%))	0/50	0/50
	TCE (1,2-epoxybutane (0.8%))	0/50	0/50
	TCE (both epichlorohydrin (0.25%) and 1,2-epoxybutane (0.25%))	0/50	0/50
1 d/wk, 89-wk exposure, Swiss rats (Van Duuren et al., 1979)			
Males (0.5mg)	Control	0/30	0/30
	TCE (unknown)	0/30	0/30
Females (0.5mg)	Control	0/30	0/30
	TCE(unknown)	0/30	0/30

[a]Henschler et al. (1984). Due to poor condition of the animals resulting from the nonspecific toxicity of high doses of TCE and/or the additives, gavage was stopped for all groups during weeks 35–40, 65, and 69–78, and all doses were reduced by a factor of 2 from the 40th week on.
[b]Van Duuren et al. (1979) observed no renal tumors in control or exposed Swiss mice.

4.4.5.3. Conclusions: Kidney Cancer in Laboratory Animals

Chronic TCE carcinogenicity bioassays have shown evidence of neoplastic lesions in the kidney in rats (mainly in males, with less evidence in females), treated via inhalation and gavage. As discussed above, individual studies have a number of limitations and have shown limited increases in kidney tumors. However, given the rarity of these tumors as assessed by historical controls and the repeatability of this result, these are considered biologically significant.

4.4.6. Role of Metabolism in TCE Kidney Toxicity

It is generally thought that one or more TCE metabolites rather than the parent compound are the active moieties for TCE nephrotoxicity. As reviewed in Section 3.3, oxidation by CYPs,

of which CYP2EI is thought to be the most active isoform, results in the production of CH, TCA, DCA, and TCOH. The GSH conjugation pathway produces metabolites such as DCVG, DCVC, dichlorovinylthiol, and NAcDCVC, although, as discussed in Section 3.3.3.2, the quantitative estimates of the amount systemically produced following TCE exposure remains uncertain. Because several of the steps for generating these reactive metabolites occur in the kidney, the GSH conjugation pathway has been thought to be responsible for producing the active moiety or moieties of TCE nephrotoxicity. A comparison of TCE's nephrotoxic effects with the effects of TCE metabolites, both in vivo and in vitro, thus provides a basis for assessing the relative roles of different metabolites. While most of the available data have been on metabolites from GSH conjugation, such as DCVC, limited information is also available on the major oxidative metabolites, TCOH and TCA.

4.4.6.1. In Vivo Studies of the Kidney Toxicity of TCE Metabolites

Studies of kidney toxicity of TCE metabolites discussed in this section are shown in Table 4-52.

4.4.6.1.1. Role of GSH conjugation metabolites of TCE

In numerous studies, DCVC has been shown to be acutely nephrotoxic in rats and mice. Mice receiving a single dose of 1 mg/kg DCVC (the lowest dose tested in this species) exhibited karyolytic proximal tubular cells in the outer stripe of the outer medulla, with some sloughing of cells into the lumen and moderate desquamation of the tubular epithelium (Eyre et al., 1995a). Higher doses in mice were associated with more severe histological changes similar to those induced by TCE, such as desquamation and necrosis of the tubular epithelium (Vaidya et al., 2003a, b; Darnerud et al., 1989; Terracini and Parker, 1965). In rats, no histological changes in the kidney were reported after single doses of 1, 5, and 10 mg/kg DCVC (Green et al., 1997a; Eyre et al., 1995b, a), but cellular debris in the tubular lumen was reported at 25 mg/kg (Eyre et al., 1995a) and slight degeneration and necrosis were seen at 50 mg/kg (Green et al., 1997a). Green et al. (1997a) reported no histological changes were noted in rats after 10 doses of 0.1–5.0 mg/kg DCVC (although increases in urinary protein and GGT were found), but some karyomegaly was noted in mice after 10 daily doses of 1 mg/kg. Therefore, mice appear more sensitive than rats to the nephrotoxic effects of acute exposure to DCVC, although the number of animals used at each dose in these studies was limited (10 or less). Although the data are not sufficient to assess the relatively sensitivity of other species, it is clear that multiple species, including rabbits, guinea pigs, cats, and dogs, are responsive to DCVC's acute nephrotoxic effects (Krejci et al., 1991; Wolfgang et al., 1989a; Jaffe et al., 1984; Terracini and Parker, 1965).

Table 4-52. Laboratory animal studies of kidney noncancer toxicity of TCE metabolites

Reference	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Kidney effect(s) discussed in Section 4.4.4
Dow and Green (2000)	F344 rats (M)	Drinking water	0, 0.5, 1 g/L TCOH, 12 wks	3/group	Increased formic acid in urine.
Jaffe et al. (1984)	Swiss-Webster mice (M)	Drinking water	0–22 mg/kg-d DCVC, 37 wks	5/group	Cytomegaly and tubular degeneration.
Mather et al. (1990)	Sprague-Dawley rats (M)	Drinking water	0–355 mg/kg-d TCA, 90 d	10/group	Increased kidney weight.
Terracini and Parker (1965)	Wistar rats (Gender not specified) Grey mice (Gender not specified)	Drinking water	0, 0.01% DCVC, 12 wks	35/group	Necrosis of tubular epithelium in mice and rats.

Very few studies are available at longer durations. Terracini and Parker (1965) gave DCVC in drinking water to rats at a concentration of 0.01% for 12 weeks (approximately 10 mg/kg-day), and reported consistent pathological and histological changes in the kidney. The progression of these effects was as follows: (1) during the first few days, completely necrotic tubules, with isolated pyknotic cells being shed into the lumen; (2) after 1 week, dilated tubules in the inner part of the cortex, lined with flat epithelial cells that showed thick basal membranes, some with big hyperchromatic nuclei; and (3) in the following weeks, increased prominence of tubular cells exhibiting karyomegaly, seen in almost all animals, less pronounced tubular dilation, and cytomegaly in the same cells showing karyomegaly. In addition, increased mitotic activity was reported the first few days, but was not evident for the rest of the experiment. Terracini and Parker (1965) also reported the results of a small experiment (13 male and 5 female rats) given the same concentration of DCVC in drinking water for 46 weeks, and observed for 87 weeks. They noted renal tubular cells exhibiting karyomegaly and cytomegaly consistently throughout the experiment. Moreover, a further group of eight female rats given DCVC in drinking water at a concentration of 0.001% (approximately 1 mg/kg-day) also exhibited similar, though less severe, changes in the renal tubules. In mice, Jaffe et al. (1984) gave DCVC in drinking water at concentrations of 0.001, 0.005, and 0.01% (estimated daily doses of 1–2, 7–13, and 17–22 mg/kg-day), and reported similar effects in all dose groups, including cytomegaly, nuclear hyperchromatism, and multiple nucleoli, particularly in the pars recta section of the kidney. Thus, effects were noted in both mice and rats under chronic exposures at doses as low as 1–2 mg/kg-day (the lowest dose tested). Therefore, while limited, the available data do not suggest differences between mice and rats to the nephrotoxic effects of DCVC under chronic exposure conditions, in contrast to the greater sensitivity of mice to acute and subchronic DCVC-induced nephrotoxicity.

Importantly, as summarized in Table 4-53, the histological changes and their location in these subchronic and chronic experiments with DCVC are quite similar to those reported in chronic studies of TCE, described above, particularly the prominence of karyomegaly and cytomegaly in the pars recta section of the kidney. Moreover, the morphological changes in the tubular cells, such as flattening and dilation, are quite similar. Similar pathology is not observed with the oxidative metabolites alone (see Section 4.4.6.1.2).

Table 4-53. Summary of histological changes in renal proximal tubular cells induced by chronic exposure to TCE, DCVC, and TCOH

Effects	TCE	DCVC	TCOH
Karyomegaly	Enlarged, hyperchromatic nuclei, irregular to oblong in shape. Vesicular nuclei containing prominent nucleoli.	Enlarged, hyperchromatic nuclei with and multiple nucleoli. Nuclear pyknosis and karyorrhexis.	None reported.
Cytomegaly	Epithelial cells were large, elongated, and flattened.	Epithelial cells were large, elongated, and flattened cells.	No report of enlarged cells.
Cell necrosis/ hyperplasia	Stratified epithelium that partially or completely filled the tubular lumens. Cells in mitosis were variable in number or absent. Cells had abundant eosinophilic or basophilic cytoplasm.	Thinning of tubular epithelium, frank tubular necrosis, re-epitheliation. Tubular atrophy, interstitial fibrosis and destruction of renal parenchyma. More basophilic and finely vacuolated.	No flattening or loss of epithelium reported. Increased tubular cell basophilia, followed by increased cellular eosinophilia, tubular cell vacuolation.
Morphology/ content of tubules	Some tubules enlarged/dilated to the extent that they were difficult to identify. Portions of basement membrane had a stripped appearance. Tubules were empty or contained —wisps of eosinophilic material."	Tubular dilation, denuded tubules. Thick basal membrane. Focal areas of dysplasia, intraluminal casts.	No tubular dilation reported. Intratubular cast formation.

Sources: NCI (1976); NTP (1990, 1988); Maltoni et al. (1988; Maltoni et al., 1986); Terracini and Parker (1965); Jaffe et al. (1985); Green et al. (2003).

Additionally, it is important to consider whether sufficient DCVC may be formed from TCE exposure to account for TCE nephrotoxicity. While direct pharmacokinetic measurements, such as the excretion of NAcDCVC, have been used to argue that insufficient DCVC would be formed to be the active moiety for nephrotoxicity (Green et al., 1997a), as discussed in Chapter 3, urinary NAcDCVC is a poor marker of the flux through the GSH conjugation pathway because of the many other possible fates of metabolites in that pathway. In another approach, Eyre et al. (Eyre et al., 1995b), using acid-labile adducts as a common internal dosimeter between TCE and DCVC, reported that a single TCE dose of 400 mg/kg in rats (similar to the lowest daily doses in the NCI and NTP rat bioassays) and 1,000 mg/kg (similar to the lowest daily doses in the NCI and NTP mouse bioassays) corresponded to a single equivalent DCVC dose of 6 and 1 mg/kg-day in rats and mice, respectively. These equivalent doses of DCVC are greater or equal to those in which nephrotoxicity has been reported in these species under chronic conditions. Therefore, assuming that this dose correspondence is accurate under chronic conditions, sufficient DCVC would be formed from TCE exposure to explain the observed histological changes in the renal tubules. Nevertheless, direct estimates of how much DCVC is formed after TCE exposure are lacking.

The Eker rat model (*Tsc-2*$^{\pm}$) is at increased risk for the development of spontaneous RCC and as such, has been used to understand the mechanisms of renal carcinogenesis (Stemmer et al., 2007; Wolf et al., 2000). One study has demonstrated similar pathway activation in Eker rats as that seen in humans with *VHL* mutations leading to RCC, suggesting that *Tsc-2* inactivation is analogous to inactivation of *VHL* in human RCC (Liu et al., 2003). Although the Eker rat model is a useful tool for analyzing the progression of renal carcinogenesis, it has some limitations in analysis of specific genetic changes, particularly given the potential for different genetic changes depending on type of exposure and tumor. The results of short-term assays to genotoxic carcinogens in the Eker rat model (Stemmer et al., 2007; Morton et al., 2002) reported limited preneoplastic and neoplastic lesions, which may be related to the increased background rate of renal carcinomas in this animal model.

Recently, Mally et al. (2006) exposed male rats carrying the Eker mutation to TCE (0–1,000 mg/kg body weight) by corn oil gavage and demonstrated no increase in renal preneoplastic lesions or tumors. Primary Eker rat kidney cells exposed to DCVC in this study did induce an increase in transformants in vitro but no DCVC-induced *VHL* or *Tsc-2* mutations were observed. In vivo exposure to TCE (5 days/week for 13 weeks), decreased body weight gain and increased urinary excretion at the two highest TCE concentrations analyzed (500 and 1,000 mg/kg body weight) but did not change standard nephrotoxicity markers (GGT, creatinine, and urinary protein). Renal tubular epithelial cellular proliferation as measured by BrdU incorporation was demonstrated at the three highest concentrations of TCE (250, 500 and 1,000 mg/kg-day). A minority of these cells also showed karyomegaly at the two higher TCE concentrations. Although renal cortical tumors were demonstrated in all TCE exposed groups,

these were not significantly different from controls (13 weeks). These studies were complemented with in vitro studies of DCVC (10–50 μM) in rat kidney epithelial (RKE) cells examining proliferation at 8, 24, and 72 hours and cellular transformation at 6–7 weeks. Treatment of RKE cells from susceptible rats with DCVC gave rise to morphologically transformed colonies consistently higher than background (Mally et al., 2006). Analyzing 10 of the renal tumors from the TCE-exposed rats and 9 of the DCVC transformants from these studies for alterations to the *VHL* gene that might lead to inactivation found no alterations to *VHL* gene expression or mutations.

One paper has linked the *VHL* gene to chemical-induced carcinogenesis. Shiao et al. (1998) demonstrated *VHL* gene somatic mutations in *N*-nitrosodimethylamine-induced rat kidney cancers that were of the clear cell type. The clear cell phenotype is rare in rat kidney cancers, but it was only the clear cell cancers that showed *VHL* somatic mutation (three of eight tumors analyzed). This provided an additional link between *VHL* inactivation and clear cell kidney cancer. However, this study examined archived formalin-fixed, paraffin-embedded tissues from previous experiments. As described previously (see Section 4.4.3), DNA extraction from this type of preparation creates some technical issues. Similarly, archived formalin-fixed, paraffin-embedded tissues from rats exposed to potassium bromide were analyzed in a later study by Shiao et al. (2002). This later study examined the *VHL* gene mutations following exposure to potassium bromide, a rat renal carcinogen known to induce clear cell renal tumors. Clear cell renal tumors are the most common form of human renal epithelial neoplasms, but are extremely rare in animals. Although F344 rats exposed to potassium bromide in this study did develop renal clear cell carcinomas, only two of nine carried the same C to T mutation at the core region of the Sp1 transcription-factor binding motif in the *VHL* promoter region, and one of four untreated animals had a C to T mutation outside the conserved core region. Mutation in the *VHL* coding region was only detected in one tumor, so although the tumors developed following exposure to potassium bromide were morphologically similar to those found in humans, no similarities were found in the genetic changes.

Elfarra et al. (1984) found that both DCVG and DCVC administered to male F344 rats by i.p. injections in isotonic saline resulted in elevations in BUN and urinary glucose excretion. Furthermore, inhibition of renal GGT activity with acivicin-protected rats from DCVG-induced nephrotoxicity. In addition, both the β-lyase inhibitor, AOAA, and the renal organic anion transport inhibitor, probenecid, provided protection from DCVC, demonstrating a requirement for metabolism of DCVG to the cysteine conjugate by the action of renal GGT and dipeptidase, uptake into the renal cell by the organic anion transporter, and subsequent activation by the β-lyase. This conclusion was supported further by showing that the methyl analog of DCVC, which cannot undergo a β-elimination reaction due to the presence of the methyl group, was not nephrotoxic.

Korrapati et al. (2005) built upon a series of investigations of hetero- (by mercuric chloride [$HgCl_2$]) and homo-(by DCVC, 15 mg/kg) protection against a lethal dose of DCVC (75 mg/kg). Priming, or preconditioning, with pre-exposure to either $HgCl_2$ or DCVC of male Swiss-Webster mice was said to augment and sustain cell division and tissue repair, hence protecting against the subsequent lethal DCVC dose (Vaidya et al., 2003b, a; 2003c). Korrapati et al. (2005) showed that a lethal dose of DCVC downregulates phosphorylation of endogenous retinoblastoma protein (pRb), which is considered critical in renal proximal tubular and mesangial cells for the passage of cells from G1 to S-phase, thereby leading to a block of renal tubule repair. Priming, in contrast, upregulated P-pRB which was sustained even after the administration of a lethal dose of DCVC, thereby stimulating S-phase DNA synthesis, which was concluded to result in tissue repair and recovery from acute renal failure and death. These studies are more informative about the mechanism of autoprotection than on the mechanism of initial injury caused by DCVC. In addition, the priming injury (not innocuous, as it caused 25–50% necrosis and elevated BUN) may have influenced the toxicokinetics of the second DCVC injection.

4.4.6.1.2. Role of oxidative metabolites of TCE

Some investigators (Green et al., 2003; Dow and Green, 2000; Green et al., 1998) have proposed that TCE nephrotoxicity is related to formic acid formation. They demonstrated that exposure to either TCOH or TCA causes increased formation and urinary excretion of formic acid (Green et al., 1998). The formic acid does not come from TCE. Rather, TCE (or a metabolite) has been proposed to cause a functional depletion of vitamin B_{12}, which is required for the methionine salvage pathway of folate metabolism. Vitamin B_{12} depletion results in folate depletion. Folate is a cofactor in one-carbon metabolism and depletion of folate allows formic acid to accumulate, and then to be excreted in the urine (Dow and Green, 2000).

TCE (1 and 5 g/L), TCA (0.25, 0.5, and 1 g/L), and TCOH (0.5 and 1.0 g/L) exposure in male Fischer rats substantially increased excretion of formic acid in urine, an effect suggested as a possible explanation for TCE-induced renal toxicity in rats (Green et al., 1998). Green et al. (2003) reported tubular toxicity as a result of chronic (1 year) exposure to TCOH (0, 0.5, and 1.0 g/L). Although TCOH causes tubular degeneration in a similar region of the kidney as TCE, there are several dissimilarities between the characteristics of nephrotoxicity between the two compounds, as summarized in Table 4-53. In particular, Green et al. (1998) did not observe TCOH causing karyomegaly and cytomegaly. These effects were seen as early as 13 weeks after the commencement of TCE exposure (NTP, 1990), with 300 ppm inhalation exposures to TCE (Maltoni et al., 1988; Maltoni et al., 1986), as well as at very low chronic exposures to DCVC (Jaffe et al., 1984; Terracini and Parker, 1965). In addition, Green et al. (2003) reported neither flattening nor loss of the tubular epithelium nor hyperplasia, but suggested that the increased early basophilia was due to newly divided cells, and therefore, represented tubular regeneration

in response to damage. Furthermore, they noted that such changes were seen with the spontaneous damage that occurs in aging rats. However, several of the chronic studies of TCE noted that the TCE-induced damage observed was distinct from the spontaneous nephropathy observed in rats. A recent in vitro study of rat hepatocytes and primary human renal proximal tubule cells from two donors measured formic acid production following exposure to CH (0.3–3 mM, 3–10 days) (Lock et al., 2007). This study observed increased formic acid production at day 10 in both human renal proximal tubule cell strains, but a similar level of formic acid was measured when CH was added to media alone. The results of this study are limited by the use of only two primary human cell strains, but suggest that exposure to CH does not lead to significant increases in formic acid production in vivo.

Interestingly, it appears that the amount of formic acid excreted reaches a plateau at a relatively low dose. Green et al. (2003) added folic acid to the drinking water of the group of rats receiving the lower dose of TCOH (18.3 mg/kg-day) in order to modulate the excretion of formic acid in that dose group, and retain the dose-response in formic acid excretion relative to the higher-dose group (54.3 mg/kg-day). These doses of TCOH are much lower than what would be expected to be formed in vivo at chronic gavage doses. For instance, after a single 500-mg/kg dose of TCE (the lower daily dose in the NTP rat chronic bioassays), Green and Prout (1985) reported excretion of about 41% of the TCE gavage dose in urine as TCOH or TCOG in 24 hours. Thus, using the measure of additional excretion after 24 hours and the TCOH converted to TCA as a lower bound as to the amount of TCOH formed by a single 500 mg/kg dose of TCE, the amount of TCOH would be about 205 mg/kg, almost fourfold greater than the high dose in the Green et al. (2003) study. By contrast, these TCOH doses are somewhat smaller than those expected from the inhalation exposures of TCE. For instance, after 6-hour exposures to 100 and 500 ppm TCE (similar to the daily inhalation exposures in Maltoni et al. (1988; 1986), male rats excreted 1.5 and 4.4 mg of TCOH over 48 hours, corresponding to 5 and 15 mg/kg for a rat weighing 0.3 kg (Kaneko et al., 1994). The higher equivalent TCOH dose is similar to the lower TCOH dose used in Green et al. (2003), so it is notable that while Maltoni et al. (1988; Maltoni et al., 1986) reported a substantial incidence of cytomegaly and karyomegaly after TCE exposure (300 and 600 ppm), none was reported in Green et al. (2003).

TCOH alone does not appear sufficient to explain the range of renal effects observed after TCE exposure, particularly cytomegaly, karyomegaly, and flattening and dilation of the tubular epithelium. However, given the studies described above, it is reasonable to conclude that TCOH may contribute to the nephrotoxicity of TCE, possibly due to excess formic acid production, because: (1) there are some similarities between the effects observed with TCE and TCOH and (2) the dose at which effects with TCOH are observed overlap with the approximate equivalent TCOH dose from TCE exposure in the chronic studies.

Dow and Green (2000) noted that TCA also induced formic acid accumulation in rats, and suggested that TCA may therefore, contribute to TCE-induced nephrotoxicity. However,

TCA has not been reported to cause any similar histologic changes in the kidney. Mather et al. (1990) reported an increase of kidney-weight to body-weight ratio in rats after 90 days of exposure to TCA in drinking water at 5,000 ppm (5 g/L) but reported no histopathologic changes in the kidney. DeAngelo et al. (1997) reported no effects of TCA on kidney weight or histopathology in rats in a 2-year cancer bioassay. Dow and Green (2000) administered TCA at quite high doses (1 and 5 g/L in drinking water), greater than the subsequent experiments of Green et al. (2003) with TCOH (0.5 and 1 g/L in drinking water), and reported similar amounts of formic acid produced (about 20 mg/day for each compound). However, cytotoxicity or karyomegaly did not appear to be analyzed. Furthermore, much more TCOH is formed from TCE exposure than TCA. Therefore, if TCA contributes substantially to the nephrotoxicity of TCE, its contribution would be substantially less than that of TCOH. Lock et al. (2007) also measured formic acid production in human renal proximal tubule cells exposed to 0.3–3 mM CH for 10 days CH. This study measured metabolism of CH to TCOH and TCA as well as formic acid production and subsequent cytotoxicity. Increased formic acid was not observed in this study, and limited cytotoxicity was observed. However, this study was performed in human renal proximal tubular cells from only two donors, and there is potential for large interindividual variability in response, particularly with CYP enzymes.

In order to determine the ability of various chlorinated hydrocarbons to induce peroxisomal enzymes, Goldsworthy and Popp (1987) exposed male F344 rats and male B6C3F$_1$ mice to TCE (1,000 mg/kg body weight) and TCA (500 mg/kg body weight) by corn oil gavage for 10 consecutive days. Peroxisomal activation was measured by palmitoyl coenzyme A (CoA) oxidase activity levels. TCE led to increased peroxisomal activation in the kidneys of both rats (300% of control) and mice (625% of control), while TCA led to an increase only in mice (280% of control). A study by Zanelli et al. (1996) exposed Sprague-Dawley rats to TCA for 4 days and measured both renal and hepatic peroxisomal and CYP enzyme activities. TCA-treated rats had increased activity in CYP 4A subfamily enzymes and peroxisomal palmitoyl-CoA oxidase. Both of these acute studies focused on enzyme activities and did not further analyze resulting histopathology.

4.4.6.2. In Vitro Studies of Kidney Toxicity of TCE and Metabolites

Generally, it is believed that TCE metabolites are responsible for the bulk of kidney toxicity observed following exposure. In particular, studies have demonstrated a role for DCVG and DCVC in kidney toxicity, though, as discussed in Section 3.3.3.2, the precise metabolic yield of these metabolites following TCE exposure remains uncertain. The work by Lash and colleagues (Cummings and Lash, 2000; Cummings et al., 2000a; Cummings et al., 2000b; Lash et al., 2000b) examined the effect of TCE and its metabolites in vitro. TCE and DCVC are toxic to primary cultures of rat proximal and distal tubular cells (Cummings et al., 2000c), while the TCE metabolites, DCVG and DCVC, have been demonstrated to be cytotoxic to rat and rabbit

kidney cells in vitro (Lash et al., 2001b; Lash et al., 2000b; Groves et al., 1993; Wolfgang et al., 1989b; Hassall et al., 1983). GSH-related enzyme activities were well maintained in the cells, whereas CYP activities were not. The enzyme activity response to DCVC was greater than the response to TCE; however, the proximal and distal tubule cells had similar responses even though the proximal tubule is the target in vivo. The authors attributed this to the fact that the proximal tubule is exposed before the distal tubule in vivo and to possible differences in uptake transporters. They did not address the extent to which transporters were maintained in the cultured cells.

In further studies, Lash et al. (2001b) assessed the toxicity of TCE and its metabolites, DCVC and DCVG, using in vitro techniques as compared to in vivo studies. Experiments using isolated cells were performed only with tissues from F344 rats, and lactate dehydrogenase (LDH) release was used as the measure of cellular toxicity. The effects were greater in males. DCVC and TCE had similar effects, but DCVG exhibited increased efficacy compared with TCE and DCVC.

In vitro mitochondrial toxicity was assessed in renal cells from both F344 rats and $B6C3F_1$ mice following exposure to both DCVC and DCVG (Lash et al., 2001b). Renal mitochondria from male rats and mice responded similarly; a greater effect was seen in cells from the female mice. These studies show DCVC to be slightly more toxic than TCE and DCVG, but species differences are not consistent with the effects observed in long-term bioassays. This suggests that in vitro data should be used with caution in risk assessment, being mindful that in vitro experiments do not account for in vivo pharmacokinetic and metabolic processes.

In LLC-PK1 cells, DCVC causes loss of mitochondrial membrane potential, mitochondrial swelling, release of cytochrome c, caspase activation, and apoptosis (Chen et al., 2001). Thus, DCVC is toxic to mitochondria, resulting in either apoptosis or necrosis. DCVC-induced apoptosis also has been reported in primary cultures of human proximal tubule cells (Lash et al., 2001a).

DCVC was further studied in human renal proximal tubule cells for alterations in gene expression patterns related to proposed modes of action in nephrotoxicity (Lock et al., 2006). In cells exposed to subtoxic levels of DCVC to better mimic workplace exposures, the expression of genes involved with apoptosis (caspase 8, FADD-like regulator) was increased at the higher dose (1 μM) but not at the lower dose (0.1 μM) of DCVC exposure. Genes related to oxidative stress response (SOD, NF-κB, p53, c-Jun) were altered at both subtoxic doses, with genes generally upregulated at 0.1 μM DCVC being downregulated at 1 μM DCVC. The results of this study support the need for further study, and highlight the involvement of multiple pathways and variability of response based on different concentrations.

Lash et al. (2007) examined the effect of modulation of renal metabolism on toxicity of TCE in isolated rat cells and microsomes from kidney and liver. Following exposure to

modulating chemicals, LDH was measured as a marker of cytotoxicity, and the presence of specific metabolites was documented (DCVG, TCA, TCOH, and CH). Inhibition of the CYP stimulated an increase of GSH conjugation of TCE and increased cytotoxicity in kidney cells. This modulation of CYP had a greater effect on TCE-induced cytotoxicity in liver cells than in kidney cells. Increases in GSH concentrations in the kidney cells led to increased cytotoxicity following exposure to TCE. Depletion of GSH in hepatocytes exposed to TCE, however, led to an increase in hepatic cytotoxicity. The results of this study highlight the role of different bioactivation pathways needed in both the kidney and the liver, with the kidney effects being more affected by the GSH conjugation pathways metabolic products.

In addition to the higher susceptibility of male rats to TCE-induced nephrocarcinogenicity and nephrotoxicity, isolated renal cortical cells from male F344 rats are more susceptible to acute cytotoxicity from TCE than cells from female rats. TCE caused a modest increase in LDH release from male rat kidney cells but had no significant effect on LDH release from female rat kidney cells. These results on male susceptibility to TCE agree with the in vivo data.

4.4.6.3. Conclusions as to the Active Agents of TCE-Induced Nephrotoxicity

In summary, the TCE metabolites, DCVC, TCOH, and TCA, have all been proposed as possible contributors to the nephrotoxicity of TCE. Both in vivo and in vitro data strongly support the conclusion that DCVC and related GSH conjugation metabolites are the active agents of TCE-induced nephrotoxicity. Of these, DCVC induces effects in renal tissues, both in vivo and in vitro, that are most similar to those of TCE, and formed in sufficient amounts after TCE exposure to account for those effects. A role for formic acid due to TCOH or TCA formation from TCE cannot be ruled out, as it is known that substantial TCOH and TCA are formed from TCE exposure, that formic acid is produced from all three compounds, and that TCOH exposure leads to toxicity in the renal tubules. However, the characteristics of TCOH-induced nephrotoxicity do not account for the range of effects observed after TCE exposure, while those of DCVC-induced nephrotoxicity do. Also, TCOH does not induce the same pathology as TCE or DCVC. TCA has also been demonstrated to induce peroxisomal proliferation in the kidney (Goldsworthy and Popp, 1987), but this has not been associated with kidney cancer. Therefore, although TCOH and possibly TCA may contribute to TCE-induced nephrotoxicity, their contribution is likely to be small compared to that of DCVC. However, as discussed in Section 3.3.3.2, the precise metabolic yield of these DCVC following TCE exposure remains uncertain.

4.4.7. Mode(s) of Action for Kidney Carcinogenicity

This section will discuss the evidentiary support for several hypothesized modes of action for kidney carcinogenicity, including mutagenicity, cytotoxicity and regenerative proliferation,

peroxisome proliferation, α2μ-related nephropathy, and formic acid-related nephropathy, following the framework outlined in the *Cancer Guidelines* (U.S. EPA, 2005b).[6] The data and conclusions for the modes of action with the greatest experimental support are summarized in Table 4-54.

4.4.7.1. Hypothesized Mode of Action: Mutagenicity

One hypothesis is that a mutagenic mode of action is operative in TCE-induced renal carcinogenesis. According to this hypothesis, the key events leading to TCE-induced kidney tumor formation constitute the following: TCE GSH conjugation metabolites (e.g., DCVG, DCVC, NAcDCVC, and/or other reactive metabolites derived from subsequent beta-lyase, flavin monooxygenases [FMO], or CYP metabolism) derived from the GSH-conjugation pathway, after being either produced in situ in or delivered systemically to the kidney, cause direct alterations to DNA (e.g., mutation, DNA damage, and/or micronuclei induction). Mutagenicity is a well-established cause of carcinogenicity.

Toxicokinetic data are consistent with these genotoxic metabolites either being delivered to or produced in the kidney. As discussed in Section 3, following in vivo exposure to TCE, the metabolites DCVG, DCVC, and NAcDCVC have all been detected in the blood, kidney, or urine of rats, and DCVG in blood and NAcDCVC in urine have been detected in humans (2006; Lash et al., 1999b; Bernauer et al., 1996; Birner et al., 1993). In addition, in vitro data have shown DCVG formation from TCE in cellular and subcellular fractions from the liver, from which it would be delivered to the kidney via systemic circulation, and from the kidney (see Tables 3-23–3-24, and references therein). Furthermore, in vitro data in both humans and rodents support the conclusion that DCVC is primarily formed from DCVG in the kidney itself, with subsequent in situ transformation to NAcDCVC by *N*-Acetyl transferase or to reactive metabolites by beta-lyase, FMO, or CYPs (see Sections 3.3.3.2.2 to 3.3.3.2.5). Therefore, it is highly likely that both human and rodent kidneys are exposed to these TCE metabolites.

[6]As recently reviewed (Guyton et al., 2008), the approach to evaluating mode of action information described in EPA's Cancer Guidelines (2005b) considers the issue of human relevance of a hypothesized mode of action in the context of hazard evaluation. This excludes, for example, consideration of toxicokinetic differences across species; specifically, the *Cancer Guidelines* state, "The toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action." In addition, information suggesting quantitative differences in the occurrence of a key event between test species and humans are noted for consideration in the dose-response assessment, but is not considered in human relevance determination. In keeping with these principles, a formal analysis of the dose-response of key events in the hypothesized modes of action is not presented unless it would aid in the overall weight of evidence analysis for carcinogenicity, as presented in Section 4.1.

Table 4-54. Summary of major mode-of-action conclusions for TCE kidney carcinogenesis

Hypothesized MOA postulated key events	*Experimental support*	Human relevance	Weight-of-evidence conclusion
Mutagenicity			
GSH conjugation-derived metabolites produced in situ *or delivered systemically to kidney.*	*Evidence that TCE or TCE metabolites induces key events:* • Multiple in vitro and in vivo studies demonstrate GSH conjugation of TCE, and availability to the kidney (see Section 3.3.3). • Uncertainties are quantitative (precise amount of flux), not qualitative. *Necessity of key events for carcinogenesis:* • Active GSTT1 alleles are associated with higher kidney cancer risk in humans following TCE exposure as compared to null genotypes (Moore et al. 2010).	Yes: demonstrated in humans in vivo and in human cells in vitro.	Highly likely that both human and rodent kidneys are exposed to the GSH-conjugation derived metabolites.
Mutagenicity induced by GSH-derived metabolites advances acquisition of the multiple critical traits contributing to carcinogenesis.	*Evidence that TCE or TCE metabolites induces key events:* • GSH conjugation derived metabolites (DCVG, DCVC, NAcDCVC) demonstrated to be genotoxic in most in vitro assays in which they have been tested, including Ames test (see Section 4.2.1.4.1). • Kidney-specific genotoxicity in rats and rabbits after in vivo administration of TCE or DCVC. Not seen in mice, but may be due to species differences in metabolism and in sensitivity to renal carcinogenesis. *Necessity of key events for carcinogenesis:* • Inconsistent results with respect to *VHL* mutation status, with some studies providing suggestive evidence of a TCE-induced kidney tumor genotype; no data regarding other specific mutations.	Yes: no basis for discounting in vitro or in vivo genotoxicity results.	Predominance of positive genotoxicity data consistent with GSH-conjugation derived metabolites causing mutations in the kidney.
Overall Conclusion	*Sufficiency of MOA for carcinogenesis:* • Mutagenicity is assumed to cause cancer, as a sufficient cause.	Yes: well established.	Data are sufficient to conclude that a mutagenic MOA is operative in TCE-induced kidney tumors. (Section 4.4.7.1).

Table 4-54. Summary of major mode-of-action conclusions for TCE kidney carcinogenesis (continued)

Hypothesized MOA postulated key events	*Experimental support*	Human relevance	Weight-of-evidence conclusion
Cytotoxicity and regenerative proliferation			
GSH conjugation-derived metabolites produced in situ *or delivered systemically to kidney.*	*Evidence that TCE or TCE metabolites induces key events:* • Multiple in vitro and in vivo studies demonstrate GSH conjugation of TCE, and availability to the kidney (see Section 3.3.3). • Uncertainties are quantitative (precise amount of flux), not qualitative. *Necessity of key events for carcinogenesis:* • Active GSTT1 alleles are associated with higher kidney cancer risk in humans following TCE exposure as compared to null genotypes (Moore et al. 2010).	Yes: demonstrated in humans in vivo and in human cells in vitro.	Highly likely that both human and rodent kidneys are exposed to the GSH-conjugation derived metabolites.
• *Cytotoxicity.* • *Compensatory cell proliferation.* • *Clonal expansion of initiated cells.*	*Evidence that TCE or TCE metabolites induces key events:* • Multiple human and laboratory animal studies demonstrating TCE to be nephrotoxic, including chronic studies (see Sections 4.4.1 and 4.4.4). • Multiple laboratory animal studies and in vitro studies in rat and human kidney cells demonstrating DCVC to be nephrotoxic (see Sections 4.4.6.1.1 and 4.4.6.2). • Some evidence that TCOH is nephrotoxic, but histological changes caused by TCE more similar to those caused by DCVC (see Section 4.4.6.1 and 4.4.6.3). • Increased DNA synthesis as measured by BrdU in Eker rats. • No increase in preneoplastic or neoplastic lesions in Eker rats exposed to TCE for 13 wk, but no data on longer durations or from other rat strains sensitive to TCE renal carcinogenesis. *Necessity of key events for carcinogenesis:* • No TCE-specific studies to establish the necessity of TCE-induced proliferation resulting from nephrotoxicity to clonal expansion and cancer.	Yes: demonstrated human nephrotoxicity of TCE in vivo and DCVC in vitro.	• TCE is nephrotoxic in humans, and DCVC is likely the predominant moiety responsible. • TCE increases cell proliferation. • Data linking TCE-induced proliferation to clonal expansion are lacking.
Overall Conclusion	*Sufficiency of MOA for carcinogenesis:* • Maximal levels of cytotoxicity are reached at doses below which the incidence of tumors is elevated, suggesting cytotoxicity is not sufficient for carcinogenesis. • While cytotoxicity and regenerative cell proliferation occur and are assumed to contribute to carcinogenesis, a more plausible MOA may involve combination of cytotoxicity with mutagenicity.	Yes: well established.	Data are consistent with hypothesis that cytotoxicity and regenerative proliferation contribute to TCE-induced kidney tumors, but data linking TCE-induced proliferation to clonal expansion are lacking. (Section 4.4.7.2)

Table 4-54. Summary of major mode-of-action conclusions for TCE kidney carcinogenesis (continued)

Hypothesized MOA postulated key events	*Experimental support*	Human relevance	Weight-of-evidence conclusion
Peroxisome proliferation activated receptor alpha activation			
• *TCE oxidative metabolites (e.g., TCA), after being produced in the liver, activate PPARα in the kidney.* • *Alterations in cell proliferation and apoptosis.* • *Clonal expansion of initiated cells.*	*Evidence that TCE or TCE metabolites induces key events:* • Increased PCO activity (marker for PPARα activation) observed in rats and mice treated with TCE or TCA. • No increases in kidney/body weight ratios (potential marker for changes in cell proliferation/apoptosis) due to oxidative metabolites. • No data on altered cell proliferation/apoptosis or clonal expansion in the kidney due to PPARα activation. *Necessity of key events for carcinogenesis:* • No PCE-specific studies. No data from other chemicals on PPARα involvement in kidney tumors.	Yes. Humans produce oxidative metabolites of TCE, PPARα is present in the human kidney.	Highly likely that PPARα is activated in the kidney, but little evidence for other key events.
Overall	*Sufficiency of MOA for carcinogenesis:* • Inadequate data to support a role for PPARα activation in renal carcinogenesis, in general, or for TCE specifically.	N/A	Little evidence that PPARα activation contributes to renal carcinogenesis.

Table 4-54. Summary of major mode-of-action conclusions for TCE kidney carcinogenesis (continued)

Hypothesized MOA postulated key events	*Experimental support*	Human relevance	Weight-of-evidence conclusion
α2μ-Globulin-related nephropathy			
• *TCE oxidative metabolites (e.g., TCOH), cause hyaline droplet accumulation and an increase in α2μ-globulin, resulting in nephrotoxicity.* • *Subsequent cytotoxicity and necrosis.* • *Sustained regenerative tubule cell proliferation.* • *Development of intralumenal granular casts from sloughed cellular debris associated with tubule dilatation and papillary mineralization.* • *Foci of tubule hyperplasia in the convoluted proximal tubules.* • *Renal tubule tumors.*	*Evidence that TCE or TCE metabolites induces key events:* • TCOH caused hyaline droplet accumulation and an increase in α2μ-globulin, but at levels insufficient to account for the observed nephropathy. • TCE is associated with small increases in kidney cancer in female rats (not consistent with α2μ-globulin hypothesis). • TCE is associated with kidney cancer in humans (not consistent with α2μ-globulin hypothesis). *Necessity of key events for carcinogenesis:* • Inadequate support that it is necessary for TCE-induced renal carcinogenesis.	No.	Unlikely that α2μ-globulin is the major cause of TCE-induced nephrotoxicity.
Overall	*Sufficiency of MOA for carcinogenesis:* • Inadequate data to support a role in TCE-induced renal carcinogenesis.	No.	Little evidence that increases in α2μ-globulin contribute to TCE-induced renal carcinogenesis.

Table 4-54. Summary of major mode-of-action conclusions for TCE kidney carcinogenesis (continued)

Hypothesized MOA postulated key events	*Experimental support*	Human relevance	Weight-of-evidence conclusion
Formic acid-related nephrotoxicity			
• *TCE oxidative metabolites (e.g., TCA or TCOH), after being produced in the liver, lead to increased formation and urinary excretion of formic acid, which causes cytotoxicity in the kidney.* • *Compensatory cell proliferation.* • *Clonal expansion of initiated.*	*Evidence that TCE or TCE metabolites induces key events:* • TCOH causes histological changes in the kidney, along with increased formic acid. • TCOH-induced kidney effects do not account for most of the kidney effects observed after TCE exposure (not consistent with formic acid hypothesis). • No data as to oxidative metabolites causing regenerative proliferation, or other key events in the kidney. *Necessity of key events for carcinogenesis:* • Inadequate data to support the necessity of formic acid formation in renal carcinogenesis.	Yes.	Unlikely that formic acid is a major contributor to TCE-induced nephrotoxicity.
Overall	*Sufficiency of MOA for carcinogenesis:* • Inadequate data supporting a sufficient role for formic acid in renal carcinogenesis, whether generally or for TCE specifically.	N/A	Unlikely that formic acid formation and its sequelae contribute to TCE-induced renal carcinogenesis.

4.4.7.1.1. Experimental support for the hypothesized mode of action

Evidence for the hypothesized mode of action for TCE includes: (1) the formation of GSH-conjugation pathway metabolites in the kidney demonstrated in TCE toxicokinetics studies and (2) the genotoxicity of these GSH-conjugation pathway metabolites demonstrated in most existing in vitro and in vivo assays of gene mutations (i.e., Ames test) and in assays of unscheduled DNA synthesis, DNA strand breaks, and micronuclei using both "standard" systems and renal cells/tissues.[7] Additional relevant data come from analyses of *VHL* mutations in human kidney tumors and studies using the Eker rat model. These lines of evidence are elaborated below.

Toxicokinetic data are consistent with these genotoxic metabolites either being delivered to or produced in the kidney. As discussed in Chapter 3, following in vivo exposure to TCE, the metabolites DCVG, DCVC, and NAcDCVC have all been detected in the blood, kidney, or urine of rats, and DCVG in blood and NAcDCVC in urine have been detected in humans (2006; Lash et al., 1999b; Bernauer et al., 1996; Birner et al., 1993). In addition, in vitro data have shown DCVG formation from TCE in cellular and subcellular fractions from the liver, from which it would be delivered to the kidney via systemic circulation, and from the kidney (see Tables 3-23–3-24, and references therein). Furthermore, in vitro data in both humans and rodents support the conclusion that DCVC is primarily formed from DCVG in the kidney itself, with subsequent in situ transformation to NAcDCVC by *N*-Acetyl transferase or to reactive metabolites by beta-lyase, FMO, or CYPs (see Sections 3.3.3.2.2 to 3.3.3.2.5). Therefore, it is highly likely that both human and rodent kidneys are exposed to these TCE metabolites.

As discussed in Section 4.2.5, DCVG, DCVC, and NAcDCVC have been demonstrated to be genotoxic in most available in vitro assays.[8] In particular, DCVC was mutagenic in the Ames test in three of the tested strains of *S. typhimurium* (TA100, TA2638, TA98) (Vamvakas et

[7]The EPA *Cancer Guidelines* (2005b, e) note reliance on "evaluation of in vivo or in vitro short-term testing results for genetic endpoints" and evidence that "the carcinogen or a metabolite is DNA-reactive and/or has the ability to bind to DNA"as part of this weight of evidence supporting a mutagenic mode of action. While evidence from hypothesis-testing experiments that mutation is an early step in the carcinogenic process is considered if available, it is not required for determination of a mutagenic mode of action; rather, reliance on short-term genotoxicity tests is emphasized. Thus, such tests are the focus of this analysis, which also includes an analysis of other available data from humans and animals. In keeping with these principles, a formal analysis of the temporal concordance of key events in the hypothesized modes of action is not presented unless it would aid in the overall weight of evidence analysis for carcinogenicity, as presented in Section 4.11.

[8]Evaluation of genotoxicity data entails a weight of evidence approach that includes consideration of the various types of genetic damage that can occur. In acknowledging that genotoxicity tests are by design complementary evaluations of different mechanisms of genotoxicity, a recent IPCS publication (Eastmond et al., 2009) notes that "multiple negative results may not be sufficient to remove concern for mutagenicity raised by a clear positive result in a single mutagenicity assay." These considerations inform the present approach. In addition, consistent with EPA's *Cancer Guidelines* (2005b, e), the approach does not address relative potency (e.g., among TCE metabolites, or of such metabolites with other known genotoxic carcinogens) per se, nor does it consider quantitative issues related to the probable production of these metabolites in vivo. Instead, the analysis of genetic toxicity data presented in Section 4.2 and summarized here focuses on the identification of a genotoxic hazard of these metabolites; a quantitative analysis of TCE metabolism to reactive intermediates, via PBPK modeling, is presented in Section 3.5.

al., 1988b; Dekant et al., 1986c) and caused dose-dependent increases in UDS in the two available assays: porcine kidney tubular epithelial cell line (Vamvakas et al., 1996) and Syrian hamster embryo fibroblasts (Vamvakas et al., 1988a). DCVC has also been shown to induce DNA strand breaks in both available studies (Robbiano et al., 2004; Jaffe et al., 1985), and induce micronucleus formation in primary kidney cells from rats and humans (Robbiano et al., 2004) but not in Syrian hamster embryo fibroblasts (Vamvakas et al., 1988a). Only one study each is available for DCVG and *N*-AcDCVC, but notably, both were positive in the Ames test (1988b; Vamvakas et al., 1987). Although the number of test systems was limited, these results are consistent.

These in vitro results are further supported by studies reporting kidney-specific genotoxicity after in vivo administration of TCE or DCVC. In particular, Robbiano et al. (1998) reported increased numbers of micronucleated cells in the rat kidney following oral TCE exposure. Oral exposure to DCVC in both rabbits (Jaffe et al., 1985) and rats (Clay, 2008) increased DNA strand breaks in the kidney. However, in one inhalation exposure study in rats, TCE did not increase DNA breakage in the rat kidney, possibly due to study limitations (limited exposure time [6 hours/day for only 5 days] and small number of animals exposed [n = 5]; Clay, (2008). One study of TCE exposure in the Eker rat, a rat model heterozygous for the tumor suppressor gene *Tsc-2*, reported no significant increase in kidney tumors as compared to controls (Mally et al., 2006). Inactivation of *Tsc-2* in this rat model is associated with spontaneous RCC with activation of pathways similar to that of *VHL* inactivation in humans (Liu et al., 2003). TCE exposure for 13 weeks (corn oil gavage) led to increased nephrotoxicity but no significant increases in preneoplastic or neoplastic lesions as compared to controls (Mally et al., 2006). This lack of increased incidence of neoplastic or preneoplastic lesions reported by Mally et al. (2006) in the tumor-prone Eker rat is similar to lack of significant short-term response exhibited by other genotoxic carcinogens in the Eker rat (Stemmer et al., 2007; Morton et al., 2002) and may be related to the increased background rate of renal carcinomas in this animal model. Mally et al. (2006) also exposed primary kidney epithelial cells from the Eker rat to DCVC in vitro and demonstrated increased transformation similar to that of other renal carcinogens (Horesovsky et al., 1994).

As discussed in Section 4.2.1.4.1, although Douglas et al. (1999) did not detect increased mutations in the kidney of *lacZ* transgenic mice exposed to TCE for 12 days, these results are not highly informative as to the role of mutagenicity in TCE-induced kidney tumors, given the uncertainties in the production in genotoxic GSH conjugation metabolites in mice and the low carcinogenic potency of TCE for kidney tumors in rodents relative to what is detectable in experimental bioassays. Limited, mostly in vitro, toxicokinetic data do not suggest that mice have less GSH conjugation or subsequent renal metabolism/bioactivation (see Section 3.3.3.2.7), but quantitatively, the uncertainties in the flux through these pathways remain significant (see Section 3.5). In additional, similar to other genotoxic renal carcinogens analyzed by NTP, there

is limited evidence of mouse kidney tumors following TCE exposure. However, given the already low incidences of kidney tumors observed in rats, a relatively small difference in potency in mice would be undetectable in available chronic bioassays. Notably, of seven chemicals categorized as direct-acting genotoxic carcinogens that induced rat renal tumors in NTP studies, only two also led to renal tumors in the mouse (tris[2,3-dibromopropyl]phosphate and ochratoxin A) (Reznik et al., 1979; Kanisawa and Suzuki, 1978), so the lack of detectable response in mouse bioassays does not preclude a genotoxic mode of action.

VHL inactivation (via mechanisms such as deletion, silencing, or mutation) observed in human renal clear cell carcinomas is the basis of a hereditary syndrome of kidney cancer predisposition and is hypothesized to be an early and causative event in this disease (e.g., 2008). Therefore, specific actions of TCE metabolites that produce or select for mutations of the *VHL* suppressor gene could lead to kidney tumorigenesis. Several studies have compared *VHL* mutation frequencies in cases with TCE exposures with those from control or background populations. Brüning et al. (1997b) and Brauch et al. (2004; 1999) reported differences between TCE-exposed and nonexposed RCC patients in the frequency of somatic *VHL* mutations, the incidence of a hot spot mutation of cytosine to thymine at nucleotide 454, and the incidence of multiple mutations. These data suggest that kidney tumor genotype data in the form of a specific mutation pattern may potentially serve to discriminate TCE-induced tumors from other types of kidney tumors in humans. If validated, this would also suggest that TCE-induced kidney tumors are dissimilar from those occurring in unexposed individuals. Thus, while not confirming a mutation mode of action, these data suggest that TCE-induced tumors may be distinct from those induced spontaneously in humans. However, it has not been examined whether a possible linkage exists between *VHL* loss or silencing and mutagenic TCE metabolites.

By contrast, Schraml et al. (1999) and Charbotel et al. (2007) reported that TCE-exposed RCC patients did not have significantly higher incidences of *VHL* mutations compared to nonexposed patients. However, details as to the exposure conditions were lacking in Schraml et al. (1999). In addition, the sample preparation methodology employed by Charbotel et al. (2007) and others (Brauch et al., 1999; Brüning et al., 1997b) often results in poor quality and/or low quantity DNA, leading to study limitations (<100% of samples were able to be analyzed). Therefore, further investigations are necessary to either confirm or contradict the validity of the genetic biomarkers for TCE-related renal tumors reported by Brüning et al. (1997b) and Brauch et al. (2004; 1999).

In addition, while exposure to mutagens is certainly associated with cancer induction (as discussed with respect to the liver in Appendix E, Sections E.3.1 and E.3.2), examination of end-stage tumor phenotype or genotype has limitations concerning determination of early key events. The mutations that are observed with the progression of neoplasia are associated with increased genetic instability and an increase in mutation rate. Further, inactivation of the *VHL* gene also occurs through other mechanisms in addition to point mutations, such as loss of heterozygosity

or hypermethylation (Nickerson et al., 2008; Kenck et al., 1996) not addressed in these studies. Recent studies examining the role of other genes or pathways suggest roles for multiple genes in RCC development (Toma et al., 2008; Furge et al., 2007). Therefore, the inconsistent results with respect to *VHL* mutation status do not constitute negative evidence for a mutational mode of action and the positive studies are suggestive of a TCE-induced kidney tumor genotype.

In sum, the predominance of positive genotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular the evidence of kidney-specific genotoxicity following in vivo exposure to TCE or DCVC), coupled with the toxicokinetic data consistent with the in situ formation of these GSH-conjugation metabolites of TCE in the kidney, is consistent with the hypothesis that a mutagenic mode of action is operative in TCE-induced kidney tumors. Mutagenicity is a well-established cause of carcinogenicity. Available data on the *VHL* gene in humans add biological plausibility to these conclusions. Quantitatively, however, as discussed in Section 3.3.3.2, the precise metabolic yield of the GSH conjugation metabolites following TCE exposure remains uncertain.

4.4.7.2. Hypothesized Mode of Action: Cytotoxicity and Regenerative Proliferation

Another hypothesis is that TCE acts by a cytotoxicity mode of action in TCE-induced renal carcinogenesis. According to this hypothesis, the key events leading to TCE-induced kidney tumor formation comprise the following: the TCE GSH-conjugation metabolite, DCVC, after being either produced in situ in or delivered systemically to the kidney, causes cytotoxicity, leading to compensatory cellular proliferation and subsequently increased mutations and clonal expansion of initiated cells.

4.4.7.2.1. Experimental support for the hypothesized mode of action

Evidence for the hypothesized mode of action consist primarily of (1) the demonstration of nephrotoxicity following TCE exposure at current occupational limits in human studies and chronic TCE exposure in animal studies; (2) the relatively high potential of the TCE metabolite DCVC to cause nephrotoxicity; and (3) toxicokinetic data demonstrating that DCVC is formed in the kidney following TCE exposure. Data on nephrotoxicity of TCE and DCVC are discussed in more detail below, while the toxicokinetic data were summarized previously in the discussion of mutagenicity. Thus, the data are consistent with the hypothesized mode of action, and therefore, do not rule out a contribution from cytotoxicity and regenerative proliferation to TCE-induced kidney carcinogenesis. However, there is a lack of experimental data supporting a causal link between TCE nephrotoxicity combined with sustained cellular proliferation and TCE-induced nephrocarcinogenicity.

There is substantial evidence that TCE is nephrotoxic in humans and laboratory animals and that its metabolite, DCVC, is nephrotoxic in laboratory animals. Epidemiological studies have consistently demonstrated increased excretion of nephrotoxicity markers (NAG, protein,

albumin) at occupational (Green et al., 2004) and higher (Bolt et al., 2004; Brüning et al., 1999a; 1999b) levels of TCE exposure. However, direct evidence of tubular toxicity, particularly in RCC cases, is not available. These studies are supported by the results of multiple laboratory animal studies. Chronic bioassays have reported very high (nearly 100%) incidences of nephrotoxicity of the proximal tubule in rats (NTP, 1990, 1988) and mice (NTP, 1990; NCI, 1976) at the highest doses tested. In vivo studies examining the effect of TCE exposure on nephrotoxicity showed increased proximal tubule damage following i.p. injection and inhalation of TCE in rats (Chakrabarti and Tuchweber, 1988) and i.p. injection in mice (Cojocel et al., 1989). Studies examining DCVC exposure in rats (Elfarra et al., 1986; Terracini and Parker, 1965) and mice (Darnerud et al., 1989; Jaffe et al., 1984) have also shown increases in kidney toxicity. The greater potency for kidney cytotoxicity for DCVC compared to TCE was shown by in vitro studies (Lash et al., 1986, 1995; Stevens et al., 1986). These studies also further confirmed the higher susceptibility of male rats or mice to DCVC-induced cytotoxicity. Cytokaryomegaly (an effect specific to TCE and not part of the chronic progressive nephropathy or the pathology that occurs in aging rat kidneys) was observed in the majority of rodent studies and may or may not progress to carcinogenesis. Finally, as discussed extensively in Section 4.4.6.1, a detailed comparison of the histological changes in the kidney caused by TCE and its metabolites supports the conclusion that DCVC is the predominant moiety responsible for TCE-induced nephrotoxicity.

Because it is known that not all cytotoxins are carcinogens (i.e., cytotoxicity is not a specific predictor of carcinogenicity), additional experimental support is required to causally link nephrotoxicity to nephrocarcinogenicity. For chemicals that bind to α2μ-globulin, a mode of action involving cell necrosis followed by subsequent regenerative proliferation has been hypothesized to cause kidney tumors in the absence of genotoxicity (Short, 1993). However, for other chemicals, toxicity and increased cell proliferation have been observed in the kidney without inducing tumors even after chronic exposure (Tennant et al., 1991). Similarly, in the liver, partial hepatectomy leading to regenerative hyperplasia does not by itself lead to increased hepatocarcinogenicity, and requires administration of a mutagen to exhibit enhanced carcinogenic effects. By analogy, a biologically plausible mode of action may involve a combination of mutagenicity and cytotoxicity, with mutagenicity increasing the rate of mutation and regenerative proliferation induced by cytotoxicity enhancing the selection, survival, or clonal expansion of mutated cells.

For TCE and kidney cancer, clearly, cytotoxicity occurs at doses below those causing carcinogenicity, as the incidence of nephrotoxicity in chronic bioassays is an order of magnitude higher than that of renal tumors. Thus, these data are consistent with cytotoxicity being a precursor to carcinogenicity (i.e., if the opposite were the case—carcinogenicity without cytoxicity—then the hypothesis would be falsified). While chronic nephrotoxicity was reported in the same bioassays showing increased kidney tumor incidences, the use of such data to inform

mode of action is indirect and associative, and do not offer a test of the hypothesis (Short, 1993). Nephrotoxicity is observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but kidney tumors are only observed at low incidences in rats at the highest tested doses (NTP, 1990; NCI, 1976). Therefore, nephrotoxicity alone appears to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis, since maximal levels of toxicity are reached before the onset of tumors. Furthermore, there are multiple mechanisms by which TCE has been hypothesized to induce cytotoxicity, including oxidative stress, disturbances in calcium ion homeostasis, mitochondrial dysfunction, and protein alkylation (Lash et al., 2000a). Some of these effects may therefore, have ancillary consequences related to tumor induction which are independent of cytotoxicity per se. Therefore, data currently cannot distinguish as to whether cytoxicity is causally related to tumorigenesis or merely associated by virtue of being a marker for a different, key causal event.

Under the hypothesized mode of action, cytotoxicity leads to the induction of repair processes and compensatory proliferation that could lead to an increased production or clonal expansion of cells previously initiated by mutations occurred spontaneously, from co-exposures, or from TCE or its metabolites. Data on compensatory cellular proliferation and the subsequent hypothesized key events in the kidney are few, with no data from rat strains used in chronic bioassays. In rats carrying the Eker mutation, Mally et al. (2006) reported increased DNA synthesis as measured by BrdU incorporation in animals exposed to the high dose of TCE (1,000 mg/kg-day) for 13 weeks, but there was no evidence of clonal expansion or tumorigenesis in the form of increased preneoplastic or neoplastic lesions as compared to controls. Therefore, in both rodent and human studies of TCE, data demonstrating a causal link between compensatory proliferation and the induction of kidney tumors are lacking.

In sum, the predominance of positive nephrotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular the evidence of kidney-specific cytotoxicity following in vivo exposure to TCE or DCVC), coupled with the toxicokinetic data consistent with the in situ formation of these GSH-conjugation metabolites of TCE in the kidney, is consistent with the hypothesis that a mode of action involving cytotoxicity and regenerative proliferation contributes to TCE-induced kidney tumors, either independently or in combination with a mutagenic mode of action. However, nephrotoxicity is not in itself predictive of tumorigenesis, and experimental data supporting for a causal link between TCE nephrotoxicity combined with sustained cellular proliferation and TCE-induced nephrocarcinogenicity are lacking. A more biologically plausible mode of action may involve a combination of mutagenicity and cytotoxicity, with mutagenicity increasing the rate of mutation and regenerative proliferation enhancing the selection, survival or clonal expansion of mutated cells. However, this hypothesis has yet to be tested experimentally.

4.4.7.3. Additional Hypothesized Modes of Action with Limited Evidence or Inadequate Experimental Support

Along with metabolites derived from GSH conjugation of TCE, oxidative metabolites are also present and could induce toxicity in the kidney. After TCE exposure, the oxidative metabolite and peroxisome proliferator, TCA, is present in the kidney and excreted in the urine as a biomarker of exposure. Hypotheses have also been generated regarding the roles of α2μ-globulin or formic acid in nephrotoxicity induced by TCE oxidative metabolites TCA or TCOH. However, the available data are limited or inadequate for supporting these hypothesized modes of action.

4.4.7.3.1. Peroxisome proliferation

Although not as well studied as the effects of GSH metabolites in the kidney, there is evidence that oxidative metabolites affect the kidney after TCE exposure. Both TCA and DCA are peroxisome proliferator activated receptor alpha (PPARα) agonists although most activity has been associated with TCA production after TCE exposure. Exposure to TCE has been found to induce peroxisome proliferation not only in the liver, but also in the kidney. Peroxisome proliferation in the kidney has been evaluated by only one study of TCE (Goldsworthy and Popp, 1987), using increases in cyanide-insensitive palmitoyl-CoA oxidation (PCO) activity as a marker. Increases in renal PCO activity were observed in rats (3.0-fold) and mice (3.6-fold) treated with TCE at 1,000 mg/kg-day for 10 days, with smaller increases in both species from TCA treatment at 500 mg/kg-day for 10 days. However, no significant increases in kidney/body weight ratios were observed in either species. There was no relationship between induction of renal peroxisome proliferation and renal tumors (i.e., a similar extent of peroxisome proliferation-associated enzyme activity occurred in species with and without TCE-induced renal tumors). However, the increased peroxisomal enzyme activities due to TCE exposure are indicative of oxidative metabolites being present and affecting the kidney. Such metabolites have been associated with other tumor types, especially liver, and whether co-exposures to oxidative metabolites and GSH metabolites contribute to kidney tumorigenicity has not been examined.

4.4.7.3.2. α2μ-Globulin-related nephropathy

Induction of α2μ-globulin nephropathy by TCE has been investigated by Goldsworthy et al. (1988), who reported that TCE did not induce increases in this urinary protein, nor did it stimulate cellular proliferation in rats. In addition, whereas kidney tumors associated with α2μ-globulin nephropathy are specific to the male rat, as discussed above, nephrotoxicity is observed in both rats and mice and kidney tumor incidence is elevated (though not always statistically significant) in both male and female rats. TCOH was recently reported to cause hyaline droplet accumulation and an increase in α2μ-globulin, but these levels were insufficient

to account for the observed nephropathy as compared to other exposures (Green et al., 2003). Therefore, it is unlikely that α2μ-globulin nephropathy contributes significantly to TCE-induced renal carcinogenesis.

4.4.7.3.3. Formic acid-related nephrotoxicity

Another mode-of-action hypothesis proposes that TCE nephrotoxicity is mediated by increased formation and urinary excretion of formic acid mediated by the oxidative metabolites TCA or TCOH (Green et al., 2003; Dow and Green, 2000; 1998). The subsequent hypothesized key events are the same as those for DCVC-induced cytotoxicity, discussed above (see Section 4.4.7.2). As discussed extensively in Section 4.4.6.1.2, these oxidative metabolites do not appear sufficient to explain the range of renal effects observed after TCE exposure, particularly cytomegaly, karyomegaly, and flattening and dilation of the tubular epithelium. Although TCOH and possibly TCA may contribute to the nephrotoxicity of TCE, perhaps due to excess formic acid production, these metabolites do not show the same range of cytotoxic effects observed following TCE exposure (see Table 4-53). Therefore, without specific evidence linking the specific nephrotoxic effects caused by TCOH or TCA to carcinogenesis, and in light of the substantial evidence that DCVC itself can adequately account for the nephrotoxic effects of TCE, the weight of evidence supports a conclusion that cytotoxicity mediated by increased formic acid production induced by oxidative metabolites TCOH and possibly TCA is not responsible for the majority of the TCE-induced cytotoxicity in the kidneys, and therefore, would not be the major contributor to the other hypothesized key events in this mode of action, such as subsequent regenerative proliferation.

4.4.7.4. Conclusions About the Hypothesized Modes of Action

4.4.7.4.1. Is the hypothesized mode of action sufficiently supported in the test animals

4.4.7.4.1.1. Mutagenicity

The predominance of positive genotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular the evidence of kidney-specific genotoxicity following in vivo exposure to TCE or DCVC), coupled with the toxicokinetic data consistent with the in situ formation of these GSH-conjugation metabolites of TCE in the kidney, supports the conclusion that a mutagenic mode of action is operative in TCE-induced kidney tumors.

4.4.7.4.1.2. Cytotoxicity

As reviewed above, in vivo and in vitro studies have shown a consistent nephrotoxic response to TCE and its metabolites in proximal tubule cells from male rats. Therefore, it has been proposed that cytotoxicity seen in this region of the kidney is a precursor to carcinogenicity. Available data are consistent with the hypothesis that a mode of action involving cytotoxicity

and regenerative proliferation contributes to TCE-induced kidney tumors, either independently or in combination with a mutagenic mode of action. However, it has not been determined whether tubular toxicity is a necessary precursor of carcinogenesis, and there is a lack of experimental support for causal links, such as compensatory cellular proliferation or clonal expansion of initiated cells, between nephrotoxicity and kidney tumors induced by TCE. Nephrotoxicity alone appears to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis, since maximal levels of toxicity are reached before the onset of tumors. A more biologically plausible mode of action may involve a combination of mutagenicity and cytotoxicity, with mutagenicity increasing the rate of mutation and regenerative proliferation enhancing the survival or clonal expansion of mutated cells. However, this hypothesis has yet to be tested experimentally.

4.4.7.4.1.3. Additional hypothese

The kidney is also exposed to oxidative metabolites that have been shown to be carcinogenic in other target organs. TCA is excreted in kidney after its metabolism from TCE and also can cause peroxisome proliferation in the kidney, but there are inadequate data to define a mode of action for kidney tumor induction based on peroxisome proliferation. TCE induced little or no α2μ-globulin and hyaline droplet accumulation to account for the observed nephropathy, so available data do not support this hypothesized mode of action. The production of formic acid following exposure to TCE and its oxidative metabolites TCOH and TCA may also contribute to nephrotoxicity; however, the available data indicate that TCOH and TCA are minor contributors to TCE-induced nephrotoxicity, and therefore, do not support this hypothesized mode of action. Because these additional mode-of-action hypotheses are either inadequately defined or are not supported by the available data, they are not considered further in the conclusions below.

4.4.7.4.2. Is the hypothesized mode of action relevant to humans

4.4.7.4.2.1. Mutagenicity

The evidence discussed above demonstrates that TCE GSH-conjugation metabolites are mutagens in microbial as well as test animal species. Therefore, the presumption that they would be mutagenic in humans. Available data on the *VHL* gene in humans add biological plausibility to this hypothesis. The few available data from human studies concerning the mutagenicity of TCE and its metabolites suggest consistency with this mode of action, but are not sufficiently conclusive to provide direct supporting evidence for a mutagenic mode of action. Therefore, this mode of action is considered relevant to humans.

4.4.7.4.2.2. Cytotoxicity

Although data are inadequate to determine that the mode of action is operative, none of the available data suggest that this mode of action is biologically precluded in humans. Furthermore, both animal and human studies suggest that TCE causes nephrotoxicity at exposures that also induce renal cancer, constituting positive evidence of the human relevance of this hypothesized mode of action.

4.4.7.4.3. Which populations or lifestages can be particularly susceptible to the hypothesized mode of action

4.4.7.4.3.1. Mutagenicity

The mutagenic mode of action is considered relevant to all populations and lifestages. According to EPA's *Cancer Guidelines* (U.S. EPA, 2005b) and *Supplemental Guidance* (U.S. EPA, 2005e), there may be increased susceptibility to early-life exposures for carcinogens with a mutagenic mode of action. Therefore, because the weight of evidence supports a mutagenic mode of action for TCE carcinogenicity and in the absence of chemical-specific data to evaluate differences in susceptibility, early-life susceptibility should be assumed and the age-dependent adjustment factors (ADAFs) should be applied, in accordance with the *Supplemental Guidance*.

In addition, because the mode of action begins with GSH-conjugation metabolites being delivered systemically or produced in situ in the kidney, toxicokinetic differences (i.e., increased production or bioactivation of these metabolites) may render some individuals more susceptible to this mode of action. However, as discussed in Section 3.3.3.2, quantitative estimates of the amount of GSH conjugation following TCE exposure remain uncertain. Toxicokinetic-based susceptibility is discussed further in Section 4.10.

In rat chronic bioassays, TCE-treated males have higher incidence of kidney tumors than similarly treated females. However, the basis for this sex difference is unknown, and whether it is indicative of a sex difference in human susceptibility to TCE-induced kidney tumors is likewise unknown. The epidemiologic studies generally do not show sex differences in kidney cancer risk. Lacking exposure-response information, it is not known if the sex-difference in one RCC case-control study (Dosemeci et al., 1999) may reflect exposure differences or susceptibility differences.

4.4.7.4.3.2. Cytotoxicity

Populations that may be more susceptible based on the toxicokinetics of the production of GSH conjugation metabolites and the sex differences observed in rat chronic bioassays are the same as for a mutagenic mode of action. No data are available as to whether other factors may lead to different populations or lifestages being more susceptible to a cytotoxic mode of action for TCE-induced kidney tumors. For instance, it is not known how the hypothesized key events in this mode of action interact with known risk factors for human RCC.

The weight of evidence sufficiently supports a mutagenic mode of action for TCE in the kidney, based on supporting data that GSH-metabolites are genotoxic and produced in sufficient quantities in the kidney to lead to tumorigenesis. Cytotoxicity and regenerative proliferation were considered as an alternate mode of action; however, there are inadequate data to support a causal association between cytotoxicity and kidney tumors. Further, hypothesized modes of action relating to peroxisomal proliferation, α2μ-globulin nephropathy and formic acid-related nephrotoxicity were considered and rejected due to limited evidence and/or inadequate experimental support.

4.4.8. Summary: TCE Kidney Toxicity, Carcinogenicity, and Mode of Action

Human studies have shown increased levels of proximal tubule damage in workers exposed to high levels of TCE (NRC, 2006). These studies analyzed workers exposed to TCE alone or in mixtures and reported increases in various urinary biomarkers of kidney toxicity or ESRD (β2-microglublin, total protein, NAG, α1-microglobulin) (Jacob et al., 2007; Radican et al., 2006; Bolt et al., 2004; Green et al., 2004; Brüning et al., 1999a; 1999b; Selden et al., 1993; Nagaya et al., 1989a). Laboratory animal studies examining TCE exposure provide additional support, as multiple studies by both gavage and inhalation exposure show that TCE causes renal toxicity in the form of cytomegaly and karyomegaly of the renal tubules in male and female rats and mice. By gavage, incidences of these effects under chronic bioassay conditions approach 100%, with male rats appearing to be more sensitive than either female rats or mice of either sex based on the severity of effects. Under chronic inhalation exposures, only male rats exhibited these effects. Further studies with TCE metabolites have demonstrated a potential role for DCVC, TCOH, and TCA in TCE-induced nephrotoxicity. Of these, DCVC induces the renal effects that are most like TCE, and it is formed in sufficient amounts following TCE exposure to account for these effects.

Kidney cancer risk from TCE exposure has been studied related to TCE exposure in cohort, case-control, and geographical studies. These studies have examined TCE in mixed exposures as well as alone. Elevated risks are observed in many of the cohort and case-control studies examining kidney cancer incidence in industries or job titles with historical use of TCE (see Table 4-39 and 4-40), particularly among subjects ever exposed to TCE (Moore et al., 2010; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Dosemeci et al., 1999) or subjects with TCE surrogate for high exposure (Moore et al., 2010; Charbotel et al., 2006; Zhao et al., 2005; Brüning et al., 2003; Raaschou-Nielsen et al., 2003). Greater susceptibility to TCE exposure and kidney cancer is observed among subjects with a functionally active GSTT polymorphism, particularly among those with certain alleles in single nucleotide polymorphisms of the cysteine conjugation β-lyase gene region (Moore et al., 2010). Although there are some controversies related to deficiencies of the epidemiological studies (Vamvakas et al., 1998; Henschler et al., 1995), many of these are overcome in later studies (Moore et al., 2010;

Charbotel et al., 2006; Brüning et al., 2003). A meta-analysis of the overall effect of TCE exposure on kidney cancer, additionally, suggests a small, statistically significant increase in risk (RRm = 1.27 95% CI: 1.13, 1.43) with an RRm estimate in the higher exposure group of 1.58, (95% CI: 1.28, 1.96), robust in sensitivity to alternatives and lacking observed statistical heterogeneity among 17 studies meeting explicitly-defined inclusion criteria.

In vivo laboratory animal studies to date suggest a small increase in renal tubule tumors in male rats and, to a lesser extent, in female rats, with no increases seen in mice or hamsters. These results are based on limited studies of both oral and inhalation routes, some of which were deemed insufficient to determine carcinogenicity based on various experimental issues. However, because of the rarity of kidney tumors in rodents, the repeatability of this finding across strains and studies supports their biological significance despite the limitations of individual studies and relatively small increases in reported tumor incidence.

Some, but not all, human studies have suggested a role for *VHL* mutations in TCE-induced kidney cancer (Charbotel et al., 2007; 2004; Brauch et al., 1999; Schraml et al., 1999; Brüning et al., 1997b). Certain aspects of these studies may explain some of these discrepant results. The majority of these studies have examined paraffinized tissue that may lead to technical difficulties in analysis, as paraffin extractions yield small quantities of often low-quality DNA. The chemicals used in the extraction process itself may also interfere with enzymes required for further analysis (PCR, sequencing). Although these studies do not clearly show mutations in all TCE-exposed individuals, or in fact in all kidney tumors examined, this does not take into account other possible means of *VHL* inactivation, including silencing or loss, and other potential targets of TCE mutagenesis were not systematically examined. A recent study by Nickerson et al. (2008) analyzed both somatic mutation and promoter hypermethylation of the *VHL* gene in clear cell-RCC frozen tissue samples using more sensitive methods. The results of this study support the hypothesis that *VHL* alterations are an early event in clear cell RCC carcinogenesis, but these alterations may not be gene mutations. No experimental animal studies have been performed examining *VHL* inactivation following exposure to TCE, although one in vitro study examined *VHL* mutation status following exposure to the TCE-metabolite DCVC (Mally et al., 2006). This study found no mutations following DCVC exposure, although this does not rule out a role for DCVC in *VHL* inactivation by some other method or *VHL* alterations caused by other TCE metabolites.

Although not encompassing all of the actions of TCE and its metabolites that may be involved in the formation and progression of neoplasia, available evidence supports the conclusion that a mutagenic mode of action mediated by the TCE GSH-conjugation metabolites (predominantly DCVC) is operative in TCE-induced kidney cancer. This conclusion is based on substantial evidence that these metabolites are genotoxic and are delivered to or produced in the kidney, including evidence of kidney-specific genotoxicity following in vivo exposure to TCE or DCVC. Cytotoxicity caused by DCVC leading to compensatory cellular proliferation is also a

potential mode of action in renal carcinogenesis. A combination of mutagenicity and cytotoxicity, with mutagenicity increasing the rate of mutation and regenerative proliferation enhancing the survival or clonal expansion of mutated cells, while biologically plausible, has yet to be tested experimentally. The additional mode-of-action hypotheses of peroxisome proliferation, accumulation of α2μ-globulin, and cytotoxicity mediated by TCE-induced excess formic acid production are not supported by the available data.

4.5. LIVER TOXICITY AND CANCER

4.5.1. Liver Noncancer Toxicity in Humans

The complex of chronic liver disease is a spectrum of effects and comprises nonalcoholic fatty liver disease (nonalcoholic steatohepatitis) and cirrhosis, more rare anomalies ones such as autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis, and hepatocellular and cholangiocarcinoma (intrahepatic bile duct cancer) (Juran and Lazaridis, 2006). Chronic liver disease and cirrhosis, excluding neoplasia, is the 12th leading cause of death in the United States in 2005 with 27,530 deaths (Kung et al., 2008) with a morality rate of 9.0 per 100,000 (Jemal et al., 2008).

Eight studies reported on liver outcomes and TCE exposure and are identified in Table 4-55. Three studies are suggestive of effects on liver function tests in metal degreasers occupationally exposed to TCE (Xu et al., 2009; Nagaya et al., 1993; Rasmussen et al., 1993b). Nagaya et al. (1993) in their study of 148 degreasers in metal parts factories, semiconductor factors, or other factories, observed total mean serum cholesterol concentration and mean serum high density lipoprotein-cholesterol (HDL-C) concentrations to increase with increasing TCE exposure, as defined by U-TTC), although a statistically significant linear trend was not found. Nagaya et al. (1993) estimated that TCE exposures were 1 ppm in the low-exposure group, 6 ppm in the moderate-exposure group, and 210 ppm in the high-exposure group. No association was noted between serum liver function tests and U-TTC, a finding not surprising given that individuals with a history of hepatobiliary disease were excluded from this study. Nagaya et al. (1993) follows 13 workers with higher U-TTC concentrations over a 2-year period; serum HDL-C and two hepatic function enzymes, GGT and aspartate aminotransferase (AST) concentrations were highest during periods of high level exposure, as indicated from U-TTC concentrations. Similarly, in a study of 95 degreasers, 70 exposed to TCE and 25 exposed to CFC113 (Rasmussen et al., 1993b), mean serum GGT concentration for subjects with the highest TCE exposure duration was above normal reference values and was about threefold higher compared to the lowest exposure group. Rasmussen et al. (1993b) estimated mean urinary TCE concentration in the highest exposure group as 7.7 mg/L with past exposures estimated as equivalent to 40–60 mg/L. Multivariate regression analysis showed a small statistically nonsignificant association due to age and a larger effect due to alcohol abuse that reduced and changed direction of a TCE exposure affect. The inclusion of CFC113-exposed subjects

introduces a downward bias since liver toxicity is not associated with CFC113 exposure (U.S. EPA, 2008b) and would underestimate any possible TCE effect. Xu et al. (2009) reported symptoms and liver function tests of 21 metal degreasers with severe hypersensitivity dermatitis (see last paragraph in this section for discussion of other liver effects in hypersensitivity dermatitis cases). TCE concentration of agent used to clean metal parts ranged from 10.2 to 63.5% with workplace ambient monitoring TWA TCE concentrations of 18–683 mg/m^3 (3–127 ppm). Exposure was further documented by urinary TCA levels in 14 of 21 cases above the recommended occupation level of 50 mg/L. The prevalence of elevated liver enzymes among these subjects was 90% (19 cases) for alanine aminotransferase (ALT), 86% (18 cases) for AST, and 76% (16 cases) for total bilirubin (Xu et al., 2009). Two studies provide evidence of plasma or serum bile acids changes among TCE-exposed degreasers. Neghab et al. (1997) in a small prevalence study of 10 healthy workers (5 unexposed controls and 5 exposed) observed statistically significantly elevated total serum bile acids, particularly deoxycholic acid and the subtotal of free bile acids, among TCE subjects at postexposure compared to their pre-exposure concentrations and serum bile acid levels correlated well with TCE exposure (r = 0.94). Total serum bile acid concentration did not change in control subjects between pre- and postexposure, nor did enzyme markers of liver function in either unexposed or exposed subjects differ between pre- and postexposure periods. However, the statistical power of this study is quite limited and the prevalence design does not include subjects who may have left employment because of possible liver problems. The paper provides minimal details of subject selection and workplace exposure conditions, except that pre-exposure testing was carried out on the 1st work day of the week (pre-exposure), repeated sampling after 2 days (postexposure), and a postexposure 8-hour TWA TCE concentration of 9 ppm for exposed subjects; no exposure information is provided for control subjects. Driscoll et al. (1992) in a study of 22 subjects (6 unexposed and 16 exposed) employed at a factory manufacturing small appliances reported statistically significant group differences in logistic regression analyses controlling for age and alcohol consumption in mean fasting plasma bile acid concentrations. Other indicators of liver function such as plasma enzyme levels were statistically significant different between exposed and unexposed subjects. Laboratory samples were obtained at the start of subject‘s work shift. Exposure data are not available on the 22 subjects and assignment of exposed and unexposed was based on work duties. Limited personal monitoring from other nonparticipating workers at this facility indicated TCE exposure as low, <5 ppm, with occasional peaks over 250 ppm, although details are lacking whether these data represent exposures of study subjects.

Table 4-55. Summary of human liver toxicity studies

Subjects	Effect	Exposure	Reference
148 male metal degreasers in metal parts, semiconductor and other factories	Serum liver function enzyme (HDL-C, AST, and GGT) concentrations did not correlated with TCE exposure assesses in a prevalence study but did correlate with TCE concentration over a 2-yr follow-up period	U-TTC levels obtained from spot urine sample obtained during working hrs used to assign exposure category included the following: High: 209 ± 99 mg/g Cr Medium: 35 ± 27 mg/g Cr Low: 5 ± 2 mg/g Cr Note: this study does not include an unexposed referent group	Nagaya et al. (1993)
95 workers (70 TCE exposed, 25 CFC113 exposed) selected from a cohort of 240 workers at 72 factors engaged in metal degreasing with chlorinated solvents	Increased serum GGT concentration with increasing cumulative exposure	4 groups (cumulative number of yr exposed over a working life): I: 0.6 (0–0.99) II: 1.9 (1–2.8) III: 4.4 (2.9–6.7) IV: 14.4 (6.8–35.6)	Rasmussen et al. (1993b)
21 metal degreasers with severe hypersensitivity dermatitis	High prevalence of serum liver function enzymes above normal levels: ALT, 19 or 21 cases; AST, 18 of 21 cases, and T-Bili, 16 of 21 cases	TWA mean ambient TCE concentration occupational setting of cases, 18 mg/m^3–683 mg/m^3 14 of 21 cases with U-TCE above recommended occupational level of 50 mg/L	Xu et al. (2009)
Five healthy workers engaged in decreasing activities in steel industry and five healthy workers from clerical section of same company	Total serum bile acid concentration increased between pre- and postexposure (2-d period)	8-hr TWA mean personal air: 8.9 ± 3.2 ppm postexposure	Neghab et al. (1997)
22 workers at a factory manufacturing small appliances	Increased in several bile acids	Regular exposure to <5 ppm TCE; peak exposure for two workers to >250 ppm	Driscoll et al. (1992)
4,489 males and female residents from 15 Superfund site and identified from ATSDR TCE Exposure Subregistry	Liver problems diagnosed with past yr	Residency in community with Superfund site identified with TCE and other chemicals	Davis et al. (2005)
Case reports from eight countries of individuals with idiosyncratic generalized skin disorders	Hepatitis in 46–94% of cases; other liver effects includes hepatomegaly and elevated liver function enzymes; and in rare cases, acute liver failure	If reported, TCE, from <50 mg/m^3 to >4,000 mg/m^3; symptoms developed within 2–5 wks of initial exposure, with some intervals up to 3 mo	Kamijima et al. (2007)
Deaths in California between 1979 and 1981 due to cirrhosis	SMR of 211 (95% CI: 136, 287) for white male sheet metal workers and SMR = 174 (95% CI: 150–197) for metal workers	Occupational title on death certificate	Leigh and Jiang (1993)

Davis et al. (2005) in their analysis of subjects from the TCE subregistry of ATSDR's National Exposure Registry examined the prevalence of subjects reporting liver problems (defined as seeking treatment for the problem from a physician within the past year) using rates for the equivalent health condition from the National Health Interview Survey (a nationwide multipurpose health survey conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention). The TCE subregistry is a cohort of exposed persons from 15 sites in 5 states. The shortest time interval from inclusion in the exposure registry and last follow-up was 5 years for one site and 10 years for seven sites. Excess in past-year liver

disorders relative to the general population persisted for much of the lifetime of follow-up. SMRs for liver problems were 3rd follow-up, SMR = 2.23 (99% CI: 1.13, 3.92); 4th follow-up, SMR = 3.25 (99% CI: 1.82, 5.32); and 5th follow-up, SMR = 2.82 (99% CI: 1.46, 4.89). Examination by TCE exposure, duration, or cumulative exposure to multiple organic solvents did not show exposure-response patterns. Overall, these observations are suggestive of liver disorders as associated with potential TCE exposure, but whether TCE caused these conditions is not possible to determine given the study's limitations. These limitations include a potential for misclassification bias, the direction of which could dampen observations in a negative direction, and lack of adjustment in statistical analyses for alcohol consumption, which could bias observations in a positive direction.

Evaluation in epidemiologic studies of risk factors for cirrhosis other than alcohol consumption and Hepatitis A, B, and C is quite limited. NRC (2006) cited a case report of cirrhosis developing in an individual exposed occupationally to TCE for 5 years from a hot-process degreaser and to 1,1,1-trichloroethane for 3 months thereafter (Thiele et al., 1982). One cohort study on cirrhosis deaths in California between 1979 and 1981 and occupational risk factors as assessed using job title observed elevated risks with occupational titles of sheet metal workers and metalworkers and cirrhosis among white males who comprised the majority of deaths (Leigh and Jiang, 1993). This analysis lacks information on alcohol patterns by occupational title in addition to specific chemical exposures. Few deaths attributable to cirrhosis are reported for nonwhite male and for both white and nonwhite female metalworkers with analyses examining these individuals limited by low statistical power. Some, but not all, TCE mortality studies report risk ratios for cirrhosis (see Table 4-56). A statistically significant deficit in cirrhosis mortality was observed in three studies (Boice et al., 2006b; Boice et al., 1999; Morgan et al., 1998) and with risk ratios including a risk of 1.0 in the remaining studies (ATSDR, 2004a; Ritz, 1999a; Blair et al., 1989, 1998; Garabrant et al., 1988). These results do not rule out an effect of TCE on liver cirrhosis since disease misclassification may partly explain observations. Available studies are based on death certificates where a high degree of underreporting, up to 50%, is known to occur (Blake et al., 1988).

Table 4-56. Selected results from epidemiologic studies of TCE exposure and cirrhosis

Study population	Exposure group	RR (95% CI)	Number of observable events	Reference
Cohort and PMR-mortality				
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	0.39 (0.16, 0.80)	7	Boice et al. (2006b)
	Low cumulative TCE score	Not reported		Zhao et al. (2005)
	Medium cumulative TCE score			
	High TCE score			
	p for trend			
View-master workers				
	Males	0.76 (0.16, 2.22)	3	ATSDR (2003b)
	Females	1.51 (0.72, 2.78)	10	
Electronic workers (Taiwan)				
	Primary liver, males	Not reported		Chang et al. (2005; 2003)
	Primary liver, females	Not reported		
Uranium-processing workers				
	Any TCE exposure	0.91 (0.63, 1.28)	33	Ritz (1999a)
	Light TCE exposure, >2 yrs duration	Not reported		
	Mod TCE exposure, >2 yrs duration	Not reported		
Aerospace workers (Lockheed)				
	TCE routine exposure	0.61 (0.39, 0.91)	23	Boice et al. (1999)
	TCE routine-intermittent	Not reported	13	
Aerospace workers (Hughes)				
	TCE subcohort	0.55 (0.30, 0.93)	14	Morgan et al. (2000, 1998)
	Low intensity (<50 ppm)	0.95 (0.43, 1.80)	9	
	High intensity (>50 ppm)	0.32 (0.10, 0.74)	5	
Aircraft maintenance workers (Hill Air Force Base, Utah)				
	TCE subcohort	1.1 (0.6, 1.9)[a]	44	Blair et al. (1998)
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	0.6 (0.2, 1.3)	10	
	5–25 ppm-yr	0.8 (0.3, 1.9)	9	
	>25 ppm-yr	1.2 (0.6, 2.4)	17	

Table 4-56. Selected results from epidemiologic studies of TCE exposure and cirrhosis (continued)

Study population	Exposure group	RR (95% CI)	Number of observable events	Reference
Aircraft maintenance workers (continued)	Females, cumulative exposure			
	0	1.0[a]		Blair et al. (1998) (continued)
	<5 ppm-yr	2.4 (1.4, 13.7)	6	
	5–25 ppm-yr	1.8 (0.2, 15.0)	1	
	>25 ppm-yr	0.6 (0.1, 4.8)	1	
	TCE subcohort	1.04 (0.56, 1.93)[a,b]	37	Radican et al. (2008)
	Males, cumulative exposure	0.87 (0.43, 1.73)	31	
	0	1.0[a,b]		
	<5 ppm-yr	0.56 (0.23, 1.40)	8	
	5–25 ppm-yr	1.07 (0.45, 2.53)	10	
	>25 ppm-yr	1.06 (0.48, 2.38)	13	
	Females, cumulative exposure	1.79 (0.54, 5.93)	6	
	0	1.00[a]		
	<5 ppm-yr	3.30 (0.88, 12.41)	4	
	5–25 ppm-yr	2.20 (0.26, 18.89)	1	
	>25 ppm-yr	0.59 (0.97, 5.10)	1	
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		Not reported		Greenland et al. (1994)
U.S. Coast Guard employees				Blair et al. (1989)
	Marine inspectors	1.36 (0.79, 2.17)	17	
	Noninspectors	0.53 (0.23, 1.05)	8	
Aircraft manufacturing plant employees (Italy)				Costa et al. (1989)
	All subjects	Not reported		
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)
	All subjects	0.86 (0.67, 1.11)	63	

[a]Referent group are subjects from the same plant or company, or internal referents.
[b]Numbers of cirrhosis deaths in Radican et al. (2008) are fewer than Blair et al. (1989) because Radican et al. (2008) excluded cirrhosis deaths due to alcohol.

A number of case reports exist of liver toxicity including hepatitis accompanying immune-related generalized skin diseases described as a variation of erythema multiforme, Stevens-Johnson syndrome, toxic epiderma necrolysis patients, and hypersensitivity syndrome (Section 4.6.1.2 describes these disorders and evidence on TCE) (Kamijima et al., 2007). Kamijima et al. (2007) reported hepatitis was seen in 92–94% of cases presenting with an immune-related generalized skin diseases of variation of erythema multiforme, Stevens-Johnson syndrome, and toxic epiderma necrolysis patients, but the estimates within the hypersensitivity syndrome group were more variable (46–94%). Many cases developed with a short time after initial exposure and presented with jaundice, hepatomegaly or hepatosplenomegaly, in addition,

to hepatitis. Hepatitis development was of a nonviral etiology, as antibody titers for Hepatitis A, B, and C viruses were not detectable, and not associated with alcohol consumption (Kamijima et al., 2007; Huang et al., 2002). Liver failure was moreover a leading cause of death among these subjects. Kamijima et al. (2007) noted the similarities between specific skin manifestations and accompanying hepatic toxicity and case presentations of TCE-related generalized skin diseases and conditions that have been linked to specific medications (e.g., carbamezepine, allupurinol, antibacterial sulfonamides), possibly in conjunction with reactivation of specific latent viruses. However, neither cytomegalovirus nor Epstein-Barr viruses are implicated in the few reports that did include examination of viral antibodies.

4.5.2. Liver Cancer in Humans

Primary hepatocellular carcinoma (HCC) and cholangiocarcinoma (intrahepatic and extrahepatic bile ducts) are the most common primary hepatic neoplasms (Blechacz and Gores, 2008; El-Serag, 2007). Primary HCC is the 5th most common of cancer deaths in males and 9th in females (Jemal et al., 2008). Age-adjusted incidence rates of HCC and intrahepatic cholangiocarcinoma (ICC) are increasing, with a twofold increase in HCC over the past 20 years. This increase is higher than expected from an expanded definition of liver cancer to include primary or secondary neoplasms since International Classification of Disease (ICD)-9, incorrect classification of hilar cholangiocarcinomas in ICD-O as ICC, or to improved detection methods (El-Serag, 2007). It is estimated that 21,370 Americans will be diagnosed in 2008 with liver and intrahepatic bile cancer; age-adjusted incidence rates for liver and intrahepatic bile duct cancer for all races are 9.9 per 100,000 for males and 3.5 per 100,000 for females (Ries et al., 2008). Survival for liver and biliary tract cancers remains poor and age-adjusted mortality rates are just slightly lower than incidence rates. While hepatitis B and C viruses and heavy alcohol consumption are believed major risk factors for HCC and ICC, these risk factors cannot fully account for roughly 10 and 20% of HCC cases (Kulkarni et al., 2004). Cirrhosis is considered a premalignant condition for HCC; however, cirrhosis is not a sufficient cause for HCC since 10–25% of HCC cases lack evidence of cirrhosis at time of detection (Kumar et al., 2007; Fattovich et al., 2004; Chiesa et al., 2000). Nonalcoholic steatohepatitis reflecting obesity and metabolic syndrome is recently suggested as contributing to liver cancer risk (El-Serag, 2007).

All cohort studies, except Zhao et al. (2005), present risk ratios (SIRs or SMRs) for liver and biliary tract cancer. More rarely reported in cohort studies are risk ratios for primary liver cancer (HCC) or for gallbladder and extrahepatic bile duct cancer. Four community studies also presented risk ratios for liver and biliary tract cancer including a case-control study of primary liver cancer of residents of Taiwanese community with solvent-contaminated drinking water wells (ATSDR, 2006a; Lee et al., 2003; Morgan and Cassady, 2002; Vartiainen et al., 1993). Several population case-control studies examine liver cancer and organic solvents or occupational job titles with possible TCE usage (Lindbohm et al., 2009; Ji and Hemminki, 2005;

Kvam et al., 2005; Weiderpass et al., 2003; Porru et al., 2001; Heinemann et al., 2000; Døssing et al., 1997; Hernberg et al., 1988; Austin et al., 1987; Hardell et al., 1984; Hernberg et al., 1984; Stemhagen et al., 1983); however, the lack of detailed exposure assessment to TCE, specifically in the population case-control studies as well as in geographic-based studies, or too few exposed cases and controls in those studies that do present some information limits their usefulness for evaluating hepatobiliary or gall bladder cancer and TCE exposure. Table 4-57 presents observations from cohort, case-control, and community studies on liver and biliary tract cancer, primary liver, and gallbladder and extrahepatic bile duct cancer and TCE.

Excess liver cancer incidence is observed in most studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis were identified (Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994) as is mortality (Radican et al., 2008; Boice et al., 2006b; ATSDR, 2004a; Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998). Risks for primary liver cancer and for gallbladder and biliary tract cancers in females were statistically significantly elevated only in Raaschou-Nielsen et al. (2003), the study with the largest number of observed cases without suggestion of exposure duration-response patterns. Cohort studies with more uncertain exposure assessment approaches, e.g., studies of all subjects working at a factory (Chang et al., 2005; Chang et al., 2003; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988), do not show association but are quite limited given their lacking attribution of who may have higher or lower exposure potentials. Ritz (1999a), the exception, found evidence of an exposure-response relationship; mortality from hepatobiliary cancer was found to increase with degree and duration of exposure and time since first exposure with a statistically significant but imprecise (wide CIs) liver cancer risk for those with the highest exposure and longest time since first exposure. This observation is consistent with association with TCE, but with uncertainty given one TCE exposed case in the highest exposure group and correlation between TCE, cutting fluids, and radiation exposures.

Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Cohort and PMR studies—incidence								
Aerospace workers (Rocketdyne)								
	Low cumulative TCE score	Not reported						Zhao et al. (2005)
	Medium cumulative TCE score	Not reported						
	High TCE score	Not reported						
	p for trend							
Danish blue-collar workers with TCE exposure								
	Males + females	1.3 (1.0, 1.6)[a]	82					Raaschou-Nielson et al. (2003)
	Males + females	1.4 (1.0, 1.8)[b]	57					
	Males, any exposure	1.1 (0.8, 1.5)[b]	41	1.1 (0.7, 1.6)	27	1.1 (0.6, 1.9)	14	
	<1-yr employment duration	1.2 (0.7, 2.1)[b]	13	1.3 (0.6, 2.5)	9	1.1 (0.3, 2.9)	4	
	1–4.9-yr employment duration	0.9 (0.5, 1.6)[b]	13	1.0 (0.5, 1.9)	9	0.8 (0.2, 2.1)	4	
	≥5-yr employment duration	1.1 (0.6, 1.7)[b]	15	1.1 (0.5, 2.1)	9	1.4 (0.5, 3.1)	6	
	Females, any exposure	2.8 (1.6, 4.6)[b]	16	2.8 (1.1, 5.8)	7	2.8 (1.3, 5.3)	9	
	<1-yr employment duration	2.5 (0.7, 6.5)[b]	4	2.8 (0.3, 10.0)	2	2.3 (0.3, 8.4)	2	
	1–4.9-yr employment duration	4.5 (2.2, 8.3)[b]	10	4.1 (1.1, 10.5)	4	4.8 (1.7, 10.4)	6	
	≥5-yr employment duration	1.1 (0.1, 3.8)[b]	2	1.3 (0.0, 7.1)	1	0.9 (0.0, 5.2)	1	

Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Biologically-monitored Danish workers								
	Males + females	2.1 (0.7, 5.0)[b]	5	1.7 (0.2, 6.0)	2	2.5 (0.5, 7.3)	3	Hansen et al. (2001)
	Males	2.6 (0.8, 6.0)[b]	5	1.8 (0.2, 6.6)	2	3.3 (0.7, 9.7)	3	
	Females		0 (0.4 exp)		0 (0.1 exp)		0 (0.3 exp)	
	Cumulative exposure (Ikeda)	Not reported						
	<17 ppm-yr							
	≥17 ppm-yr							
	Mean concentration (Ikeda)	Not reported						
	<4 ppm							
	4+ ppm							
	Employment duration	Not reported						
	<6.25 yrs							
	≥6.25							

Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Aircraft maintenance workers from Hill Air Force Base								
	TCE subcohort	Not reported	9	Not reported				Blair et al. (1998)
	Males, cumulative exposure							
	0	1.0[c]		1.03				
	<5 ppm-yr	0.6 (0.1, 3.1)	3	1.2 (0.1, 2.1)	2			
	5–25 ppm-yr	0.6 (0.1, 3.8)	2	1.0 (0.1, 16.7)	1			
	>25 ppm-yr	1.1 (0.2, 4.8)	4	2.6 (0.3, 25.0)	3			
	Females, cumulative exposure		0		0			
Biologically-monitored Finnish workers								
	All subjects	1.89 (0.86, 3.59)[b]	9	2.27 (0.74, 5.29)	5	1.56 (0.43, 4.00)	4	Anttila et al. (1995)
	Mean air-TCE (Ikeda extrapolation from U-TCA)							
	<6 ppm	Not reported		1.64 (0.20, 5.92)	2			
	6+ ppm			2.74 (0.33, 9.88)	2			
Biologically-monitored Swedish workers								
	Males	1.41 (0.38, 3.60)[b]	4					Axelson et al. (1994)
	Females	Not reported						
Cohort and PMR-mortality								
Computer manufacturing workers (IBM), New York		Not reported	1					Clapp and Hoffman (2008)

Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Aerospace workers (Rocketdyne)								
	Any TCE (utility/eng flush)	1.28 (0.35, 3.27)	4					Boice et al. (2006b)
	Low cumulative TCE score	Not reported						Zhao et al. (2005)
	Med cumulative TCE score							
	High TCE score							
	p for trend							
View-Master workers								
	Males	2.45 (0.50, 7.12)[d]	3	1.01 (0.03, 5.63)[d]	1	8.41 (1.01, 30.4)[d]	2	ATSDR (2003b)
	Females		0 (2.61 exp)		0 (1.66 exp)		0 (0.95 exp)	
Electronic workers (Taiwan)								
	Primary liver, males	Not reported			0 (0.69 exp)			Chang et al. (2005; 2003)
	Primary liver, females	Not reported			0 (0.57 exp)			

Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Uranium-processing workers								
	Any TCE exposure	Not reported						Ritz (1999a)
	Light TCE exposure, >2 yr-duration	0.93 (0.19, 4.53)[e]	3					
	Mod TCE exposure, >2 yr-duration	4.97 (0.48, 51.1)[e]	1					
	Light TCE exposure, >5 yr-duration	2.86 (0.48, 17.3)[f]	3					
	Mod TCE exposure, >5 yr-duration	12.1 (1.03, 144)[f]	1					
Aerospace workers (Lockheed)								
	TCE routine exposure	0.54 (0.15, 1.38)	4					Boice et al. (1999)
	TCE routine-intermittent							
	0 yr	1.00[c]	22					
	Any exposure	Not reported	13					
	<1 yr	0.53 (0.18, 1.60)	4					
	1–4 yrs	0.52 (0.15, 1.79)	3					
	≥5 yrs	0.94 (0.36, 2.46)	6					
	p for trend	>0.20						

Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Aerospace workers (Hughes)								
	TCE subcohort	0.98 (0.36, 2.13)	6					Morgan et al. (2000, 1998)
	Low intensity (<50 ppm)[e]	1.32 (0.27, 3.85)	3					
	High intensity (>50 ppm)[e]	0.78 (0.16, 2.28)	3					
	TCE subcohort (Cox analysis)							
	Never exposed	1.00[c]	14					
	Ever exposed	1.48 (0.56, 3.91)[g,h]	6					
	Cumulative							
	Low	2.12 (0.59, 7.66)[h]	3					
	High	1.19 (0.34, 4.16)[h]	3					
	Peak							
	No/low	1.00[c]	17					
	Medium/high	0.98 (0.29, 3.35)[h]	3					

Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Aircraft maintenance workers (Hill Air Force Base, Utah)								Blair et al. (1998)
	TCE subcohort	1.3 (0.5, 3.4)[c]	15	1.7 (0.2, 16.2)3	4			
	Males, cumulative exposure							
	0	1.0[c]						
	<5 ppm-yr	1.1 (0.3, 4.1)	6					
	5–25 ppm-yr	0.9 (0.2, 4.3)	3					
	>25 ppm-yr	0.7 (0.2, 3.2)	3					
	Females, cumulative exposure							
	0	1.0[c]						
	<5 ppm-yr	1.6 (0.2, 18.2)	1					
	5–25 ppm-yr		0					
	>25 ppm-yr	2.3 (0.3, 16.7)	2					
	TCE subcohort	1.12 (0.57, 2.19)[c,i]	31	1.25 (0.31, 4.97)[c,i]	8			Radican et al. (2008)
	Males, cumulative exposure	1.36 (0.59, 3.11)[c]	28	2.72 (0.34, 21.88)[c]	8			
	0	1.0[c]		1.03				
	<5 ppm-yr	1.17 (0.45, 3.09)	10	3.28 (0.37, 29,45)	4			
	5–25 ppm-yr	1.16 (0.39, 3.46)	6		0			
	>25 ppm-yr	1.72 (0.68, 4.38)	12	4.05 (0.45, 36.41)	4			

Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Aircraft maintenance workers (continued)	Females, cumulative exposure	0.74 (0.18, 2.97)[c]	3		0			Radican et al. (2008) (continued)
	0	1.03						
	<5 ppm-yr	0.69 (0.08, 5.74)	1					
	5–25 ppm-yr		0					
	>25 ppm-yr	0.98 (0.20, 4.90)	2					
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		0.54 (0.11, 2.63)[j]	9					Greenland et al. (1994)
U.S. Coast Guard employees								
	Marine inspectors	1.12 (0.23, 3.26)	3					Blair et al. (1989)
	Noninspectors	Not reported	0 (2 exp)					
Aircraft manufacturing plant employees (Italy)								
	All subjects	0.70 (0.23, 1.64)	5					Costa et al. (1989)
Aircraft manufacturing plant employees (San Diego, California)								
	All subjects	0.94 (0.40, 1.86)	8					Garabrant et al. (1988)
Case-control studies								
Residents of community with contaminated drinking water (Taiwan)								
	Village of residency, males							Lee et al. (2003)
	Upstream	1.00						
	Downstream	2.57 (1.21, 5.46)	26					

Table 4-57. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	Reference
Geographic studies								
Residents in two study areas in Endicott, New York		0.71 (0.09, 2.56)	<6					ATSDR (2006a)
Residents in 13 census tracts in Redlands, California		1.29 (0.74, 2.05)[k]	28					Morgan and Cassidy (2002)
Finnish residents								
	Residents of Hausjarvi	0.7 6 (0.3, 1.4)	7					Vartiainen et al. (1993)
	Residents of Huttula	0.6 (0.2, 1.3)	6					

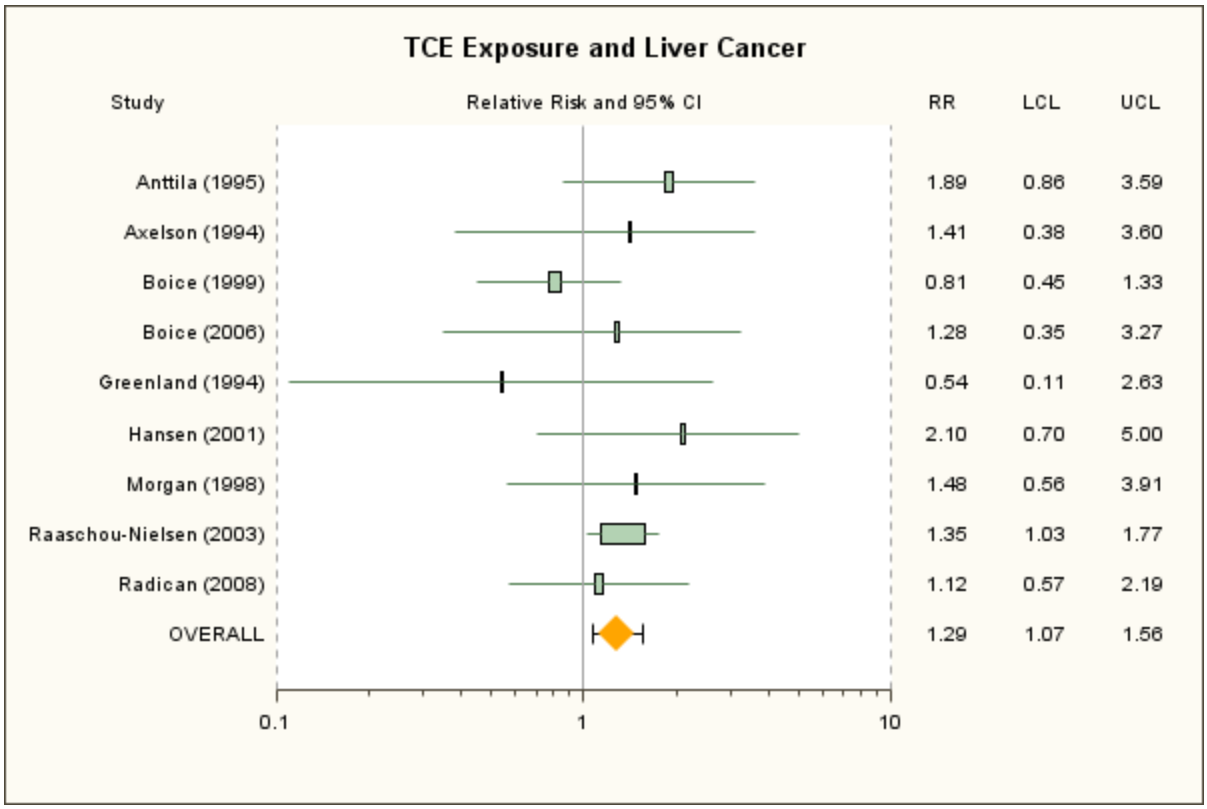
[a]ICD-7, 155 and 156; primary liver (155.0), gallbladder, and biliary passages (155.1), and liver secondary and unspecified (156).
[b]ICD-7, 155; primary liver, gallbladder, and biliary passages.
[c]Internal referents, workers without TCE exposure.
[d]PMR.
[e]Logistic regression analysis with a 0-year lag for TCE exposure.
[f]Logistic regression analysis with a 15-year lag for TCE exposure.
[g]Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex, and decade in Environmental Health Strategies (1997).
[h]Morgan et al. (1998) do not identify if SIR is for liver and biliary passage or primary liver cancer; identified as primary liver in NRC (2006).
[i]Radican et al. (2008) provide results for TCE exposure for follow-up through 1990, comparing the Poisson model rate ratios as reported by Blair et al. (1998) with Cox model hazard ratios. RR from Cox model adjusted for age and gender for liver and intrahepatic bile duct cancer was 1.2 (95% CI: 0.5, 3.4) and for primary liver cancer was 1.3 (95% CI: 0.1, 12.0).
[j]OR.
[k]99% CIs.

exp = expected

Observations in these studies provide some evidence of susceptibility of liver, gallbladder, and biliary tract; these observations are consistent with pharmacokinetic processing of TCE and the extensive intra- and extrahepatic recirculation of metabolites. Magnitude of risk of gallbladder and biliary tract cancer is slightly higher than that for primary liver cancer in Raaschou-Nielsen et al. (2003), the study with the most cases. Observations in Blair et al. (1998), Hansen et al. (2001), and Radican et al. (2008), three smaller studies, suggest slightly larger risk ratios for primary liver cancer compared to gallbladder and biliary tract cancer. Overall, these studies are not highly informative for cross-organ comparison of relative magnitude of susceptibility.

The largest geographic studies (Lee et al., 2003; Morgan and Cassady, 2002) are also suggestive of association with the risk ratio (mortality OR) in Lee et al. (2003) as statistically significantly elevated. The geographic studies do not include a characterization of TCE exposure to individual subjects other than residency in a community with groundwater contamination by TCE with potential for exposure misclassification bias dampening observations; these studies lack characterization of TCE concentrations in drinking water and exposure characteristics such as individual consumption patterns. For this reason, observations in Morgan and Cassidy (2002) and Lee et al. (2003) are noteworthy, particularly if positive bias leading to false positive finding is considered minimal, and the lack of association with liver cancer in the two other community studies (ATSDR, 2006a; Vartiainen et al., 1993) does not detract from Morgan and Cassidy (2002) or Lee et al. (2003). Lee et al. (2003), however, do not address possible confounding related to hepatitis viral infection status, a risk factor for liver cancer, or potential misclassification due to the inclusion of secondary liver cancer among the case series, factors which may amplify observed association.

Meta-analysis is adopted as a tool for examining the body of epidemiologic evidence on liver cancer and TCE exposure, to identify possible sources of heterogeneity and as an additional means to identify cancer hazard. The meta-analyses of the overall effect of TCE exposure on liver (and gall bladder/biliary passages) cancer suggest a small, statistically significant increase in risk. The summary estimate from the primary random effects meta-analysis of the 9 (all cohort) studies is 1.29 (95% CI: 1.07, 1.56) (see Figure 4-3). The study of Raaschou-Nielsen et al. (2003) contributes about 57% of the weight; its removal from the analysis decreases somewhat the RRm estimate and is no longer statistically significant (RRm = 1.22; 95% CI: 0.93, 1.61). The summary estimate was not overly influenced by any other single study, nor was it overly sensitive to individual RR estimate selections. There is no evidence of publication bias in this data set, and no observable heterogeneity (I^2 = 0%) across the study results.



Random effects model; fixed effect model same. The summary estimate is in the bottom row, represented by the diamond. Symbol sizes reflect relative weights of the studies.

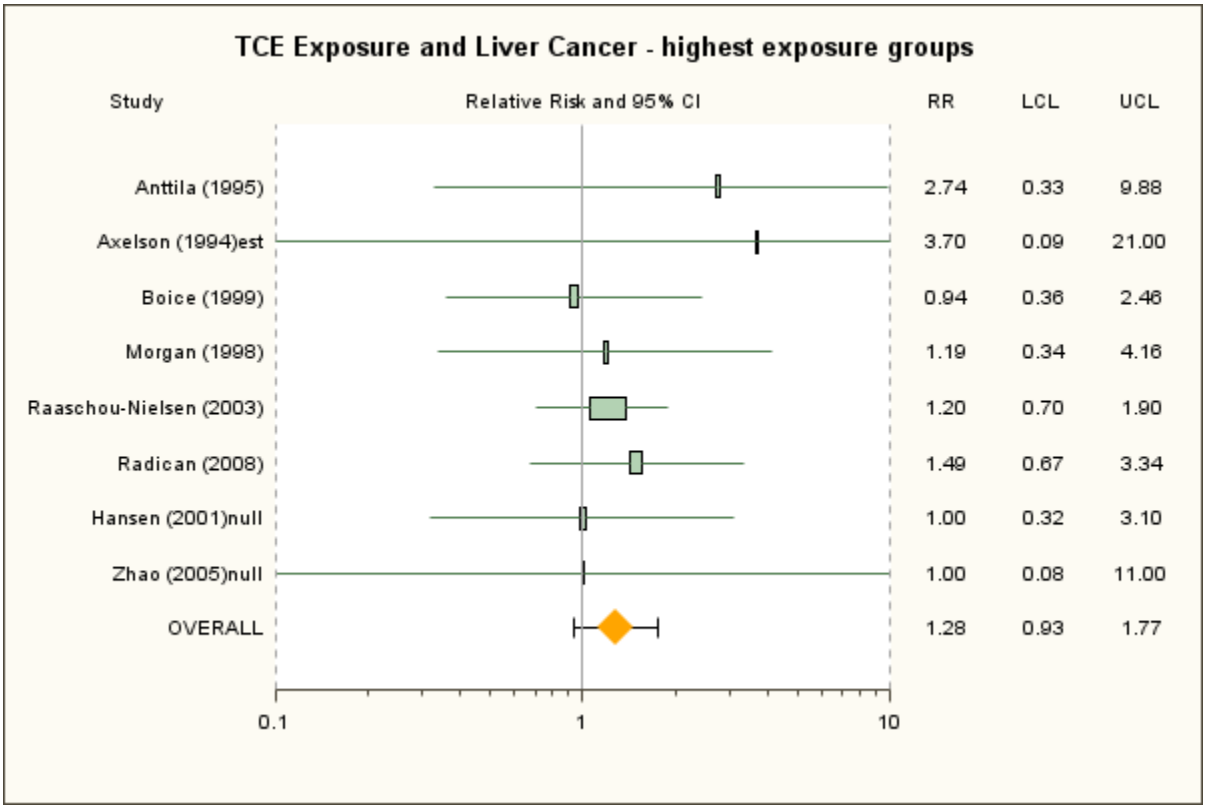
Figure 4-3. Meta-analysis of liver and biliary tract cancer and overall TCE exposure.

Examination of sites individually (i.e., primary liver and intrahepatic bile ducts separate from the combined liver and gallbladder/biliary passage grouping) resulted in the RRm estimate for liver cancer alone (for the three studies for which the data are available; for the other studies, results for the combined grouping were used) slightly lower than the one based entirely on results from the combined cancer categories and was just short of statistical significance (1.25; 95% CI: 0.99, 1.57). This result is driven by the fact that the risk ratio estimate from the large Raaschou-Nielsen et al. (2003) study decreased from 1.35 for liver and gall bladder/biliary passage cancers combined to 1.28 for liver cancer alone.

The RRm estimate from the random effects meta-analysis of liver cancer in the highest exposure groups in the six studies that provide risk estimates associated with highest exposure.

Primary liver cancer is 1.32 (95% CI: 0.93, 1.86), slightly lower than the RRm estimate for liver and gallbladder/biliary cancer and any TCE exposure of 1.33 (95% CI: 1.09, 1.64), and not statistically significant (see Figure 4-4). Again, the RRm estimate of the highest-exposure groups is dominated by one study (Raaschou-Nielsen et al., 2003). Two studies lack reporting of liver cancer risk associated with highest exposure, so consideration of reporting bias (considered the primary analysis) lead to a result of 1.28 (95% CI: 0.93, 1.77), similar to that estimated in the

more restricted set of studies presenting risk ratios association with highest exposure groups in published papers.



Random effects model; fixed effect model same. The summary estimate is in the bottom row, represented by the diamond. Symbol sizes reflect relative weights of the studies. Assumed null RR estimates for Hansen and Zhao (see Appendix C text).

Figure 4-4. Meta-analysis of liver cancer and TCE exposure—highest exposure groups.

Different exposure metrics are used in the various studies, and the purpose of combining results across the different highest exposure groups is not to estimate an RRm associated with some level of exposure, but rather to examine impacts of combining RR estimates that should be less affected by exposure misclassification. In other words, the highest exposure category is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any vs. none) exposure comparison. Thus, if TCE exposure increases the risk of liver and gallbladder/biliary cancer, the effects should be more apparent in the highest exposure groups. The findings of a lower RRm associated with highest exposure group reflects observations in Radican et al. (2008) and Raaschou-Nielsen et al. (2003), the study contributing greatest weight to the meta-analysis, that

RR estimates for the highest-exposure groups, although >1.0, are less than the RR estimates with any TCE exposure.

Thus, while the finding of an elevated and statistically significant RRm for liver and gallbladder/biliary cancer and any TCE exposure provides evidence of association, the statistical significance of the summary estimates is dependent on one study, which provides the majority of the weight in the meta-analyses. Furthermore, combining results from the highest-exposure groups yields lower RRm estimates than for an overall effect. These results do not rule out an effect of TCE on liver cancer, because the liver cancer results are relatively underpowered with respect to numbers of studies and number of cases; overall, the meta-analysis provides only minimal support for association between TCE exposure and liver and gallbladder/biliary cancer.

NRC (2006) deliberations on TCE commented on two prominent evaluations of the then-current TCE epidemiologic literature using meta-analysis techniques, Wartenberg et al. (2000) and Kelsh et al. (2005), submitted by Exponent-Health Sciences to NRC during their deliberations and published afterwards in the open literature as Alexander et al. (2007a) adding the than-published study of Boice et al. (2006b). NRC (2006) found weaknesses in the techniques used in Wartenberg et al. (2000) and the Exponent analyses. EPA staff conducted their analysis according to NRC (2006) suggestions for transparency, systematic review criteria, and examination of both cohort and case-control studies. The EPA analysis of liver cancer considered a similar set of studies as Alexander et al. (2007a), although treatment of these studies differs between analyses. Alexander et al. (2007a) in their Table 2, for example, present RRm estimates, grouping of studies with differing exposure potentials, for example, including liver and biliary cancer risk estimates for all subjects, those exposed and unexposed to TCE, in Boice et al.(1999), Blair et al. (1998), Morgan et al. (1998), and Boice et al. (2006b), with biomarker studies (Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994). The inclusion of risk estimates for subjects who have little to no TCE exposure over background levels has the potential to introduce misclassification bias and dampen observed risk ratios. Potential bias from exposure misclassification may be substantial in Alexander et al. (2007a) since the percentage of TCE exposed subjects to all cohort subjects in the four studies was 3, 23, 51 and 68% in Boice et al. (1999), Morgan et al. (1998), Blair et al. (1998), and Boice et al. (2006b), respectively, and is a likely alternative explanation for observed inconsistency across occupational groups reported by the authors. Another difference between the EPA and previous meta-analyses is their treatment of Ritz (1999a), included in Wartenberg et al. (2000), Kelsh et al. (2005), and Alexander et al. (2007a), but not in this analysis. For a grouping of studies with subcohorts most similar to those in EPA‘s analysis, summary liver and gall bladder/biliary tract cancer risk estimates for overall TCE exposure for TCE subcohorts is of a similar magnitude as that observed in EPA‘s updated and expanded analysis, Wartenberg et al. (2000), 1.1 (95% CI: 0.3, 4.8) for incidence and 1.1 (95% CI: 0.7, 1.7) for mortality, Kelsh et al. (2005), 1.32 (95% CI: 1.05, 1.66) and Alexander et al. (2007a), 1.30 (95% CI: 1.09–1.55).

4.5.3. Experimental Studies of TCE in Rodents—Introduction

The previous sections have described available human data for TCE-induced noncancer effects (e.g., disturbances in bile production) and whether an increased risk of liver cancer in humans has been established from analysis of the epidemiological literature. A primary concern for effects on the liver comes from a large database in rodents indicating that, not only TCE, but also a number of its metabolites are capable of inducing hepatocellular adenomas and carcinomas in rodent species. Thus, many of rodent bioassays have focused on the study of liver cancer for TCE and its metabolites and possible early effects specifically that may be related to tumor induction.

This section describes the hazard data for TCE effects in the rodent liver and inferences from studies of its metabolites. For more detailed descriptions of the issues providing context for these data in terms the state of the science of liver physiology (see Section E.1), cancer (see Section E.3), liver cancer (see Section E.3), and the mode of action of liver cancer and other TCE-induced effects (see Section E.3.4), please see Appendix E. A more comprehensive review of individual studies of TCE-induced liver effects in laboratory animals is also provided in Section E.2 that includes detailed analyses of the strengths and the limitations of these studies. Issues have been raised regarding the relevance of mouse liver tumor data to human liver cancer risk that are addressed in Sections E.3.2 and E.3.3. Given that activation of the PPARα receptor has received great attention as a potential mode of action for TCE-induced liver tumors, the current status of that hypothesis is reviewed in Section E.3.4.1. Finally, comparative studies of TCE metabolites and the similarities and differences of such study results are described in summary sections of Appendix E (i.e., Section E.2.4) as well as discussions of proposed modes of action for TCE-induced liver cancer (i.e., Sections E.2.4 and E.3.4.2).

A number of acute and subchronic studies have been undertaken to describe the early changes in the rodent liver after TCE administration, with the majority using the gavage route of administration. Several key issues affect the interpretation of these data. The few drinking water studies available for TCE have recorded a significant loss of TCE through volatilization in drinking water solutions and thus, this route of administration is generally not used. Some short-term studies of TCE have included detailed examinations, while others have reported primarily liver weight changes as a marker of TCE response. The matching and recording of age, but especially initial and final body weight, for control and treatment groups is of particular importance for studies using liver weight gain as a measure of TCE response as differences in these parameters affect TCE-induced liver weight gain. Most data are for TCE exposures of at least 10–42 days. For many of the subchronic inhalation studies (Kjellstrand et al., 1983a; Kjellstrand et al., 1983b; Kjellstrand et al., 1981b), issues associated with whole-body exposures make determination of dose levels more difficult. The focus of the long-term studies of TCE is primarily detection and characterization of liver tumor formation.

For gavage experiments, death due to gavage errors and specifically from use of this route of administration, especially at higher TCE exposure concentrations, has been a recurring problem, especially in rats. Unlike inhalation exposures, the effects of vehicle can also be an issue for background liver effects in gavage studies. Concerns regarding effects of oil vehicles, especially corn oil, have been raised (Charbonneau et al., 1991; Kim et al., 1990a). Several oral studies in particular document that use of corn oil as the vehicle for TCE gavage dosing induces a different pattern of toxicity, especially in male rodents (see Merrick et al., 1989, Section E.2.2.1). Several studies also report the effects of corn oil on hepatocellular DNA synthesis and indices of lipid peroxidation (Rusyn et al., 1999; Channel et al., 1998). For example, Rusyn et al. (1999) report that a single dose of dietary corn oil increases hepatocyte DNA synthesis 24 hours after treatment by ~3.5-fold of control, activates of NF-κB to a similar extent ~2 hours after treatment almost exclusively in Kupffer cells, and induces an approximate three- to fourfold increase of control NF-κB in hepatocytes after 8 hours and an increase in tumor necrosis factor (TNF)-α mRNA between 8 and 24 hours after a single dose in female rats.

In regard to studies that have used the i.p. route of administration, as noted by Kawamoto et al. (1988b), injection of TCE may result in paralytic ileus and peritonitis and that subcutaneous treatment paradigm will result in TCE not immediately being metabolized but retained in the fatty tissue. Wang and Stacey (1990) state that "intraperitoneal injection is not particularly relevant to humans" and suggest that intestinal interactions require consideration in responses such as increase serum bile acid.

While studies of TCE metabolites have been almost exclusively conducted via drinking water, and thus, have avoided vehicle effects and gavage error, they have issues of palatability at high doses and decreased drinking water consumption as a result that raises issues not only of the resulting internal dose of the agent, but also of effects of drinking water reduction.

Although there are data for both mice and rats for TCE exposure and studies of its metabolites, the majority of the available information has been conducted in mice. This is especially the case for long-term studies of DCA and TCA in rats. There is currently one study each available for TCA and DCA in rats and both were conducted with such few numbers of animals that the ability to detect and discern whether there was a treatment-related effect are very limited (DeAngelo et al., 1997, 1996; Richmond et al., 1995).

With regard to the sensitivity of studies used to detect a response, there are issues regarding not only the number of animals used, but also the strain and weight of the animals. For some studies of TCE strains were used that have less background rate of liver tumor development and carcinogenic response. As for the $B6C3F_1$ mouse, the strain most used in the bioassays of TCE metabolites, the susceptibility of the $B6C3F_1$ to hepatocarcinogenicity has made the strain a sensitive biomarker for a variety of hepatocarcinogens. Moreover, Leakey et al. (2003a) demonstrated that increased body weight at 45 weeks of life is an accurate predictor of large background tumor rates. Unfortunately a 2-year study of CH (George et al.,

2000) and the only available 2-year study of TCA (DeAngelo et al., 2008), which used the same control animals, were both conducted in B6C3F$_1$ mice that grew very large (~50 g) and prone to liver cancer (64% background incidence of hepatocellular adenomas and carcinomas) and premature mortality. Thus, these bioassays are of limited value for determination of the dose-response for carcinogenicity.

Finally, as discussed below, the administration of TCE to laboratory animals as well as environmental exposure of TCE in humans are effectively co-exposure studies. TCE is metabolized to a number of hepatoactive as well as hepatocarcinogenic agents. A greater variability of response is expected than from exposure to a single agent, making it particularly important to look at the TCE database in a holistic fashion rather than the results of a single study, especially for quantitative inferences. This approach is particularly useful given that the number of animals in treatment groups in a variety of TCE and TCE metabolite studies have been variable and small for control and treatment groups. Thus, their statistical power was limited not only for detection of statistically significant changes, but also, in many cases, to be able to determine whether there is not a treatment related effect (i.e., Type II error for power calculation). Section E.2.4.2 provides detailed analyses of the database for liver weight induction by TCE and its metabolites in mice and the results of those analyses are described below. Specifically, the relationship of liver weight induction, but also other endpoints such as peroxisomal enzyme activation and increases in DNA synthesis to liver tumor responses are also addressed as well.

4.5.4. TCE-Induced Liver Noncancer Effects

A number of effects have been studied as indicators of TCE effects on the liver but also as proposed events whose sequellae could be associated with resultant liver tumors after chronic TCE exposure in rodents. Similar effects have been studied in rodents exposed to TCE metabolites, which may be useful for determining not only whether such effects are associated with liver tumors induced by these metabolites but also if they are similar to what has been observed for TCE. Summaries of the laboratory animal studies of TCE noncancer effects in the liver are provided in Table 4-58 (oral studies) and Table 4-59 (inhalation studies), along with the types of effects discussed in the subsections below for each study.

Table 4-58. Oral studies of TCE-induced liver effects in mice and rats

Reference[a]	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Section(s) where noncancer liver effects are discussed
Berman et al. (1995)	F344 rats (F)	Corn oil gavage	0, 150, 500, 1,500, or 5,000 mg/kg for 1 d 0, 50, 150, 500, or 1,500 mg/kg-d for 14 d	8/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology E.2.1.11. Berman et al. (1995)
Buben and O'Flaherty (1985)	**Swiss-Cox mice (M)**	**Corn oil gavage**	**0, 100, 200, 400, 800, 1,600, 2,400, or 3.200 mg/kg-d, 5 d/wk for 6 wks**	**12–15/ group**	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology E.2.2.7. Buben and O'Flaherty (Buben and O'Flaherty, 1985)
Channel et al. (1998)	$B6C3F_1$/CrlBR mice (M)	Corn oil gavage	0 (water), 0 (corn oil), 400, 800, or 1,200 mg/kg-d, 5 d/wk for up to 8 wks	77/group	4.5.4.2 Cytotoxicity and histopathology 4.5.4.3 Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis 4.5.4.4 Peroxisome proliferation and related effects 4.5.4.5 Oxidative stress E.2.2.8. Channel et al. (1998)
Dees and Travis (1993)	$B6C3F_1$ mice (M and F)	Corn oil gavage	0, 100, 250, 500, or 1,000 mg/kg-d for 10 d	5/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology 4.5.4.3 Measures of DNA Synthesis E.2.1.9. Dees and Travis (1993)
Elcombe et al. (1985)	$B6C3F_1$ and Alderley Park (Swiss) mice (M) Osborne-Mendel and Alderley Park (Wistar) rats (M)	Corn oil gavage	0, 500, 1,000, or 1,500 mg/kg-d for 10 d	6–10/ group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology 4.5.4.3 Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis 4.5.4.4 Peroxisome proliferation and related effects E.2.1.8. Elcombe et al. (1985)
Goel et al. (1992)	Swiss albino mice (M)	Groundnut oil gavage	0, 500, 1,000, or 2,000 mg/kg-d, 5 d/wk for 28 d	6/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology 4.5.4.4 Peroxisome proliferation and related effects E.2.2.2. Goel et al. (1992)

Table 4-58. Oral studies of TCE-induced liver effects in mice and rats (continued)

Reference[a]	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Section(s) where noncancer liver effects are discussed
Goldsworthy and Popp (1987)	F344 rats (M) B6C3F$_1$ mice (M)	Corn oil or methyl cellulose gavage	1,000 mg/kg-d for 10 d	5–7/group	4.5.4.1 Liver weight 4.5.4.4 Peroxisome proliferation and related effects E.2.1.7. Goldsworthy and Popp (1987)
Laughter et al. (2004)	Sv/129 and PPARα-null mice (M)	Methyl-cellulose gavage	0–1,500 mg/kg-d for 3 d; and 5 d/wk for 3 wks	4–5/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology 4.5.4.3 Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis 4.5.4.4 Peroxisome proliferation and related effects E.2.1.13. Laughter et al. (2004)
Melnick et al. (1987)	F344 rats (M)	Micro-encapsulated in feed Corn oil gavage	0, 0.055, 1.10, 2.21, or 4.41% in feed for 14 d, equivalent to 0, 600, 1.300, 2.200, or 4.800 mg/kg-d	10/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology 4.5.4.4 Peroxisome proliferation and related effects E.2.1.12. Melnick et al. (1987)
Merrick et al. (1989)	B6C3F$_1$ mice (M and F)	Corn oil and 20% Emulphor in water gavage	Males: 0, 600, 1,200, or 2,400 mg/kg-d Females: 0, 450, 900, or 1,800 mg/kg-d	12/group	4.5.4.1 Liver weight 4.5.4.2 Cytotoxicity and histopathology E.2.2.1. Merrick et al. (1989)
Mirsalis et al. (1989)	B6C3F$_1$ mice (M and F) F344 rats (M)	Corn oil gavage	0, 50, 200, or 1,000 mg/kg (single dose)	3/group	4.5.4.3 Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis E.2.4.1. Summary of Results for Short-term Effects of TCE
Nakajima et al. (2000)	Sv/129 and PPARα-null mice (M and F)	Corn oil gavage	0 or 750 mg/kg-d for 14 d	6/sex/ group	4.5.4.1 Liver weight 4.5.4.4 Peroxisome proliferation and related effects E.2.1.10. Nakajima et al. (2000)
NTP (1990)	B6C3F$_1$ mice (M and F) F334/N rats (M and F)	Corn oil gavage	Mice: 0, 375–6,000 mg/kg-d, 5 d/wk, 13 wks Rats: 0, 62.5–1,000 mg/kg-d, 5 d/wk, 13 wks	10/group	4.5.4.2 Cytotoxicity and histopathology E.2.2.12.1 13-wk studies
NTP (1990)	B6C3F$_1$ mice (M and F) F334/N rats (M and F)	Corn oil gavage	Mice: 0, or 1,000 mg/kg-d, 5 d/wk, 103 wks Rats: 0, 500, or 1,000 mg/kg-d, 5 d/wk, 103 wks	50/group	4.5.4.2 Cytotoxicity and histopathology E.2.2.12.2 2-yr studies

Table 4-58. Oral studies of TCE-induced liver effects in mice and rats (continued)

Reference[a]	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Section(s) where noncancer liver effects are discussed
Nunes et al. (2001)	Sprague-Dawley rats (M)	Corn oil gavage	2,000 mg/kg-d on d 10–16 (with and without lead carbonate pretreatment for 9 d)	10/group	4.5.4.1. Liver weight 4.5.4.2. Cytotoxicity and histopathology E.2.1.4. Nunes et al. (2001)
Tao et al. (2000)	B6C3F$_1$ mice (F)	Corn oil gavage	1,000 mg/kg-d for 5 d	4–6/group	4.5.4.1. Liver weight E.2.1.5. Tao et al., (2000)
Tucker et al. (1982)	CD-1 mice (M and F)	Drinking water with 1% Emulphor	0 (untreated), 0 (vehicle), 0.1, 1.0, 2.5, or 5 mg/mL for 4 or 6 mo M: 0, 0, 18.4, 216.7, 393.0, or 660.2 mg/kg-d F: 0, 0, 17.9, 193.0, 437.1, or 793.3 mg/kg-d	140/group untreated and TCE-treated 260/group vehicle-treated	4.5.4.1. Liver weight E.2.1.6. Tucker et al. (1982)

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

Table 4-59. Inhalation and i.p. studies of TCE-induced liver effects in mice and rats

Reference[a]	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Section(s) where noncancer liver effects are discussed
Hamdan and Stacey (1993)	Sprague-Dawley rats (M)	i.p. in corn oil	0 or 131 mg/kg	6/group	4.5.4.6. Bile production E.2.6. Serum Bile Acid Assays
Kaneko et al. (2000)	MRL-lpr/lpr mice (M)	Inhalation	0, 500, 1,000, or 2,000 ppm, 4 hrs/d, 6 d/wk, for 8 wks	5/group	4.5.4.2. Cytotoxicity and histopathology
Kjellstrand et al. (1981b)	NMRI mice. Sprague-Dawley rats Mongolian gerbils	Inhalation	150 ppm continuous for 2–30 d	4–12/group	4.5.4.1. Liver weight E.2.2.3. Kjellstrand et al., (1981b)
Kjellstrand et al. (1983b)	wild, C57Bl, DBA, B6CBA, A/sn, NZB, and NMRI mice (M and F)	Inhalation	150 ppm continuous for 30 d	6/group	4.5.4.1. Liver weight E.2.2.5. Kjellstrand et al., (1983b)
Kjellstrand et al. (1983a)	**NMRI mice (M and F)**	**Inhalation**	**0–3,600 ppm, variable time periods of 1–24 hrs/d, for 30 or 120 d.**	**10–20/ group**	**4.5.4.1. Liver weight** **4.5.4.2. Cytotoxicity and histopathology** **E.2.2.6. Kjellstrand et al., (1983a)**
Kumar et al. (2001a)	Wistar rats (M)	Inhalation	376 ppm, 4 hrs/d, 5 d/wk, 8–24 wks	6/group	4.5.4.2. Cytotoxicity and histopathology E.2.2.10. Kumar et al.(2001b)
Okino et al. (1991)	Wistar rats (M)	Inhalation	0, 500 (8 hrs), 2,000 (2 or 8 hrs), or 8,000 ppm (2 hrs) (single exposure)	5/group	4.5.4.2. Cytotoxicity and histopathology E.2.1.3. Okino et al. (1991)
Ramdhan et al. (2008)	SV/129 mice (M) CYP2E1-null mice (M)	Inhalation	0, 1,000, or 2,000 ppm, 8 hrs/d, 7 d	6/group	4.5.4.2. Cytotoxicity and histopathology 4.5.6.2.1. Hepatomegally- qualitative and quantitative comparisons 4.5.6.2.2. Cytotoxicity E.2.1.14. Ramdhan et al. (2008)

Table 4-59. Inhalation and i.p. studies of TCE-induced liver effects in mice and rats (continued)

Reference[a]	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Section(s) where noncancer liver effects are discussed
Ramdhan et al. (2010)	Sv/129, PPARα-null, and hPPARα mice (M)	Inhalation	0, 1,000, or 2,000 ppm, 8 hrs/d, 7 d	6/group]	4.5.4.1. Liver weight 4.5.4.2. Cytotoxicity and histopathology 4.5.6.2.1. Hepatomegally- qualitative and quantitative comparisons 4.5.6.2.2. Cytotoxicity 4.5.7.2. Peroxisome Proliterator Activated Receptor Alpha (PPARα) Receptor Activation E.2.1.15. Ramdhan et al. (2010)
Toraason et al. (1999)	Fischer rats (M)	i.p. in Alkamuls/ water	0, 100, 500, or 1,000 mg/kg	6/group	4.5.4.5. Oxidative stress E.2.4.3. Summary of TCE Subchronic and Chronic Studies E.3.4.2.3. Oxidative Stress
Wang and Stacey (1990)	Sprague-Dawley rats (M)	i.p. in corn oil Inhalation	i.p.: 0, 1.3–1,314 mg/kg-d for 3 d Inhalation: 0, 200, or 1,000 ppm, 6 hrs/d for 28 d	4–6/group	4.5.4.6. Bile production E.2.2. Subchronic and Chronic Studies of TCE
Watanabe and Fukui (2000)	ddY mice (M)	i.p. in corn oil	0, 158 mg/kg (single dose)	4/group	4.5.4.4. Peroxisome proliferation and related effects
Woolhiser et al. (2006)	**Sprague-Dawley rats (F)**	**Inhalation**	**0, 100, 300, or 1,000 ppm, 6 hrs/d, 5 d/wk, for 4 wks**	**16/group**	4.5.4.1. Liver weight E.2.2.4. Woolhiser et al. (2006)

[a]**Bolded study(ies)** carried forward for consideration in dose-response assessment (see Chapter 5).

4.5.4.1. Liver Weight

Increases in liver weight in mice, rats, and gerbils have been reported as a result of acute short-term, and subchronic TCE treatment by inhalation and oral routes of exposure (Laughter et al., 2004; Nunes et al., 2001; Nakajima et al., 2000; Tao et al., 2000; Berman et al., 1995; Dees and Travis, 1993; Goel et al., 1992; Merrick et al., 1989; Goldsworthy and Popp, 1987; Melnick et al., 1987; Buben and O'Flaherty, 1985; Elcombe et al., 1985; Kjellstrand et al., 1983a; Kjellstrand et al., 1983b; Tucker et al., 1982; Kjellstrand et al., 1981b). The extent of TCE-induced liver weight gain is dependent on species, strain, gender, nutrition status, duration of exposure, route of administration, vehicle used in oral studies, and concentration of TCE administered. Of great importance to the determination of the magnitude of response is whether the dose of TCE administered also affects whole-body weight, and thus, liver weight and the percentage liver/body weight ratio. Therefore, studies that employed high enough doses to induce whole-body weight loss generally showed a corresponding decrease in percentage liver/body weight at such doses and "flattening" of the dose-response curve, while studies that did not show systemic toxicity reported liver/body weight ratios generally proportional to dose. Chronic studies, carried out for longer durations, that examine liver weight are few and often confounded by the presence of preneoplastic foci or tumors that also affect liver weight after an extended period of TCE exposure. The number of studies that examine liver weight changes in the rat are much fewer than for mouse. Overall, the database for mice provides data for examination of the differences in TCE-induced effects from differing exposure levels, durations of exposure, vehicle, strain, and gender. One study provided a limited examination of TCE-induced liver weight changes in gerbils.

TCE-induced increases in liver weight have been reported to occur quickly. Kjellstrand et al. (1981b) reported liver weight increases after 2 days of inhalation exposure in NMRI mice, Laughter et al. (2004) reported increased liver weight in SV129 mice in their 3-day study (see below), and Tao et al. (2000) reported a increased in percentage liver/body weight ratio in female B6C3F_1 mice for after 5 days. Elcombe et al. (1985) and Dees and Travis (1993) reported gavage results in mice and rats after 10 days of exposure to TCE, which showed TCE-induced increases in liver weight. Tucker et al. (1982) reported that 14 days of exposure to 24 and 240 mg/kg TCE via gavage to induce a dose-related increase in liver weight in male CD-1 mice but did not show the data.

For mice, the inhalation studies of Kjellstrand et al. provided the most information on the affect of duration of exposure, dose of exposure, strain tested, gender, initial weight, and variability in response between experiments on TCE-induced liver weight increases. These experiments also provided results that were independent of vehicle effect. Although the determination of the exact magnitude of response is limited by experimental design, Kjellstrand et al. (1981b) reported that in NMRI mice, continuous TCE inhalation exposure induced increased percentage liver/body weight by 2 days and that by 30 days (the last recorded

data point) the highest percentage liver/body weight ratio was reported (~1.75-fold over controls) in both male and female mice. Kjellstrand et al. (1983b) exposed seven different strains of mice (wild, C57BL, DBA, B6CBA, A/sn, NZB, NMRI) to 150 ppm TCE for 30 days and demonstrated that strain, gender, and toxicity, as reflected by changes in whole-body weight, affected the percentage liver/body weight ratios induced by 30 days of continuous TCE exposure. In general for the seven strains of mice examined, female mice had the less variable increases in TCE-induced liver weight gain across duplicate experiments than male mice. For instance, in strains that did not exhibit changes in body weight (reflecting systemic toxicity) in either gender (wild-type and DBA), 150 ppm TCE exposure for 30 days induced 1.74–1.87-fold of control percentage liver/body weight ratios in female mice and 1.45–2.00-fold of control percentage liver/body weight ratios in male mice. The strain with the largest TCE-induced increase in percentage liver/body weight increase was the NZB strain (~2.08-fold of control for females and 2.34–3.57-fold of control for males). Kjellstrand et al. (1983a) provided dose-response information for the NMRI strain of mice (A Swiss-derived strain) that indicated dose-related increases in percentage liver/body weight ratios between 37 and 300 ppm TCE exposure for 30 days. The 150 ppm dose was reported to induce a 1.66- and 1.69-fold increases in percentage liver/body weight ratios in male and female mice, respectively. Interestingly, they also reported similar liver weight increases among groups with the same cumulative exposure, but with different daily exposure durations (1 hour/day at 3,600 ppm to 24 hours/day at 150 ppm for 30 days).

Not only have most gavage experiments been carried out in male mice, which Kjellstrand et al. (1983b) had demonstrated to have more variability in response than females, but also vehicle effects were noted to occur in experiments that examined them. Merrick et al. (1989) reported that corn oil induced a similar increase in percentage liver/body weight ratios in female mice fed TCE in Emulphor and corn oil for 4 weeks; male mice TCE administered in the corn oil vehicle induced a greater increase in liver weight than Emulphor but less mortality at a high does.

Buben and O'Flaherty (1985) treated male, outbred Swiss-Cox mice for 6 weeks at doses ranging from 100 to 3,200 mg/kg-day, and reported increased liver/body-weight ratios at all tested doses (1.12–1.75-fold of controls). Given the large strain differences observed by Kjellstrand et al. (1983b), the use of predominantly male mice, and the effects of vehicle in gavage studies, interstudy variability in dose-response relationships is not surprising.

Dependence of PPARα activation for TCE-liver weight gain has been investigated in PPARα null mice by Nakajima et al. (2000), Laughter et al. (2004), and Ramdhan et al. (2010), the latter of which also investigated PPARα null mice with human PPARα inserted. Nakajima et al. (2000) reported that after 2 weeks of 750 mg/kg TCE exposure to carefully matched SV129 wild-type or PPARα-null male and female mice (n = 6 group), there was a reported 1.50-fold increase in wild-type and 1.26-fold of control percentage liver/body weight ratio in PPARα-null

male mice. For female mice, there was ~1.25-fold of control percentage liver/body weight ratios for both wild-type and PPARα-null mice. Thus, TCE-induced liver weight gain was not dependent on a functional PPARα receptor in female mice and some portion of it may have been in male mice. Both wild-type male and female mice were reported to have similar increases in the number of peroxisome in the pericentral area of the liver and TCE exposure and, although increased twofold, were still only ~4% of cytoplasmic volume. Female wild-type mice were reported to have less TCE-induced elevation of very long chain acyl-CoA synthetase, D-type peroxisomal bifunctional protein, mitochondrial trifunctional protein α subunits α and β, and CYP 4A1 than males mice, even though peroxisomal volume was similarly elevated in male and female mice. The induction of PPARα protein by TCE treatment was also reported to be slightly less in female than male wild-type mice (2.17- vs. 1.44-fold of control induction, respectively). Thus, differences between genders in this study were for increased liver weight were not associated with differences in peroxisomal volume in the hepatocytes but there was a gender-related difference in induction of enzymes and proteins associated with PPARα.

The study of Laughter et al. (2004) used SV129 wild-type and PPARα-null male mice treated with three daily doses of TCE in 0.1% methyl cellulose for either 3 days (1,500 mg/kg TCE) or 3 weeks (0, 10, 50, 125, 500, 1,000, or 1,500 mg/kg TCE 5 days/week). However, the paradigm is not strictly comparable to other gavage paradigms due to the different dose vehicle and the documented impacts of vehicles such as corn oil on TCE-induced effects. In addition, no initial or final body weights of the mice were reported and thus, the influence of differences in initial body weight on percentage liver/body weight determinations could not be ascertained. While control wild-type and PPARα-null mice were reported to have similar percentage liver/body weight ratios (i.e., ~4.5%) at the end of the 3-day study, at the end of the 3-week experiment, the percentage liver/body weight ratios were reported to be larger in the control PPARα-null male mice (5.1%). TCE treatment for 3 days was reported for percentage liver/body weight ratio to be 1.4-fold of control in the wild-type mice and 1.07-fold of control in the null mice. After 3 weeks of TCE exposure at varying concentrations, wild-type mice were reported to have percentage liver/body weight ratios that were within ~2% of control values with the exception of the 1,000 and 1,500 mg/kg treatment groups (~1.18- and 1.30-fold of control, respectively). For the PPARα-null mice, the variability in percentage liver/body weight ratios was reported to be greater than that of the wild-type mice in most of the TCE groups and the baseline levels of percentage liver/body weight ratio for control mice 1.16-fold of that of wild-type mice. TCE exposure was apparently more toxic in the PPARα-null mice. Decreased survival at the 1,500 mg/kg TCE exposure level resulted in the prevention of recording of percentage liver/body weight ratios for this group. At 1,000 mg/kg TCE exposure level, there was a reported 1.10-fold of control percentage liver/body weight ratio in the PPARα-null mice. None of the increases in percentage liver/body weight in the null mice were reported to be statistically significant by Laughter et al. (2004). However, the power of the study was limited

due to low numbers of animals and increased variability in the null mice groups. The percentage liver/body weight ratio after TCE treatment reported in this study was actually greater in the PPARα-null mice than the wild-type male mice at the 1,000 mg/kg TCE exposure level (5.6 ± 0.4 vs. 5.2 ± 0.5%, for PPARα-null and wild-type mice, respectively) resulting in a 1.18-fold of wild-type and 1.10-fold of PPARα-null mice. Although the results reported in Laughter et al. (2004) for DCA and TCA were not conducted in experiments that used the same paradigm, the TCE-induced increase in percentage liver/body weight more closely resembled the dose-response pattern for DCA than for DCA wild-type SV129 and PPARα-null mice.

Ramdhan et al. (2010) examined TCE-induced hepatice steatosis and toxicity using male wild type, PPARα-null, and human PPARα inserted ("humanized") mice exposed to high inhalation concentrations of TCE for 7 days. Significant differences were observed among control mice for each genotype with reduced body weight in untreated humanized mice. Liver/body weight ratios were 11% higher in untreated PPARα-null mice than wild type mice. Higher levels of liver triglycerides and hepatic steatosis were reported in the untreated humanized mice and PPARα null mice than wild type mice. Background expression of a number of genes and protein expression levels were significantly different between the untreated strains. In particular, human PPARα protein levels were >10-fold greater in the humanized mice than mouse PPARα in untreated wild type mice. Insertion of human PPARα in the null mice did not return the mice to a normal state. Both PPARα null and humanized mice were more susceptible to TCE toxicity. Hepatomegally was induced in all strains to a similar extent after TCE exposure. However, urinary TCA concentrations were reported to be significantly lower and TCOH levels significantly higher in TCE-treated PPARα-null mice in comparison to treated wild type mice. This difference was not related to changes in expression of metabolic enzymes.

No study examined strain differences among rats, and cross-study comparisons are confounded by heterogeneity in the age of animals, dosing regimen, and other design characteristics that may affect the degree of response. For rats, TCE-induced percentage liver/body weight ratios were reported to range from 1.16- to 1.46-fold of control values depending on the study paradigm. The studies that employed the largest range of exposure concentrations (Berman et al., 1995; Melnick et al., 1987) examined four doses in the rat. In general, there was a dose-related increase in percentage liver/body weight in the rat, especially at doses that did not cause concurrent decreased survival or significant body weight loss. For gerbils, Kjellstrand et al. (1981b) reported a similar value of ~1.25-fold of control percentage liver/body weight as for Sprague-Dawley rats exposed to 150 ppm TCE continuously for 30 days. Woolhiser et al. (2006) also reported inhalation TCE exposure to increase the percentage liver/body weight ratios in female Sprague-Dawley rats, although this strain appeared to be less responsive that others tested for induction of hepatomegaly from TCA exposure and to also be less prone to spontaneous liver cancer.

The size of the liver is under tight control and after cessation of a mitogenic stimulus or one inducing hepatomegaly, the liver will return to its preprogrammed size (see Appendix E). The increase in liver weight from TCE-exposure also appears to be reversible. Kjellstrand et al. (1981b) reported a reduction in liver weight gain increases after cessation of TCE exposure for 5 or 30 days in male and female mice. However, experimental design limitations precluded discernment of the magnitude of decrease. Kjellstrand et al. (1983a) reported that mice exposed to 150 ppm TCE for 30 days and then examined 120 days after the cessation of exposure had liver weights were 1.09-fold of control for TCE-exposed female mice and the same as controls for TCE-exposed male mice. However, the livers were not the same as untreated liver in terms of histopathology. The authors reported that "after exposure to 150 ppm for 30 days, followed by 120 days of rehabilitation, the morphological picture was similar to that of the air-exposure controls except for changes in cellular and nuclear sizes." Qualitatively, the reduction in liver weight after treatment cessation is consistent with the report of Elcombe et al. (1985) in Alderly Park mice. The authors report that the reversibility of liver effects after the administration of TCE to Alderly Park mice for 10 consecutive days. Effects upon liver weight, DNA concentration, and tritiated thymidine incorporation 24 and 48 hours after the last dose of TCE were reported to still be apparent. However, 6 days following the last dose of TCE, all of these parameters were reported to return to control values with the authors not showing the data to support this assertion. Thus, cessation of TCE exposure would have resulted in a 75% reduction in liver weight by 4 days in mice exposed to the highest TCE concentration. Quantitative comparisons are not possible because Elcombe et al. (1985) did not report data for these results (e.g., how many animals, what treatment doses, and differences in baseline body weights) and such a large decrease in such a short period of time needs to be verified.

4.5.4.2. Cytotoxicity and Histopathology

Acute exposure to TCE appears to induce low cytotoxicity below subchronically lethal doses. Relatively high doses of TCE appear necessary to induce cytotoxicity after a single exposure with two available studies reported in rats. Okino et al. (1991) reported small increases in the incidence of hepatocellular necrosis in male Wistar rats exposed to 2,000 ppm (8 hours) and 8,000 ppm (2 hours), but not at lower exposures. In addition, "swollen" hepatocytes were noted at the higher exposure when rats were pretreated with ethanol or phenobarbital. Serum transaminases increased only marginally at the 8,000-ppm exposure, with greater increases with pretreatments. Berman et al. (1995) reported hepatocellular necrosis, but not changes in serum markers of necrosis, after single gavage doses of 1,500 and 5,000 mg/kg TCE in female F344 rats. However, they did not report any indications of necrosis after 14 days of treatment at 50–1,500 mg/kg-day nor the extent of necrosis.

At acute and subchronic exposure periods to multiple doses, the induction of cytotoxicity, though usually mild, appears to differ depending on rodent species, strain, dosing vehicle, and

duration of exposure, and the extent of reporting to vary between studies. For instance, Elcombe et al. (1985) and Dees and Travis (1993), which used the B6C3F$_1$ mouse strain and corn oil vehicle, reported only slight or mild necrosis after 10 days of treatment with TCE at doses up to 1,500 mg/kg-day. Elcombe et al. (1985) also reported cell hypertrophy in the centrilobular region. Dees and Travis (1993) reported some loss of vacuolization in hepatocytes of mice treated at 1,000 mg/kg-day. Laughter et al. (2004) reported that "wild-type" SV129 mice exposed to 1,500 mg/kg TCE exposure for 3 weeks exhibited mild granuloma formation with calcification or mild hepatocyte degeneration, but gave no other details or quantitative information as to the extent of the lesions or what parts of the liver lobule were affected. The authors noted that "wild-type mice administered 1,000 and 1,500 mg/kg exhibited centrilobular hypertrophy" and that "the mice in the other groups did not exhibit any gross pathological changes" after TCE exposure. Channel et al. (1998) reported no necrosis in B6C3F$_1$ mice treated with 400–1,200 mg/kg-day TCE by corn oil gavage for 2 days to 8 weeks.

However, as stated above, Merrick et al. (1989) reported that corn oil resulted in more hepatocellular necrosis, as described by small focal areas of 3–5 hepatocytes, in male B6C3F$_1$ mice than use of Emulphor as a vehicle for 4-week TCE gavage exposures. Necrotic hepatocytes were described as surrounded by macrophages and polymorphonuclear cells. The authors reported that visible necrosis was observed in 30–40% of male mice administered TCE in corn oil but not that there did not appear to be a dose-response. For female mice, the extent of necrosis was reported to be 0 for all control and TCE treatment groups using either vehicle. Serum enzyme activities for ALT, AST, and LDH (markers of liver toxicity) showed that there was no difference between vehicle groups at comparable TCE exposure levels for male or female mice. Except for LDH levels in male mice exposed to TCE in corn oil, there was not a correlation with the extent of necrosis and the patterns of increases in ALT and AST enzyme levels.

Ramdhan et al. (2008) assessed TCE-induced hepatotoxicity by measuring plasma ALT and AST activities and histopathology in Sv/129 mice treated by inhalation exposure, which are not confounded by vehicle effects. Despite high variability and only six animals per dose group, all three measures showed statistically significant increases at the high dose of 2,000 ppm (8 hours/day for 7 days), although a nonstatistically significant elevation is evident at the low dose of 1,000 ppm. Even at the highest dose, cytotoxicity was not severe, with ALT and AST measures increased twofold or less and an average histological score <2(range 0–4).

Using the same paradigm, Ramdhan et al. (2010) also reported increased in AST and ALT liver injury biomarkers to be significantly increased in all exposed mice (Sv/129 wild type, PPARα-null, and humanized PPARα mice) relative to controls (41–74 and 36–79% higher, for ALT and AST, respectively). Mean levels within each treatment group were higher, though not statistically significantly different, with exposure to 2,000 vs. 1,000 ppm TCE. Steatosis scores were reported to be significantly higher in the 2,000 vs. 1,000 ppm TCE exposures to

PPARα-null mice. The authors reported steatosis scored to be significantly correlated with liver triglyceride levels of all mice examined in the study (r = 0.75). Macrovesicular steatosis was reported to occur more frequently in hPPARα than PPARα-null mice. Necrosis scores were reported to be significantly higher in TCE-exposed mice relative to controls in all three genotype mice and to be significantly higher with 2,000 vs. 1,000 ppm TCE exposure in wild type mice and hPPARα mice. Inflammation scores were reported to be significantly higher with exposed group than control with 2,000 ppm TCE exposure than controls for each genotype group with a difference between the 2,000 ppm and 1,000 ppm exposure groups in wild type mice.

Kjellstrand et al. (1983a) exposed male and female NRMI mice to 150 ppm for 30–120 days. Kjellstrand et al. (1983a) reported more detailed light microscopic findings from their study and stated that

> After 150 ppm exposure for 30 days, the normal trabecular arrangement of the liver cells remained. However, the liver cells were generally larger and often displayed a fine vacuolization of the cytoplasm. The nucleoli varied slightly to moderately in size and shape and had a finer, granular chromatin with a varying basophilic staining intensity. The Kupffer cells of the sinusoid were increased in cellular and nuclear size. The intralobular connective tissue was infiltrated by inflammatory cells. There was not sign of bile stasis. Exposure to TCE in higher or lower concentrations during the 30 days produced a similar morphologic picture. After intermittent exposure for 30 days to a time-weighted-average concentration of 150 ppm or continuous exposure for 120 days, the trabecular cellular arrangement was less well preserved. The cells had increased in size and the variations in size and shape of the cells were much greater. The nuclei also displayed a greater variation in basophilic staining intensity, and often had one or two enlarged nucleoli. Mitosis was also more frequent in the groups exposed for longer intervals. The vacuolization of the cytoplasm was also much more pronounced. Inflammatory cell infiltration in the interlobular connective tissue was more prominent. After exposure to 150 ppm for 30 days, followed by 120 days of rehabilitation, the morphological picture was similar to that of the air-exposure controls except for changes in cellular and nuclear sizes.

Although not reporting comparisons between male and female mice in the results section of the paper for TCE-induced histopathological changes, the authors stated in the discussion section that ―However, livemass increase and the changes in liver cell morphology were similar in TCE-exposed male and female mice.” Kjellstrand et al. (1983a) did not present any quantitative data on the lesions they described, especially in terms of dose-response. Most of the qualitative description presented was for the 150-ppm exposure level and the authors suggest that lower concentrations of TCE give a similar pathology as those at the 150-ppm level, but do not present data to support that conclusion. Although stating that Kupffer cells were reported to be increased in cellular and nuclear size, no differential staining was applied light microscopy sections to distinguish Kupffer from endothelial cells lining the hepatic sinusoid in this study. Without differential staining, such a determination is difficult at the light microscopic level.

Indeed, Goel et al. (1992) describe proliferation of "sinusoidal endothelial cells" after 1,000 and 2,000 mg/kg-day TCE exposure for 28 days in male Swiss mice. They reported that histologically, "the liver exhibits swelling, vacuolization, widespread degeneration/necrosis of hepatocytes as well as marked proliferation of endothelial cells of hepatic sinusoids at 1,000 and 2,000 mg/kg TCE doses." Only one figure is given, at the light microscopic level, in which it is impossible to distinguish endothelial cells from Kupffer cells and no quantitative measures or proliferation were examined or reported to support the conclusion that endothelial cells are proliferating in response to TCE treatment. Similarly, no quantitative analysis regarding the extent or location of hepatocellular necrosis was given. The presence or absence of inflammatory cells were not noted by the authors as well. In terms of white blood cell count, the authors note that it was slightly increased at 500 mg/kg-day but decreased at 1,000 and 2,000 mg/kg-day TCE, perhaps indicating macrophage recruitment from blood to liver and kidney, which was also noted to have pathology at these concentrations of TCE.

The inflammatory cell infiltrates described in the Kjellstrand et al. (1983a) study are consistent with invasion of macrophages and well as polymophorphonuclear cells into the liver, which could activate resident Kupffer cells. Although not specifically describing the changes as consistent with increased polyploidization of hepatocytes, the changes in cell size and especially the continued change in cell size and nuclear staining characteristics after 120 days of cessation of exposure are consistent with changes in polyploidization induced by TCE. Of note is that in the histological description provided by the authors, although vacuolization is reported and consistent with hepatotoxicity or lipid accumulation, which is lost during routine histological slide preparation, there is no mention of focal necrosis or apoptosis resulting from these exposures to TCE.

Buben and O'Flaherty (1985) reported liver degeneration "as swollen hepatocytes" and to be common with treatment of TCE to Male Swiss-Cox mice after 6 weeks. They reported that "Cells had indistinct borders; their cytoplasm was clumped and a vesicular pattern was apparent. The swelling was not simply due to edema, as wet weight/dry weight ratios did not increase." Karyorrhexis (the disintegration of the nucleus) was reported to be present in nearly all specimens and suggestive of impending cell death. No Karyorrhexis, necrosis, or polyploidy was reported in controls, but a low score Karyorrhexis was given for 400 mg/kg TCE and a slightly higher one given for 1,600 mg/kg TCE. Central lobular necrosis was reported to be present only at the 1,600 mg/kg TCE exposure level and was assigned a low score. Polyploidy was described as characteristic in the central lobular region but with low scores for both 400 mg/kg and 1,600 mg/kg TCE exposures. The authors reported that "hepatic cells had two or more nuclei or had enlarged nuclei containing increased amounts of chromatin, suggesting that a regenerative process was ongoing" and that there were no fine lipid droplets in TCE exposed animals. The finding of "no polyploidy" in control mouse liver in the study of Buben and O'Flaherty (1985) is unexpected given that binucleate and polyploid hepatocytes are a common

finding in the mature mouse liver. It is possible that the authors were referring to unusually high instances of "polyploidy" in comparison to what would be expected for the mature mouse. The score given by the authors for polyploidy did not indicate a difference between the two TCE exposure treatments and that it was of the lowest level of severity or occurrence. No score was given for centrolobular hypertrophy, although the DNA content and liver weight changes suggested a dose-response. The "karyrrhexis" described in this study could have been a sign of cell death associated with increased liver cell number or dying of maturing hepatocytes associated with the increased ploidy, and suggests that TCE treatment was inducing polyploidization. Consistent with enzyme analyses, centrilobular necrosis was only seen at the highest dose and with the lowest qualitative score, indicating that even at the highest dose there was little toxicity.

At high doses, Kaneko et al. (2000) reported sporadic necrosis in male Mrl-lpr/lpr mice, which are "genetically liable to autoimmune disease," exposed to 500–2,000 ppm, 4 hours/day, 6 days/week, for 8 weeks (n = 5). Dose-dependent mild inflammation and associated changes were reported to be found in the liver. The effects on hepatocytes were reported to be minimal by the authors with 500 ppm TCE inducing sporadic necrosis in the hepatic lobule. Slight mobilization and activation of sinusoid lining cells were also noted. These pathological features were reported to increase with dose.

NTP (1990), which used the $B6C3F_1$ mouse strain, reported centrilobular necrosis in 6/10 male and 1/10 female $B6C3F_1$ mice treated at a dose of 6,000 mg/kg-day for up to 13 weeks (all of the male mice and 8 of the 10 female mice died in the first week of treatment). At 3,000 mg/kg-day exposure level, although centrilobular necrosis was not observed, 2/10 males had multifocal areas of calcification in their livers, which the authors suggest is indicative of earlier hepatocellular necrosis. However, only 3/10 male mice at this dose survived to the end of the 13-week study.

For the NTP (1990) 2-year study, $B6C3F_1$ mice were reported to have no treatment-related increase in necrosis in the liver. A slight increase in the incidence of focal necrosis was noted TCE-exposed male mice (8 vs. 2%) with a slight reduction in fatty metamorphosis in treated male mice (zero treated vs. two control animals) and, in female mice, a slight increase in focal inflammation (29 vs. 19% of animals) and no other changes. Therefore, this study did not show concurrent evidence of liver toxicity with TCE-induced neoplasia after 2 years of TCE exposure in mice.

For the more limited database in rats, there appears to be variability in reported TCE-induced cytotoxicity and pathology. Nunes et al. (2001) reported no gross pathological changes in rats gavaged with corn oil or with corn oil plus 2,000 mg/kg TCE for 7 days. Goldsworthy and Popp (1987) gave no descriptions of liver histology in this report for TCE-exposed animals or corn-oil controls. Kjellstrand et al. (1981b) also did not provide histological descriptions for livers of rats in their inhalation study.

Elcombe et al. (1985) provided a description of the histopathology at the light microscopy level in Osborne-Mendel rats and Alderly Park rats exposed to TCE via gavage for 10 days. However, they did not provide a quantitative analysis or specific information regarding the variability of response between animals within group and there was no indication by the authors regarding how many rats were examined by light microscopy. Hematoxylin and eosin sections from Osborne-Mendel rats were reported to show that:

> Livers from control rats contained large quantities of glycogen and isolated inflammatory foci, but were otherwise normal. The majority of rats receiving 1,500 mg/kg body weight TCE showed slight changes in centrilobular hepatocytes. The hepatocytes were more eosinophilic and contained little glycogen. At lower doses these effects were less marked and were restricted to fewer animals. No evidence of treatment-related hepatotoxicity (as exemplified by single cell or focal necrosis) was seen in any rat receiving TCE. H&E [hematoxylin and eosin] sections from Alderly Park Rats showed no signs of treatment-related hepatotoxicity after administration of TCE. However, some signs of dose-related increase in centrilobular eosinophilia were noted.

Thus, both mice and rats were reported to exhibit pericentral hypertrophy and eosinophilia as noted from the histopathological examination in Elcombe et al. (1985).

Berman et al. (1995) reported that for female rats exposed to TCE for 14 days hepatocellular necrosis was noted to occur in the 1,500 and 5,000 mg/kg groups in 6/7 and 6/8 female rats, respectively, but not to occur in lower doses. The extent of necrosis was not noted by the authors for the two groups exhibiting a response after 1 day of exposure. Serum enzyme levels, indicative of liver necrosis, were not presented and because only positive results were presented in the paper, presumed to be negative. Therefore, the extent of necrosis was not of a magnitude to affect serum enzyme markers of cellular leakage.

Melnick et al. (1987) reported that the only treatment-related lesion observed microscopically in rats from either dosed-feed or gavage groups was individual cell necrosis of the liver with the frequency and severity of this lesion similar at each dosage levels of TCE microencapsulated in the feed or administered in corn oil. The severity for necrosis was only mild at the 2.2 and 4.8 g/kg feed groups and for the six animals in the 2.8 g/kg group corn oil group. The individual cell necrosis was reported to be randomly distributed throughout the liver lobule with the change to not be accompanied by an inflammatory response. The authors also reported that there was no histologic evidence of cellular hypertrophy or edema in hepatic parenchymal cells. Thus, although there appeared to be TCE-treatment related increases in focal necrosis after 14 days of exposure, the extent was mild even at the highest doses and involved few hepatocytes.

For the 13-week NTP study (1990), only control and high dose F344/N rats were examined histologically. Pathological results were reported to reveal that 6/10 males and 6/10 female rats had pulmonary vasculitis at the highest concentration of TCE. This change was

also reported to have occurred in 1/10 control male and female rats. Most of those animals were also reported to have had mild interstitial pneumonitis. The authors reported that viral titers were positive during this study for Sendai virus.

Kumar et al. (2001b) reported that male Wistar rats exposed to 376 ppm, 4 hours/day, 5 days/week for 8–24 weeks showed evidence of hepatic toxicity. The authors stated that, "after 8 weeks of exposure enlarged hepatocytes, with uniform presence of fat vacuoles were found in all of the hepatocytes affecting the periportal, midzonal, and centriolobular areas, and fat vacuoles pushing the pyknosed nuclei to one side of hepatocytes. Moreover, congestion was not significant. After exposure of 12 and 24 weeks, the fatty changes became more progressive with marked necrosis, uniformly distributed in the entire organ." No other description of pathology was provided in this report. In regard to the description of fatty change, the authors only did conventional H&E staining of sections with no precautions to preserve or stain lipids in their sections. However, as noted below, the NCI study also reports long-term TCE exposure in rats to result in hepatocellular fatty metamorphosis. The authors provided a table with histological scoring of simply + or – for minimal, mild, or moderate effects and did not define the criteria for that scoring. There is also no quantitative information given as to the extent, nature, or location of hepatocellular necrosis. The authors reported that "no change was observed in glutamic oxoacetate transaminase and glutamic pyruvated transaminase levels of liver in all of the three groups. The GSH level was significantly decreased while "total sulphydryl" level was significantly increased during 8, 12, and 24 weeks of TCE exposure. The acid and ALPs were significantly increased during 8, 12, and 24 weeks of TCE exposure." The authors presented a series of figures, which were poor in quality, to demonstrate histopathological TCE-induced changes. No mortality was observed from TCE exposure in any group, despite the presence of liver necrosis.

Thus, in this limited database that spans durations of exposure from days to 24 weeks and uses differing routes of administration, generally high doses for long durations of exposure are required to induce hepatotoxicity from TCE exposure in the rat. The focus of 2-year bioassays in rats has been the detection of a cancer response with little or no reporting of noncancer pathology in most studies. Henschler et al. (1984) and Fukuda et al. (1983) do not report noncancer histopathology, but both reported rare biliary-cell-derived tumors in rats in relatively insensitive assays. For male rats, noncancer pathology in the NCI (1976) study was reported to include increased fatty metamorphosis after TCE exposure and angiectasis or abnormally enlarged blood vessels. Angiectasis can be manifested by hyperproliferation of endothelial cells and dilatation of sinusoidal spaces. For the NTP (1990) study, there was little reporting of non-neoplastic pathology or toxicity and no report of liver weight at termination of the study. In the NTP (1988) study, the 2-year study of TCE exposure reported no evidence of TCE-induced liver toxicity described as non-neoplastic changes in ACI, August, Marshal, and Osborne-Mendel rats. Interestingly, for the control animals of these four strains, there was, in general, a low

background level of focal necrosis in the liver of both genders. Obviously, the negative results in this bioassay for cancer are confounded by the killing of a large portion of the animals accidently by experimental error, but TCE-induced overt liver toxicity was not reported.

In sum, the cytotoxic effects in the liver of TCE treatment appear to include little or no necrosis in the rodent liver, but rather, a number of histological changes such as mild focal hepatocyte degeneration at high doses, cellular "swelling" or hypertrophy, and enlarged nuclei. Histological changes consistent with increased polyploidization and specific descriptions of TCE-induced polyploidization have been noted in several experiments. Several studies noted proliferation of nonparenchymal cells after TCE exposure as well. These results are more consistently reported in mice, but also have been reported in some studies at high doses in rats, for which fewer studies are available. In addition, the increase in cellular and nuclear sizes appeared to persist after cessation of TCE treatment. In neither rats nor mice is there evidence that TCE treatment results in marked necrosis leading to regenerative hyperplasia.

4.5.4.3. Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis

The increased liver weight observed in rodents after TCE exposure may result from either increased numbers of cells in the liver, increased size of cells in the liver, or a combination of both. Studies of TCE in rodents have looked at whole-liver DNA content of TCE-treated animals to determine whether the concentration of DNA/g of liver decreases as an indication of hepatocellular hypertrophy (Dees and Travis, 1993; Buben and O'Flaherty, 1985; Elcombe et al., 1985). While the slight decreases observed in some studies are consistent with hypertrophy, the large variability in controls and lack of dose-response limits the conclusions that can be drawn from these data. In addition, multiple factors beyond hypertrophy affect DNA concentration in whole-liver homogenates, including changes in ploidy and the number of hepatocytes and nonparenchymal cells.

The incorporation of tritiated thymidine or BrdU has also been analyzed in whole-liver DNA and in individual hepatocytes as a measure of DNA synthesis. Such DNA synthesis can occur from either increased numbers of hepatocytes in the liver or increased polyploidization. Section E.1.1 describes polyploidization in human and rodent liver and its impacts on liver function, while Sections E.3.1.4 and E.3.3.1 discuss issues of target cell identification for liver cancer and changes in ploidy as a key even in liver cancer using animals models, respectively. Along with changes in cell size (hypertrophy), cell number (cellular proliferation), and the DNA content per cell (cell ploidy), the rate of apoptosis has also been noted or specifically examined in some studies of TCE and its metabolites. All of these phenomena have been identified in proposed hypotheses as key events possibly related to carcinogenicity. In particular, changes in cell proliferation and apoptosis have been postulated to be part of the mode of action for PPARα-agonists by Klaunig et al. (2003) (see Section E.3.4).

In regard to early changes in DNA synthesis, the data for TCE are very limited Mirsalis et al. (1989) reported measurements of in vivo-in vitro hepatocyte DNA repair and S-phase DNA synthesis in primary hepatocytes from male F344 rats and male and female B6C3F$_1$ mice administered single doses of TCE by gavage in corn oil. They reported negative results 2–12 hours after treatment of 50–1,000 mg/kg TCE in rats and mice (male and female) for UDS and repair using three animals per group. After 24 and 48 hours of 200 or 1,000 mg/kg TCE in male mice (n = 3) and after 48 hours of 200 (n = 3) or 1,000 (n = 4) mg/kg TCE in female mice, similar values of 0.30–0.69% of hepatocytes were reported as undergoing DNA synthesis in primary culture. Only the 1,000 mg/kg TCE dose in male mice at 48 hours was reported to give a result considered to be positive (~2.2% of hepatocytes), but no statistical analyses were performed on these measurements. These results are limited by both the number of animals examined and the relevance of the paradigm.

As noted above, TCE treatment in rodents has been reported to result in hepatocellular hypertrophy and increased centrilobular eosinophilia. Elcombe et al. (1985) reported a small decrease in DNA content with TCE treatment (consistent with hepatocellular hypertrophy) that was not dose-related, increased tritiated thymidine incorporation in whole mouse liver DNA that was that was treatment- but not dose-related (i.e., a two-, two-, and fivefold of control in mice treated with 500, 1,000, and 1,500 mg/kg TCE), and slightly increased numbers of mitotic figures that were treatment but not dose-related and not correlated with DNA synthesis as measured by thymidine incorporation. Elcombe et al. (1985) reported no difference in response between 500 and 1,000 mg/kg TCE treatments for tritiated thymidine incorporation. Dees and Travis (1993) also reported that incorporation of tritiated thymidine in DNA from mouse liver was elevated after TCE treatment with the mean peak level of tritiated thymidine incorporation occurred at 250 mg/kg TCE treatment level and remaining constant for the 500 and 1,000 mg/kg treated groups. Dees and Travis (1993) specifically report that mitotic figures, although very rare, were more frequently observed after TCE treatment, found most often in the intermediate zone, and found in cells resembling mature hepatocytes. They reported that there was little tritiated thymidine incorporation in areas near the bile duct epithelia or close to the portal triad in liver sections from both male and female mice. Channel et al. (1998) reported proliferating cell nuclear antigen (PCNA) positive cells, a measure of cells that have undergone DNA synthesis, was elevated only on day 10 (out of the 21 studied) and only in the 1,200 mg/kg-day TCE exposed group with a mean of ~60 positive nuclei per 1,000 nuclei for six mice (~6%). Given that there was little difference in PCNA positive cells at the other TCE doses or time points studied, the small number of affected cells in the liver could not account for the increase in liver size reported in other experimental paradigms at these doses. The PCNA positive cells as well as "mitotic figures" were reported to be present in centrilobular, midzonal, and periportal regions with no observed predilection for a particular lobular distribution. No data were shown regarding any quantitative estimates of mitotic figures and whether they correlated with PCNA

results. Thus, whether the DNA synthesis phases of the cell cycle indicated by PCNA staining were indentifying polyploidization or increased cell number cannot be determined.

For both rats and mice, the data from Elcombe et al. (1985) showed that tritiated thymidine incorporation in total liver DNA observed after TCE exposure did not correlate with mitotic index activity in hepatocytes. Both Elcombe et al. (1985) and Dees and Travis (1993) reported small mitotic indices and evidence of periportal hepatocellular hypertrophy from TCE exposure. Neither mitotic index nor tritiated thymidine incorporation data support a correlation with TCE-induced liver weight increase in the mouse, but rather that the increase is most likely due to hepatocellular hypertrophy. If higher levels of hepatocyte replication had occurred earlier, such levels were not sustained by 10 days of TCE exposure. These data suggest that increased tritiated thymidine levels were targeted to mature hepatocytes and in areas of the liver where greater levels of polyploidization occur (see Section E.1.1). Both Elcombe et al. (1985) and Dees and Travis (1993) show that tritiated thymidine incorporation in the liver was approximately twofold greater than controls between 250 and 1,000 mg/kg TCE, a result consistent with a doubling of DNA. Thus, given the normally quiescent state of the liver, the magnitude of this increase over control levels, even if a result of proliferation rather than polyploidization, would be confined to a very small population of cells in the liver after 10 days of TCE exposure.

Laughter et al. (2004) reported that there was an increase in DNA synthesis after aqueous gavage exposure to 500 and 1,000 mg/kg TCE given as three boluses/day for 3 weeks with BrdU given for the last week of treatment. An examination of DNA synthesis in individual hepatocytes was reported to show that 1 and 4.5% of hepatocytes had undergone DNA synthesis in the last week of treatment for the 500 and 1,000 mg/kg doses, respectively. Again, this level of DNA synthesis is reported for a small percentage of the total hepatocytes in the liver and not reported to be a result of regenerative hyperplasia.

Finally, Dees and Travis (1993) and Channel et al. (1998) reported evaluating changes in apoptosis with TCE treatment. Dees and Travis (1993) enumerated identified by either hematoxylin and eosin or feulgen staining in male and female mice after 10 days of TCE treatment by. Only zero or one apoptosis was observed per 100 high power (400 ×) fields in controls and all dose groups except for those given 1,000 mg/kg-day, in which eight or nine apoptoses per 100 fields were reported. None of the apoptoses were in the intermediate zones where mitotic figures were observed, and all were located near the central veins. This is the same region where one would expect endogenous apoptoses as hepatocytes —stream" from the portal triad toward the central vein (Schwartz-Arad et al., 1989). In addition, this is the same region where Buben and O'Flaherty (1985) noted necrosis and polyploidy. By contrast, Channel et al. (1998) reported no significant differences in apoptosis at any treatment dose (400–1,200 mg/kg-day) examined after any time from 2 days to 4 weeks.

4.5.4.4. Peroxisomal Proliferation and Related Effects

Numerous studies have reported that TCE administered to mice and rats by gavage leads to proliferation of peroxisomes in hepatocytes. Some studies have measured changes in the volume and number of peroxisomes as measures of peroxisome proliferation, while others have measured peroxisomal enzyme activity such as catalase and cyanide-insensitive PCO. Like liver weight, the determination of a baseline level of peroxisomal volume, number, or enzyme activity can be variable and have great effect on the ability to determine the magnitude of a treatment-related effect.

Elcombe et al. (1985) reported increases in the percentage of the cytoplasm occupied by peroxisomes in B6C3F_1 and Alderley Park mice treated for 10 days at 500–1,500 mg/kg-day. Although the increase over controls appeared larger in the B6C3F_1 strain, this is largely due to the twofold smaller control levels in that strain, as the absolute percentage of peroxisomal volume was similar between strains after treatment. All of these results showed high variability, as evidenced from the reported SDs. Channel et al. (1998) found a similar absolute percentage of peroxisomal volume after 10 days treatment in the B6C3F_1 mouse at 1,200 mg/kg-day TCE but with the percentage in vehicle controls similar to the Alderley-Park mice in the Elcombe et al. (1985) study. Interestingly, Channel et al. (1998) found that the increase in peroxisomes peaked at 10 days, with lower values after 6 and 14 days of treatment. Furthermore, the vehicle control levels also varied almost twofold depending on the number of days of treatment. Nakajima et al. (2000) treated male wild-type SV129 mice at 750 mg/kg-day for 14 days, and found even higher baseline values for the percentage of peroxisomal volume, but with an absolute level after treatment similar to that reported by Channel et al. (1998) in B6C3F_1 mice treated at 1,200 mg/kg-day TCE for 14 days. Nakajima et al. (2000) also noted that the treatment-related increases were smaller for female wild-type mice, and that there were no increases in peroxisomal volume in male or female PPARα-null mice, although vehicle control levels were slightly elevated (not statistically significant). Only Elcombe et al. (1985) examined peroxisomal volume in rats, and reported smaller treatment-related increases in two strains (OM and AP), but higher baseline levels. In particular, at 1,000 mg/kg-day, after 10 days of treatment, the percentage peroxisomal volume was similar in OM and AP rats, with similar control levels as well. While the differences from treatment were not statistically significant, only five animals were used in each group, and variability, as can be seen by the SDs, was high, particularly in the treated animals.

The activities of a number of different hepatic enzymes have also been as markers for peroxisome proliferation and/or activation of PPARα. The most common of these are catalase and cyanide-insensitive PCO. In various strains of mice (B6C3F_1, Swiss albino, SV129 wild-type) treated at doses of 500–2,000 mg/kg-day for 10–28 days, increases in catalase activity have tended to be more modest (1.3–1.6-fold of control) as compared to increases in PCO (1.4–7.9-fold of control) (Laughter et al., 2004; Nakajima et al., 2000; Watanabe and Fukui, 2000;

Goel et al., 1992; Goldsworthy and Popp, 1987; Elcombe et al., 1985). In rats, Elcombe et al. (1985) reported no increases in catalase or PCO activity in Alderley-Park rats treated at 1,000 mg/kg-day TCE for 10 days. In F344 rats, Goldsworthy and Popp (1987) and Melnick et al. (1987) reported increases of up to 2-fold in catalase and 4.1-fold in PCO relative to controls treated at 600–4,800 mg/kg-day for 10–14 days. The changes in catalase were similar to those in mice at similar treatment levels, with 1.1–1.5-fold of control enzyme activities at doses of 1,000–1,300 mg/kg-day (Melnick et al., 1987; Elcombe et al., 1985). However, the changes in PCO were smaller, with 1.1–1.8-fold of control activity at these doses, as compared to 6.3–7.9-fold of control in mice (Goldsworthy and Popp, 1987; Melnick et al., 1987).

In SV129 mice, Nakajima et al. (2000) and Laughter et al. (2004) investigated the dependence of these changes on PPARα by using a null mouse. Nakajima et al. (2000) reported that neither male nor female wild-type or PPARα null mice had significant increases in catalase after 14 days of treatment at 750 mg/kg-day. However, given the small number of animals (four per group) and the relatively small changes in catalase observed in other (wild-type) strains of mice, this study had limited power to detect such changes. Several other markers of peroxisome proliferation, including acyl-CoA oxidase and CYP4A1 (PCO was not investigated), were induced by TCE in male wild-type mice, but not in male null mice or female mice of either type. Unfortunately, none of these markers have been investigated using TCE in female mice of any other strain, so it is unclear whether the lack of response is characteristic of female mice in general, or just in this strain. Interestingly, as noted above, liver/body weight ratio increases were observed in both sexes of the null mice in this study. Laughter et al. (2004) only quantified activity of the peroxisome proliferation marker, PCO, in their study, and found in null mice a slight decrease (0.8-fold of control) at 500 mg/kg-day TCE and an increase (1.5-fold of control) at 1,500 mg/kg-day TCE after 3 weeks of treatment, with neither statistically significant (4–5 mice per group). However, baseline levels of PCO were almost 2-fold higher in the null mice, and the treated wild-type and null mice differed in PCO activity by only about 1.5-fold.

In sum, oral administration of TCE for up to 28 days causes proliferation of peroxisomes in hepatocytes along with associated increases in peroxisomal enzyme activities in both mice and rats. Male mice tend to be more sensitive in that at comparable doses, rats and female mice tend to exhibit smaller responses. For example, for peroxisomal volume and PCO, the fold-increase in rats appears to be lower by three- to sixfold than that in mice, but, for catalase, the changes were similar between mice in F344 rats. No inhalation or longer-term studies were located, and only one study examined these changes at more than one time-point. Therefore, little is known about the route-dependence, time course, and persistence of these changes. Finally, two studies in PPARα-null mice (Laughter et al., 2004; Nakajima et al., 2000) found diminished responses in terms of increased peroxisomal volume and peroxisomal enzyme activities as compared to wild-type mice, although there was some confounding due to baseline differences between null and wild-type control mice in several measures.

4.5.4.5. Oxidative Stress

Several studies have attempted to study the possible effects of "oxidative stress" and DNA damage resulting from TCE exposures. The effects of induction of metabolism by TCE, as well as through co-exposure to ethanol, have been hypothesized to increase levels of "oxidative stress" as a common effect for both exposures (see Sections E.3.4.2.3 and E.4.2.4). Oxidative stress has been hypothesized to be a key event or mode of action for peroxisome proliferators as well, but has been found to be correlated with neither cell proliferation nor carcinogenic potency of peroxisome proliferators (see Section E.3.4.1.1). As a mode of action, it is not defined or specific, as the term "oxidative stress" is implicated as part of the pathophysiologic events in a multitude of disease processes and is part of the normal physiologic function of the cell and cell signaling.

In regard to measures of oxidative stress, Rusyn et al. (2006) noted that although an overwhelming number of studies draw a conclusion between chemical exposure, DNA damage, and cancer based on detection of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a highly mutagenic lesion, in DNA isolated from organs of in vivo treated animals, a concern exists as to whether increases in 8-OHdG represent damage to genomic DNA, a confounding contamination with mitochondrial DNA, or an experimental artifact. As noted in Sections E.2.1.1 and E.2.2.11, studies of TCE which employ the i.p. route of administration can be affected by inflammatory reactions resulting from that routes of administration and subsequent toxicity that can involve oxygen radical formation from inflammatory cells. Finally, as described in Section E.2.2.8, the study by Channel et al. (1998) demonstrated that corn oil as vehicle had significant effects on measures of "oxidative stress" such as thiobarbiturate acid-reactive substances (TBARS).

The TBARS results presented by Channel et al. (1998) indicate suppression of TBARS with increasing time of exposure to corn oil alone with data presented in such a way for 8-OHdG and total free radical changes that the pattern of corn oil administration was obscured. It was not apparent from that study that TCE exposure induced oxidative damage in the liver.

Toraason et al. (1999) measured 8-OHdG and a "free radical-catalyzed isomer of arachidonic acid and marker of oxidative damage to cell membranes, 8-epi-prostaglandin F2α (8-epiPGF)," excretion in the urine and TBARS (as an assessment of malondialdehyde and marker of lipid peroxidation) in the liver and kidney of male Fischer rats exposed to single i.p. injections in of TCE in Alkamuls vehicle. Using this paradigm, 500-mg/kg TCE was reported to induce Stage II anesthesia and 1,000 mg/kg TCE was reported to induce Level III or IV (absence of reflex response) anesthesia and burgundy-colored urine with 2/6 rats at 24 hours comatose and hypothermic. The animals were sacrificed before they could die and the authors suggested that they would not have survived another 24 hours. Thus, using this paradigm, there was significant toxicity and additional issues related to route of exposure. Urine volume declined significantly during the first 12 hours of treatment and while water consumption was not measured, it was

suggested by the authors to be decreased due to the moribundity of the rats. Given that this study examined urinary markers of "oxidative stress," the effects on urine volume and water consumption, as well as the profound toxicity induced by this exposure paradigm, limit the interpretation of the study. The issues of bias in selection of the data for this analysis, as well as the issues stated above for this paradigm limit interpretation of these data while the authors suggest that evidence of oxidative damage was equivocal.

4.5.4.6. Bile Production

Effects of TCE exposure in humans and in experimental animals is presented in Section E.2.6. Serum bile acids (SBA) have been suggested as a sensitive indicator of hepatotoxicity to a variety of halogenated solvents with an advantage of increased sensitivity and specificity over conventional liver enzyme tests that primarily reflect the acute perturbation of hepatocyte membrane integrity and "cell leakage" rather than liver functional capacity (i.e., uptake, metabolism, storage, and excretion functions of the liver) (Neghab et al., 1997; Bai et al., 1992a) While some studies have reported negative results, a number of studies have reported elevated SBA in organic solvent-exposed workers in the absence of any alterations in normal liver function tests. These variations in results have been suggested to arise from failure of some methods to detect some of the more significantly elevated SBA and the short-lived and reversible nature of the effect (Neghab et al., 1997). Neghab et al. (1997) reported that occupational exposure to 1,1,2-trichloro-1,2,2-trifluoroethane and TCE has resulted in elevated SBA and that several studies have reported elevated SBA in experimental animals to chlorinated solvents such as carbon tetrachloride, chloroform, hexachlorobutadiene, tetrachloroethylene, 1,1,1-trichloro-ethane, and TCE at levels that do not induce hepatotoxicity (Hamdan and Stacey, 1993; Bai et al., 1992b; Bai et al., 1992a; Wang and Stacey, 1990). Toluene, a nonhalogenated solvent, has also been reported to increase SBA in the absence of changes in other hepatobiliary functions (Neghab and Stacey, 1997). Thus, disturbance in SBA appears to be a generalized effect of exposure to chlorinated solvents and nonchlorinated solvents and not specific to TCE exposure.

Wang and Stacey (1990) administered TCE in corn oil via i.p. injection to male Sprague-Dawley rats with liver enzymes and SBA examined 4 hours after the last TCE treatment. The limitations of i.p injection experiments have already been discussed. While reporting no overt liver toxicity, there was, generally, a reported dose-related increase in cholic acid, chenodeoxycholic acid, deoxycholic acid, taurocholic acid, tauroursodeoxycholic acid with cholic acid, and taurochlolic acid increased at the lowest dose. The authors reported that "examination of liver sections under light microscopy yielded no consistent effects that could be ascribed to trichloroethylene." In the same study, a rats were also exposed to TCE via inhalation and using this paradigm, cholic acid and taurocholic acid were also significantly elevated but the large variability in responses between rats and the low number of rats tested in this paradigm limit its ability to determine quantitative differences between groups. Nevertheless, without the

complications associated with i.p. exposure, inhalation exposure of TCE at relatively low exposure levels that were not associated with other measures of toxicity *were* associated with increased SBA level.

Hamdan and Stacey (1993) administered TCE in corn oil (1 mmol/kg) in male Sprague-Dawley rats and followed the time-course of SBA elevation, TCE concentration, and TCOH in the blood up to 16 hours. Liver and blood concentration of TCE were reported to peak at 4 hours, while those of TCOH peaked at 8 hours after dosing. TCE levels were not detectable by 16 hours in either blood or liver, while those of TCOH were still elevated. Elevations of SBA were reported to parallel those of TCE with cholic acid, and taurochloate acid was reported to show the highest levels of bile acids. The authors stated that liver injury parameters were checked and were found to be unaffected by TCE exposure, but did not provide the data. Thus, it was TCE concentration and not that of its metabolite that was most closely related to changes in SBA after a single exposure and the effect appeared to be reversible. In an in vitro study by Bai and Stacey (1993), TCE was studied in isolated rat hepatocytes with TCE reported to cause a dose-related suppression of initial rates of cholic acid and taurocholic acid, but with no significant effects on enzyme leakage and intracellular calcium contents, further supporting a role for the parent compound in this effect.

4.5.4.7. Summary: TCE-Induced Noncancer Effects in Laboratory Animals

In laboratory animals, TCE leads to a number of structural changes in the liver, including increased liver weight, small transient increases in DNA synthesis, cytomegaly in the form of "swollen" or enlarged hepatocytes, increased nuclear size probably reflecting polyploidization, and proliferation of peroxisomes. Liver weight increases proportional to TCE dose are consistently reported across numerous studies, and appear to be accompanied by periportal hepatocellular hypertrophy. There is also evidence of increased DNA synthesis in a small portion of hepatocytes at around 10 days in vivo exposure. The lack of correlation of hepatocellular mitotic figures with whole-liver DNA synthesis or DNA synthesis observed in individual hepatocytes supports the conclusion that cellular proliferation is not the predominant cause of increased DNA synthesis. The lack of correlation of whole-liver DNA synthesis and those reported for individual hepatocytes suggests that nonparenchymal cells also contribute to such synthesis. Indeed, nonparenchymal cell activation or proliferation has been noted in several studies. Moreover, the histological descriptions of TCE exposed liver are consistent with, and in some cases specifically note, increased polyploidy after TCE exposure. Interestingly, changes in TCE-induced hepatocellular ploidy, as indicated by histological changes in nuclei, have been noted to remain after the cessation of exposure. In regard to apoptosis, TCE has been reported to either not change apoptosis or to cause a slight increase at high doses. Some studies have also noted effects from dosing vehicle alone (such as corn oil in particular) not only on liver pathology, but also on DNA synthesis.

Available data also suggest that TCE does not induce substantial cytotoxicity, necrosis, or regenerative hyperplasia, as only isolated, focal necroses and mild to moderate changes in serum and liver enzyme toxicity markers having been reported. Data on peroxisome proliferation, along with increases in a number of associated biochemical markers, show effects in both mice and rats. These effects are consistently observed across rodent species and strains, although the degree of response at a given mg/kg-day dose appears to be highly variability across strains, with mice on average appearing to be more sensitive.

In addition, like humans, laboratory animals exposed to TCE have been observed to have increased serum bile acids, though the toxicological importance of these effects is unclear.

4.5.5. TCE-Induced Liver Cancer in Laboratory Animals

For 2-year or lifetime studies of TCE exposure, a consistent hepatocarcinogenic response has been observed using mice of differing strains and genders and from differing routes of exposure. However, some rat studies have been confounded by mortality from gavage error or the toxicity of the dose of TCE administered. In some studies, a relative insensitive strain of rat has been used. However, in general, it appears that the mouse is more sensitive than the rat to TCE-induced liver cancer. Three studies had results that the authors considered to be negative for TCE-induced liver cancer in mice, but have either design and/or reporting limitations, or are in strains and paradigms with apparent low ability for liver cancer induction or detection. Findings from these studies are shown in Tables 4-60 through 4-65, and discussed below.

4.5.5.1. Negative or Inconclusive Studies of Mice and Rats

Fukuda et al. (1983) reported a 104-week inhalation bioassay in female Crj:CD-1 (ICR) mice and female Crj:CD (Sprague-Dawley) rats exposed to 0-, 50-, 150-, and 450-ppm TCE (n = 50). There were no reported incidences of mice or rats with liver tumors for controls indicative of relatively insensitive strains and gender used in the study for liver effects. While TCE was reported to induce a number of other tumors in mice and rats in this study, the incidence of liver tumors was <2% after TCE exposure. Of note is the report of cystic cholangioma reported in one group of rats.

Table 4-60. Summary of liver tumor findings in gavage studies of TCE by NTP (1990)[a]

Sex	Dose (mg/kg)[b]	Adenoma (overall; terminal[c])	Adenocarcinoma (overall; terminal[c])
1/d, 5 d/wk, 103-wk study, F344/N rats			
Male	0	NA[d]	0/49
	500	NA	0/49
	1,000	NA	1/49
Female	0	NA	0/50
	500	NA	1/48
	1,000	NA	1/48
1/d, 5 d/wk, 103-wk study, B6C3F$_1$ mice			
Male	0	7/48; 6/33	8/48; 6/33
	1,000	14/50; 6/16	31/50; 14/16[f]
Female	0	4/48; 4/32	2/48; 2/32
	1,000	16/49; 11/23[e]	13/49; 8/23[g]

[a]Liver tumors not examined in 13-week study, so data shown only for 103-week study.
[b]Corn oil vehicle.
[c]Terminal values not available for rats.
[d]Data not available.
[e]$p < 0.003$.
[f]$p < 0.001$.
[g]$p \leq 0.002$.

Table 4-61. Summary of liver tumor findings in gavage studies of TCE by NCI (1976)

Sex	Dose (mg/kg)[a]	Hepatocarcinoma
1/d, 5 d/wk, 2-yr study, Osborne-Mendel rats		
Males	0	0/20
	549	0/50
	1,097	0/50
Females	0	0/20
	549	1/48
	1,097	0/50
1/d, 5 d/wk, 2-yr study, B6C3F$_1$ mice		
Males	0	1/20
	1,169	26/50[b]
	2,339	31/48[b]
Females	0	0/20
	869	4/50
	1,739	11/47[b]

[a]Treatment period was 48 week for rats, 66 week for mice. Doses were changed several times during the study based on monitoring of body weight changes and survival. Dose listed here is the TWA dose over the days on which animals received a dose.
[b]$p < 0.01$.

Table 4-62. Summary of liver tumor incidence in gavage studies of TCE by NTP (1988)

Sex	Dose (mg/kg)[a]	Adenoma	Adenocarcinoma
1/d, 5 d/wk, 2-yr study, ACI rats			
Male	0	0/50	1/50
	500	0/49	1/49
	1,000	0/49	1/49
Female	0	0/49	2/49
	500	0/46	0/46
	1,000	0/39	0/39
1/d, 5 d/wk, 2-yr study, August rats			
Male	0	0/50	0/50
	500	0/50	1/50
	1,000	0/48	1/48
Female	0	0/48	2/48
	500	0/48	0/48
	1,000	0/50	0/50
1/d, 5 d/wk, 2-yr study, Marshall rats			
Male	0	1/49	1/49
	500	0/50	0/50
	1,000	0/47	1/47
Female	0	0/49	0/49
	500	0/48	0/48
	1,000	0/46	0/46
1/d, 5 d/wk, 2-yr study, Osborne-Mendel rats			
Male	0	1/50	1/50
	500	1/50	0/50
	1,000	1/49	2/49
Female	0	0/50	0/50
	500	0/48	2/48
	1,000	0/49	2/49

[a]Corn oil vehicle.

Table 4-63. Summary of liver tumor findings in inhalation studies of TCE by Maltoni et al. (1988; 1986)[a]

Sex	Concentration (ppm)	Hepatoma
7 hrs/d, 5 d/wk, 8-wk exposure, observed for lifespan, Swiss mice		
Male	0	1/100
	100	3/60
	600	4/72
Female	0	1/100
	100	1/60
	600	0/72
7 hrs/d, 5 d/wk, 78-wk exposure, observed for lifespan, Swiss mice		
Male	0	4/90
	100	2/90
	300	8/90
	600	13/90
Female	0	0/90
	100	0/90
	300	0/90
	600	1/90
7 hrs/d, 5 d/wk, 78-wk exposure, observed for lifespan, $B6C3F_1$ mice[b]		
Male	0	1/90
	100	1/90
	300	3/90
	600	6/90
Female	0	3/90
	100	4/90
	300	4/90
	600	9/90

[a]Three inhalation experiments in this study found no hepatomas: BT302 (8-week exposure to 0, 100, or 600 ppm in Sprague-Dawley rats); BT303 (8-week exposure to 0, 100, or 600 ppm in Swiss mice); and BT304 (78-week exposure to 0, 100, 300, or 600 ppm in Sprague-Dawley rats).
[b]Female incidences are from experiment BT306, while male incidences are from experiment BT306bis, which was added to the study because of high, early mortality due to aggressiveness and fighting in males in experiment BT306.

Table 4-64. Summary of liver tumor findings in inhalation studies of TCE by Henschler et al. (1980)[a] and Fukuda et al. (1983)

Sex	Concentration (ppm)	Adenomas	Adenocarcinomas
6 hrs/d, 5 d/wk, 18-mo exposure, 30-mo observation, Han:NMRI mice (Henschler et al., 1980)			
Males	0	1/30[b]	1/30
	100	2/29[b]	0/30
	500	0/29	0/30
Females	0	0/29	0/29
	100	0/30	0/30
	500	0/28	0/28
6 hrs/d, 5 d/wk, 18-mo exposure, 36-mo observation, Han:WIST rats (Henschler et al., 1980)			
Males	0	1/29	0/29
	100	1/30	0/30
	500	0/30	0/30
Females	0	0/28	0/28
	100	1/30	1/30
	500	2/30	0/30
7 hrs/d, 5 d/wk, 2-yr study, Crj:CD (Sprague-Dawley) rats (Fukuda et al., 1983)			
Females	0	0/50	0/50
	50	1/50	0/50
	150	0/47	0/47
	450	0/51	1/50
7 hrs/d, 5 d/wk, 2-yr study, Crj:CD (ICR) mice (Fukuda et al., 1983)			
Females	0	0/49	0/49
	50	0/50	0/50
	150	0/50	0/50
	450	1/46	0/46

[a]Henschler et al. (1980) observed no liver tumors in control or exposed Syrian hamsters.
[b]One additional hepatic tumor of undetermined class not included.

Table 4-65. Summary of liver tumor findings in gavage studies of TCE by Henschler et al. (1984)[a]

Sex (TCE concentration)	TCE (Stabilizers if present)	Benign[b]	Malignant[c]
5 d/wk, 18-mo exposure, 24-mo observation, Swiss mice (Henschler et al., 1984)			
Males (2.4g/kg body weight)	Control (none)	5/50	0/50
	TCE (triethanolamine)	7/50	0/50
	TCE (industrial)	9/50	0/50
	TCE (epichlorohydrin (0.8%))	3/50	1/50
	TCE (1,2-epoxybutane [0.8%])	4/50	0/50
	TCE (both epichlorohydrin [0.25%] and 1,2-epoxybutane [0.25%])	5/50	0/50
Females (1.8 g/kg body weight)	Control (none)	1/50	0/50
	TCE (triethanolamine)	7/50	0/50
	TCE (industrial)	9/50	0/50
	TCE (epichlorohydrin [0.8%])	3/50	0/50
	TCE (1,2-epoxybutane [0.8%])	2/50	0/50
	TCE (both epichlorohydrin [0.25%] and 1,2-epoxybutane [0.25%])	4/50	1/50

[a]Henschler et al. (1984) due to poor condition of the animals resulting from the nonspecific toxicity of high doses of TCE and/or the additives, gavage was stopped for all groups during week 35–40, 65 and 69–78, and all doses were reduced by a factor of 2 from the 40th week on.
[b]Includes hepatocellular adenomas, hemangioendothelioma, cholangiocellular adenoma.
[c]Includes HCC, malignant hemangiosarcoma, cholangiocellular carcinoma.

Henschler et al. (1980) exposed NMRI mice and WIST random bred rats to 0-, 100-, and 500-ppm TCE for 18 months (n = 30). Control male mice were reported to have one HCC and one hepatocellular adenoma with the incidence rate unknown. In the 100 ppm group, two hepatocellular adenomas and one mesenchymal liver tumor were reported. No liver tumors were reported at any dose of TCE in female mice or controls. For male rats, only one hepatocellular adenomas at 100 ppm was reported. For female rats, no liver tumors were reported in controls, but one adenoma and one cholangiocarcinoma was reported at 100 ppm, and at 500 ppm, two cholangioadenomas, a relatively rare biliary tumor, were reported. The difference in survival in mice, did not affect the power to detect a response, as was the case for rats. However, the low number of animals studied, abbreviated exposure duration, low survival in rats, and absent background response (suggesting low intrinsic sensitivity to this endpoint) suggest a study of limited ability to detect a TCE carcinogenic liver response. Of note is that despite their limitations, both Fukuda et al. (1983) and Henschler et al. (1980) report rare biliary cell derived tumors in TCE-exposed rats.

Van Duuren et al. (1979), exposed mice to 0.5 mg/mouse to TCE via gavage once a week in 0.1 mL trioctanion (n = 30). Inadequate design and reporting of this study limit that ability to use the results as an indicator of TCE carcinogenicity.

The NCI (1976) study of TCE was initiated in 1972 and involved the exposure of Osborne-Mendel rats to varying concentrations of TCE. A low incidence of liver tumors was reported for controls and carbon tetrachloride positive controls in rats from this study. The authors concluded that due to mortality, "the test is inconclusive in rats." They note the insensitivity of the rat strain used to the positive control of carbon tetrachloride exposure.

The NTP (1990) study of TCE exposure in male and female F344/N rats, and $B6C3F_1$ mice (500 and 1,000 mg/kg for rats) is limited in the ability to demonstrate a dose-response for hepatocarcinogenicity. For rats, the NTP (1990) study reported no treatment-related, non-neoplastic liver lesions in males and a decrease in basophilic cytological change in female rats. The results for detecting a carcinogenic response in rats were considered to be equivocal because both groups receiving TCE showed significantly reduced survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the high-dose group) of death by gavage error.

The NTP (1988) study of TCE exposure in four strains of rats to "diisopropylamine-stabilized TCE" was also considered inadequate for either comparing or assessing TCE-induced liver carcinogenesis in these strains of rats because of chemically induced toxicity, reduced survival, and incomplete documentation of experimental data. TCE gavage exposures of 0, 500, or 1,000 mg/kg-day (5 days/week, for 103 weeks) male and female rats were also marked by a large number of accidental deaths (e.g., for high-dose male Marshal rats, 25 animals were accidentally killed).

Maltoni et al. (1988; 1986) reported the results of several studies of TCE via inhalation and gavage in mice and rats. A large number of animals were used in the treatment groups but the focus of the study was detection of a neoplastic response with only a generalized description of tumor pathology phenotype and limited reporting of non-neoplastic changes in the liver. Accidental death by gavage error was reported not to occur in this study. With regard to effects of TCE exposure on rat survival, "a nonsignificant excess in mortality correlated to TCE treatment was observed only in female rats (treated by ingestion with the compound)."

For rats, Maltoni et al. (1986) reported four liver angiosarcomas (one in a control male rat, one both in a TCE-exposed male and female at 600 ppm TCE for 8 weeks, and one in a female rat exposed to 600-ppm TCE for 104 weeks), but the specific results for incidences of hepatocellular "hepatomas" in treated and control rats were not given. Although the Maltoni et al. (1986) concluded that the small number was not treatment related, the findings were brought forward because of the extreme rarity of this tumor in control Sprague-Dawley rats, untreated or treated with vehicle materials. In rats treated for 104 weeks, there was no report of a TCE treatment-related increase in liver cancer in rats. This study only presented data for positive findings so it did not give the background or treatment-related findings in rats for liver tumors in this study. Thus, the extent of background tumors and sensitivity for this endpoint cannot be determined. Of note is that the Sprague-Dawley strain used in this study was also noted in the

Fukuda et al. (1983) study to be relatively insensitive for spontaneous liver cancer and to also be negative for TCE-induced hepatocellular liver cancer induction in rats. However, like Fukuda et al. (1983) and Henschler et al. (1980), which reported rare biliary tumors in insensitive strains of rat for hepatocellular tumors, Maltoni et al. (1986) reported a relatively rare tumor type, angiosarcoma, after TCE exposure in a relatively insensitive strain for ―hepatomas.‖ As noted above, many of the rat studies were limited by premature mortality due to gavage error or premature mortality (NTP, 1990, 1988; Henschler et al., 1980; NCI, 1976), which was reported not occur in Maltoni et al. (1986).

4.5.5.2. Positive TCE Studies of Mice

In the NCI (1976) study of TCE exposure in $B6C3F_1$ mice, TCE was reported to increase the incidence of HCCs in both doses and both genders of mice (~1,170 and 2,340 mg/kg for males and 870 and 1,740 mg/kg for female mice). HCC diagnosis was based on histologic appearance and metastasis to the lung. The tumors were described in detail and to be heterogeneous ―as described in the literature‖ and similar in appearance to tumors generated by carbon tetrachloride. The description of liver tumors in this study and tendency to metastasize to the lung are similar to descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation exposure.

The NTP (1990) study of TCE exposure in male and female $B6C3F_1$ mice (1,000 mg/kg for mice) reported decreased latency of liver tumors, with animals first showing carcinomas at 57 weeks for TCE-exposed animals and 75 weeks for control male mice. The administration of TCE was also associated with increased incidence of HCC (tumors with markedly abnormal cytology and architecture) in male and female mice. Hepatocellular adenomas were described as circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent but the tumors lacked typical lobular organization. HCCs had markedly abnormal cytology and architecture with abnormalities in cytology cited as including increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies, and variations in nuclear appearance. Furthermore, in many instances several or all of the abnormalities were present in different areas of the tumor and variations in architecture with some of the HCCs having areas of trabecular organization. Mitosis was variable in amount and location. Therefore, the phenotype of tumors reported from TCE exposure was heterogeneous in appearance between and within tumors. However, because it consisted of a single-dose group in addition to controls, this study is of limited utility for analyzing the dose-response for hepatocarcinogenicity. There was also little reporting of non-neoplastic pathology or toxicity and no report of liver weight at termination of the study.

Maltoni et al. (1986) reported the results of several studies of TCE in mice. A large number of animals were used in the treatment groups but the focus of the study was detection of a neoplastic response with only a generalized description of tumor pathology phenotype given and limited reporting of non-neoplastic changes in the liver. There was no accidental death by gavage error reported to occur in mice, but a "nonsignificant" excess in mortality correlated to TCE treatment was observed in male $B6C3F_1$ mice. TCE-induced effects on body weight were reported to be absent in mice except for one experiment (BT 306 bis) in which a slight nondose-correlated decrease was found in exposed animals. "Hepatoma" was the term used to describe all malignant tumors of hepatic cells, of different subhistotypes, and of various degrees of malignancy and were reported to be unique or multiple, and have different sizes (usually detected grossly at necropsy) from TCE exposure. In regard to phenotype, tumors were described as usual type observed in Swiss and $B6C3F_1$ mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens and to frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. Swiss mice from this laboratory were reported to have a low incidence of hepatomas without treatment (1%). The relatively larger number of animals used in this bioassay (n = 90–100), in comparison to NTP standard assays, allows for a greater power to detect a response.

TCE exposure for 8 weeks via inhalation at 100 or 600 ppm may have been associated with a small increase in liver tumors in male mice in comparison to concurrent controls during the life span of the animals. In Swiss mice exposed to TCE via inhalation for 78 weeks, there a reported increase in hepatomas associated with TCE treatment that was dose-related in male but not female Swiss mice. In $B6C3F_1$ mice exposed via inhalation to TCE for 78 weeks, increases in hepatomas were reported in both males and females. However, the experiment in males was repeated with $B6C3F_1$ mice from a different source, since in the first experiment, more than half of the mice died prematurely due to excessive fighting. Although the mice in the two experiments in males were of the same strain, the background level of liver cancer was significantly different between mice from the different sources (1/90 vs. 19/90), though the early mortality may have led to some censoring. The finding of differences in response in animals of the same strain but from differing sources has also been reported in other studies for other endpoints. However, for both groups of male $B6C3F_1$ mice, the background rate of liver tumors over the lifetime of the mice was no greater than about 20%.

There were other reports of TCE carcinogenicity in mice from chronic exposures that were focused primarily on the detection of liver tumors, with limited reporting of tumor phenotype or non-neoplastic pathology. Herren-Freund et al. (1987) reported that male $B6C3F_1$ mice given 40 mg/L TCE in drinking water had increased tumor response after 61 weeks of exposure. However, concentrations of TCE fell by about half at this dose of TCE during the twice a week change in drinking water solution so the actual dose of TCE the animals received was <40 mg/L. The percentage liver/body weight was reported to be similar for control and

TCE-exposed mice at the end of treatment. However, despite difficulties in establishing accurately the dose received, an increase in adenomas per animal and an increase in the number of animals with HCCs were reported to be associated with TCE exposure after 61 weeks of exposure and without apparent hepatomegaly. Anna et al. (1994) reported tumor incidences for male $B6C3F_1$ mice receiving 800 mg/kg-day TCE via gavage (5 days/week for 76 weeks). All TCE-treated mice were reported to be alive after 76 weeks of treatment. Although the control group contained a mixture of exposure durations (76–134 weeks) and concurrent controls had a very small number of animals, TCE treatment appeared to increase the number of animals with adenomas and the mean number of adenomas and carcinomas, but with no concurrent TCE-induced cytotoxicity.

4.5.5.3. Summary: TCE-Induced Cancer in Laboratory Animals

Chronic TCE bioassays have consistently reported increased liver tumor incidences in both sexes of $B6C3F_1$ mice treated by inhalation and gavage exposure in a number of bioassays. The only inhalation study of TCE in Swiss mice also showed an effect in males. Data in the rat, while not reporting statistically significantly increased risks, are not entirely adequate due to low numbers of animals, inadequate reporting, use of insensitive bioassays, increased systemic toxicity, and/or increased mortality. Notably, several studies in rats noted a few very rare types of liver or biliary tumors (cystic cholangioma, cholangiocarcinoma, or angiosarcomas) in treated animals.

4.5.6. Role of Metabolism in Liver Toxicity and Cancer

It is generally thought that TCE oxidation by CYPs is necessary for induction of hepatotoxicity and hepatocarcinogenicity (Bull, 2000). Direct evidence for this hypothesis is limited, e.g., the potentiation of hepatotoxicity by pretreatment with CYP inducers such as ethanol and phenobarbital (Okino et al., 1991; Nakajima et al., 1988). Rather, the presumption that CYP-mediated oxidation is necessary for TCE hepatotoxicity and hepatocarcinogenicity is largely based on similar effects (e.g., increases in liver weight, peroxisome proliferation, and hepatocarcinogenicity) having been observed with TCE‘s oxidative metabolites. The discussion below focuses the similarities and differences between the major effects in the liver of TCE and of the oxidative metabolites CH, TCA, and DCA. In addition, CH is largely converted to TCOH, TCA, and possibly DCA. DCA has been used in human clinical practice for a variety of severe illnesses and no data on liver effects in humans have been reported (U.S. EPA, 2003b). However, as noted in EPA (2003b), data on DCA in humans are scarce and complicated by the fact that available studies have predominantly focused on individuals who have a pre-existing (usually severe) disease.

4.5.6.1. Pharmacokinetics of CH, TCA, and DCA from TCE Exposure

As discussed in Chapter 3, in vivo data confirm that CH and TCA, are oxidative metabolites of TCE, with available data on TCA incorporated into the PBPK modeling. In addition, there are indirect data suggesting the formation of DCA. However, direct in vivo evidence of the formation of DCA is confounded by its rapid clearance at low concentrations, and analytical artifacts in its detection in vivo that have yet to be entirely resolved. PBPK modeling (see Section 3.5) predicts that the proportions of TCE metabolized to CH and TCA varies considerably in mice (ranging from 15 to 97 and 4 to 38%, respectively) and rats (ranging 7–75 and 0.5–22%, respectively). Therefore, a range of smaller concentrations of TCA or CH may be relevant for comparisons with TCE-induced liver effects. For example, for 1,000 mg/kg-day oral doses of TCE, the relevant comparisons would be approximately 0.25–1.5 g/L in drinking water for TCA and CH. For DCA, a corresponding range is harder to determine and has been suggested to be an upper limit of about 12% following oral exposures (Barton et al., 1999). This is consistent with the range estimated from PBPK modeling attributing all of the "unracked" oxidation (i.e., not producing TCOH or TCA) to DCA (95% CI: 0.2–16%, see Figure 3-22).

Two studies have used analytic methods for DCA that are considered more reliable and less confounded by artifactual formation. Kim et al. (2009), which was published too late to be incorporated into the PBPK model, used an empirical pharmacokinetic model to analyze data on male B6C3F$_1$ mice exposed to a single dose of 2,100 mg/kg TCE by gavage. Peak levels of TCA and DCA were found to be 64 and 18 ng/mL, respectively, a difference of more than threefold. The kinetic rate constant they estimated for TCE → DCA were more than five orders of magnitude smaller than the kinetic rate constant estimated for TCE → TCA. These data all suggest that DCA is a minor metabolite of TCE as compared to TCA at high doses of around 2,000 mg/kg. Delinsky et al. (2005) reported that in male Sprague-Dawley rats, after a single 2,000 mg/kg dose by gavage, peak levels of DCA were 39.5 ng/mL. Delinsky et al. (2005) did not report TCA levels for comparison. The only data available in rats in this range of gavage doses (coincidentally also in male Sprague-Dawley rats) reported peak levels of TCA of 24 and 60 mg/mL at gavage doses of 600 and 3,000 mg/kg, respectively (Larson and Bull, 1992b). This suggests a difference between DCA and TCA levels in rats exposed to TCE of about 1,000-fold, albeit with more uncertainty as compared to Kim et al. (2009), in which both were measured simultaneously in the same animals. However, liver toxicity in both rats and mice is evident at much lower doses, so additional data are needed to inform whether the relative amount of TCA and DCA changes at lower exposures.

4.5.6.2. Comparisons Between TCE and TCA, DCA, and CH Noncancer Effects

4.5.6.2.1. Hepatomegaly—qualitative and quantitative comparisons

As discussed above, TCE causes hepatomegaly in rats, mice, and gerbils under both acute and chronic dosing. Data from a few available studies suggest that oxidative metabolism is important for mediating these effects. Buben and O'Flaherty (1985) collected limited pharmacokinetic data in a sample of the same animals for which liver weight changes were being assessed. While liver weight increases had similarly strong correlations with applied dose and urinary metabolites for doses up to 1,600 mg/kg-day (R^2 of 0.97 for both), above that dose, the linear relationship was maintained with urinary metabolites but not with applied dose. Ramdhan et al. (2008) conducted parallel experiments at TCE 1,000 and 2,000 ppm (8 hours/day, 7 days) in wild-type and CYP2E1-null mice, which did not exhibit increased liver/body weight ratios with TCE treatment and excreted twofold lower amounts of oxidative metabolites TCA and TCOH in urine as compared to wild-type mice. However, among control mice, those with the null genotype had 1.32-fold higher absolute liver weights and 1.18-fold higher liver/body weight ratios than wild-type mice, reducing the sensitivity of the experiment, particularly with only six mice per dose group.

Ramdhan et al. (2010) reported that stated that urinary TCA levels in wild type mice were incorrectly reported by Ramdhan et al. (2008) but were corrected in this study. The authors reported no differences in urinary volume by genotype or exposure but did not show the data. TCA and TCOH were detected in all exposed mice with no significant differences between the 1,000 and 2,000 ppm TCE levels. TCA concentrations were reported to be significantly lower and TCOH levels significantly higher in PPARα-null mice relative to wild type mice, with no differences in genotype between the sum of total TCA and TCOH concentrations between genotypes. The authors reported that they measured hepatic protein expression of CYP2E1 and ALDH2 enzymes and did not observe a significant difference among controls (data not shown) and that TCE exposure did not alter hepatic CYP2E1 expression but did decrease ALDH2 expression to a comparable extent in all mouse lines (data not shown). Thus, changes in urinary TCA levels in the differing strains were not related to changes in expression of these metabolic enzymes.

As stated above, hepatomegally was increased by TCE exposure in all three strains. TCE at both 1,000 and 2,000 ppm significantly increased liver weight in the three mouse lines to a similar extent (i.e., 38 and 49% in wild type mice, 20 and 37% in PPAR-null mice, and 28 and 32% in hPPARα mice). The increases were not statistically significant between doses within each strain. Liver/body weight ratios were also significantly increased with TCE exposure at 1,000 and 2,000 ppm relative to controls (i.e., 38 and 43% in wild type mice, 24 and 36% in PPARα-null mice, and 27 and 39% in hPPARα mice, respectively). The difference between 2,000 and 1,000 ppm TCE exposure was statistically significant in PPARα-null mice. As to the nature of the hepatomegally induced under these conditions, hepatic triglyceride levels were

reported to be significantly correlated with liver/body weight ratios of all mice used in the study ($r = 0.54$).

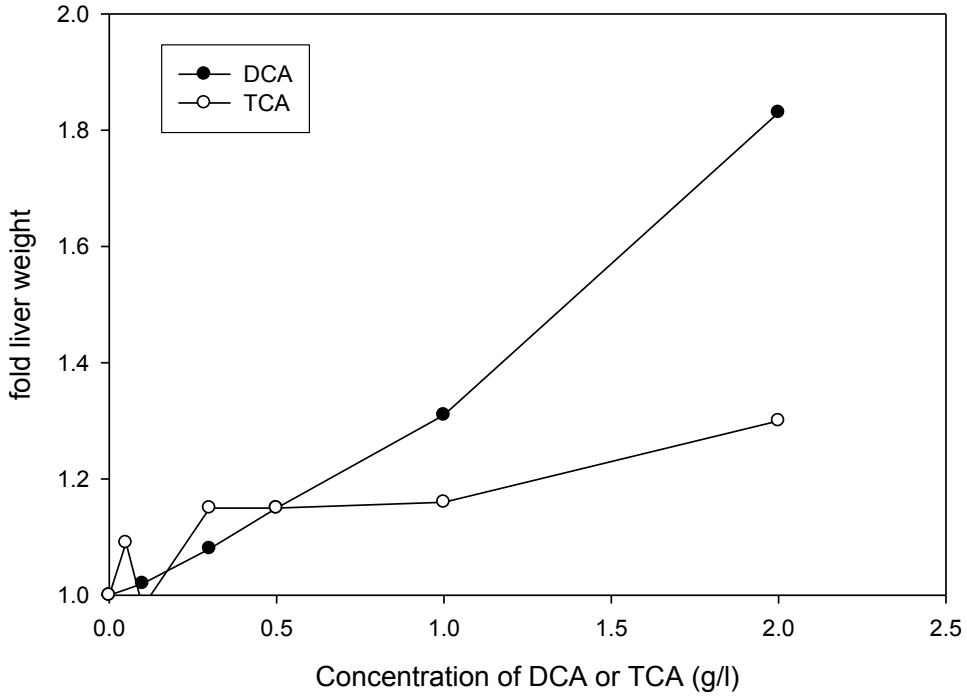
With respect to oxidative metabolites themselves, data from CH studies are not informative—either because data were not shown (Sanders et al., 1982a) or, because at the time points measured, liver weight increases were substantially confounded by foci and carcinogenic lesions (Leakey et al., 2003b). TCA and DCA have both been found to cause hepatomegaly in mice and rats, with mice being more sensitive to this effect. DCA also increases liver/body weight ratios in dogs, but TCE and TCA have not been tested in this species (Cicmanec et al., 1991).

As noted above, TCE-induced changes in liver weight appear to be proportional to the exposure concentration across route of administration, gender and rodent species. As an indication of the potential contribution of TCE metabolites to this effect, a quantitative comparison of the shape of the dose-response curves for liver weight induction for TCE and its metabolites is informative. The analysis below was reported in Evans et al. (2009).

A number of short-term (<4 weeks) studies of TCA and DCA in drinking water have attempted to measure changes in liver weight induction, with the majority of these studies being performed in male $B6C3F_1$ mice. Studies conducted from 14 to 30 days show a consistent increase in percentage liver/body weight induction by TCA or DCA. However, as stated in many of the discussions of individual studies (see Appendix E), there is a limited ability to detect a statistically significant change in liver weight change in experiments that use a relatively small number of animals or do not match control and treatment groups for age and weight. The experiments of Buben and O'Flaherty (1985) used 12–14 mice per group, giving them a greater ability to detect a TCE-induced dose-response. However, many experiments have been conducted with 4–6 mice per dose group. For example, the data from DeAngelo et al. (2008) for TCA-induced percentage liver/body weight ratio increases in male $B6C3F_1$ mice were only derived from five animals per treatment group after 4 weeks of exposure. The 0.05 and 0.5 g/L exposure concentrations were reported to give a 1.09- and 1.16-fold of control percentage liver/body weight ratios, which were consistent with the increases noted in the cross-study database above. However, a power calculation shows that the Type II error (which should be >50% and thus, greater than the chances of "flipping a coin") was only a 6 and 7% and therefore, the designed experiment could accept a false null hypothesis. In addition, some experiments took greater care to age and weight match the control and treatment groups before the start of treatment.

Therefore, given these limitations and the fact that many studies used a limited range of doses, an examination of the combined data from multiple studies (Kato-Weinstein et al., 2001; Parrish et al., 1996; Carter et al., 1995; Sanchez and Bull, 1990; DeAngelo et al., 1989, 2008) can best inform/discern differences in DCA and TCA dose-response relationships for liver weight induction (described in more detail in Section E.2.4.2). The dose-response curves for

similar concentrations of DCA and TCA are presented in Figure 4-5 for durations of exposure from 14 to 28 days in the male B6C3F$_1$ mouse, which was the most common sex and strain used. As noted in Appendix E, there appears to be a linear correlation between dose in drinking water and liver weight induction up to 2 g/L of DCA. However, the shape of the dose-response curve for TCA appears to be quite different. Lower concentrations of TCA induce larger increase that does DCA, but the TCE response reaches an apparent plateau while that of DCA continues to increase the response. TCA studies did not show significant duration-dependent difference in liver weight induction in this duration range. Short-duration studies (10–42 days) were selected because: (1) in chronic studies, liver weight increases are confounded by tumor burden; (2) multiple studies are available; and (3) TCA studies do not show significant duration-dependent differences in this duration range.

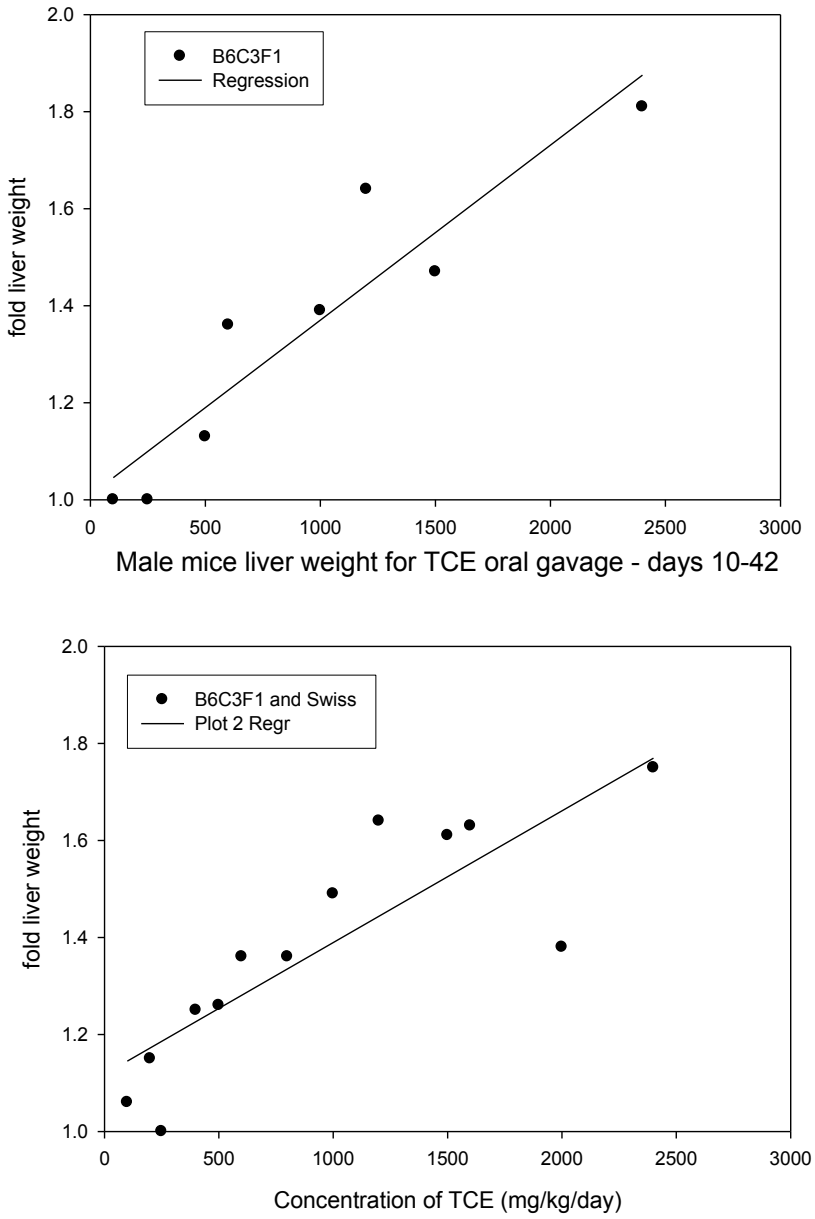


Sources: (Kato-Weinstein et al., 2001; Parrish et al., 1996; Carter et al., 1995; Sanchez and Bull, 1990; DeAngelo et al., 1989, 2008)).

Figure 4-5. Comparison of average fold-changes in relative liver weight to control and exposure concentrations of 2 g/L or less in drinking water for TCA and DCA in male B6C3F$_1$ mice for 14–30 days.

Of interest is the issue of how the dose-response curves for TCA and DCA compare to that of TCE in a similar model and dose range. Since TCA and DCA have strikingly different dose-response curves, which one, if either, best fits that of TCE and thus, can give insight as to which is causative agent for TCE‘s effects in the liver? The carcinogenicity of chronic TCE

exposure has been predominantly studies in two mouse strains, Swiss and B6C3F$_1$, both of which reportedly developed liver tumors. Rather than administered in drinking water, oral TCE studies have been conducted via gavage and generally in corn oil for 5 days of exposure per week. Factors adding to the increased difficulty in establishing the dose-response relationship for TCE across studies and for comparisons to the DCA and TCA database include vehicle effects, the difference between daily and weekly exposures, the dependence of TCE effects in the liver on its metabolism to a variety of agents capable inducing effects in the liver, differences in response between strains, and the inherent increased variability in use of the male mouse model. In particular, these factors would add variability to any effort at a combined analysis, and make a consistent dose-response pattern more difficult to discern. Nonetheless, despite such differences in exposure route, vehicle, etc., a consistent pattern of dose-response emerges from combining the available TCE data. The effects of oral exposure to TCE from 10 to 42 days on liver weight induction is shown below in Figure 4-6 using the data of Elcombe et al. (1985), Dees and Travis (1993), Goel et al. (1992), Merrick et al. (1989), Goldsworthy and Popp (1987), and Buben and O'Flaherty (1985). Oral TCE administration in male B6C3F$_1$ and Swiss mice appeared to induce a dose-related increase in percentage liver/body weight that was generally proportional to the increase in magnitude of dose, though as expected, with more variability than observed for a similar exercise for DCA or TCA in drinking water. Some of the variability is due to the inclusion of the 10-day studies, since as discussed in Section E.2.4.2, there was a greater increase in TCE-induced liver weight at 28–42 days of exposure Swiss mice than the 10-day data in B6C3F$_1$ mice, and Kjellstrand et al. (1981b) noted that TCE-induced liver weight increases are still increasing at 10 days inhalation exposure. A strain difference is not evident between the Swiss and B6C3F$_1$ males, as both the combined TCE data and that for only B6C3F$_1$ mice show similar correlation with the magnitude of dose and magnitude of percentage liver/body weight increase. The correlation coefficients for the linear regressions presented for the B6C3F$_1$ data are $R^2 = 0.861$ and for the combined data sets is $R^2 = 0.712$. Comparisons of the slopes of the dose-response curves suggest a greater consistency between TCE and DCA than between TCE and TCA. There did not appear to be evidence of a plateau with higher TCE doses, and the degree of fold-increase rises to higher levels with TCE than with TCA in the same strain of mouse.

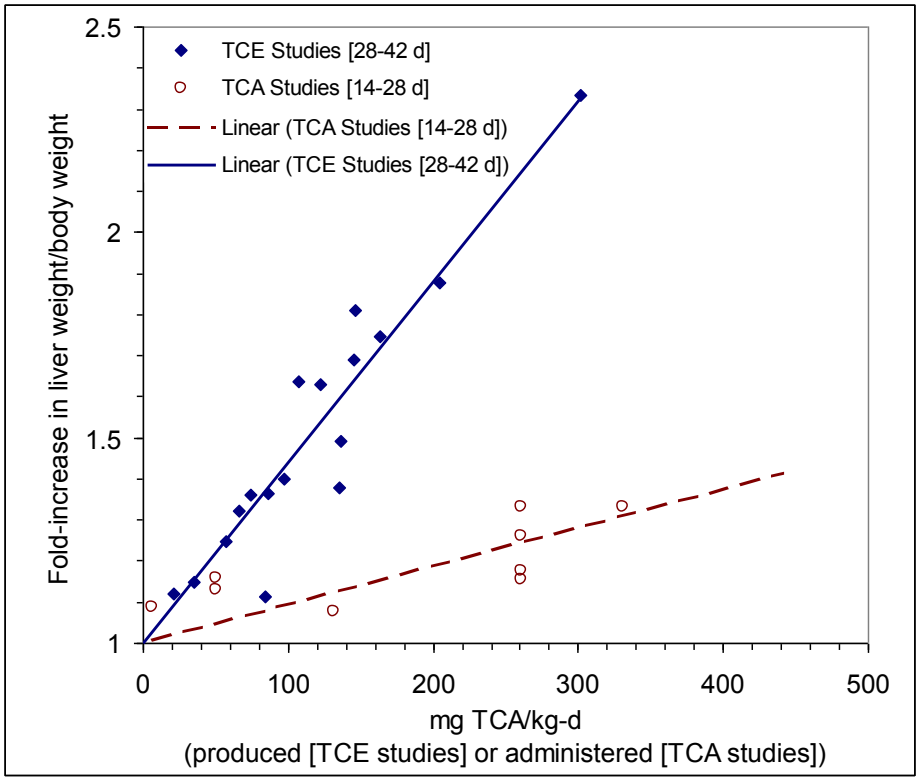


Sources: Dees and Travis (1993); Merrick et al. (1989); Goldsworthy and Popp (1987); Elcombe et al. (1985)

Figure 4-6. Comparisons of fold-changes in average relative liver weight and gavage dose of (top panel) male B6C3F$_1$ mice for 10–28 days of exposure and (bottom panel) in male B6C3F$_1$ and Swiss mice.

A more direct comparison would be on the basis of dose rather than drinking water concentration. The estimations of internal dose of DCA or TCA from drinking water studies, while varying considerably (DeAngelo et al., 2008; DeAngelo et al., 1989), nonetheless suggest that the doses of TCE used in the gavage experiments were much higher than those of DCA or TCA. However, only a fraction of ingested TCE is metabolized to DCA or TCA, as, in addition

to oxidative metabolism, TCE is also cleared by GSH conjugation and by exhalation. While DCA dosimetry is highly uncertain (see Sections 3.3 and 3.5), the mouse PBPK model, described in Section 3.5 was calibrated using extensive in vivo data on TCA blood, plasma, liver, and urinary excretion data from inhalation and gavage TCE exposures, and makes robust predictions of the rate of TCA production. If TCA were predominantly responsible for TCE-induced liver weight increases, then replacing administered TCE dose (e.g., mg TCE/kg/day) by the rate of TCA produced from TCE (mg TCA/kg/day) should lead to dose-response curves for increased liver weight consistent with those from directly administered TCA. Figure 4-7 shows this comparison using the PBPK model-based estimates of TCA production for four TCE studies from 28 to 42 days in the male NMRI, Swiss, and B6C3F$_1$ mice (Goel et al., 1992; Merrick et al., 1989; Buben and O'Flaherty, 1985; Kjellstrand et al., 1983a) and four oral TCA studies in B6C3F$_1$ male mice at ≤2 g/L drinking water exposure (2008; Kato-Weinstein et al., 2001; Parrish et al., 1996; DeAngelo et al., 1989) from 14 to 28 days of exposure. The selection of the 28–42 day data for TCE was intended to address the decreased opportunity for full expression of response at 10 days. PBPK modeling predictions of daily internal doses of TCA in terms of mg/kg-day produced via TCE metabolism would indeed be lower than the TCE concentrations in terms of mg/kg-day given orally by gavage. The predicted internal dose of TCA from TCE exposure studies are of a comparable range to those predicted from TCA drinking water studies at exposure concentrations in which palpability has not been an issue for estimation of internal dose. Thus, although the TCE data are for higher exposure concentrations, they are predicted to produce comparable levels of TCA internal dose estimated from direct TCA administration in drinking water.



Abscissa for TCE studies consists of the median estimates of the internal dose of TCA predicted from metabolism of TCE using the PBPK model described in Section 3.5 of the TCE risk assessment. Lines show linear regression with intercept fixed at unity. All data were reported fold-change in mean liver weight/body weight ratios, except for Kjellstrand et al. (1983a), which were the fold-change in the ratio of mean liver weight to mean body weight. In addition, in Kjellstrand et al. (1983a), some systemic toxicity as evidence by decreased total body weight was reported in the highest-dose group.

Sources: Kjellstrand et al. (1983a); Goel et al. (1992); Merrick et al. (1989; Maltoni et al., 1988); Buben and O'Flaherty (1985); DeAngelo et al. (1999); DeAngeleo et al. (2008); Kato-Weinstein et al. (2001); Parrish et al. (1996)

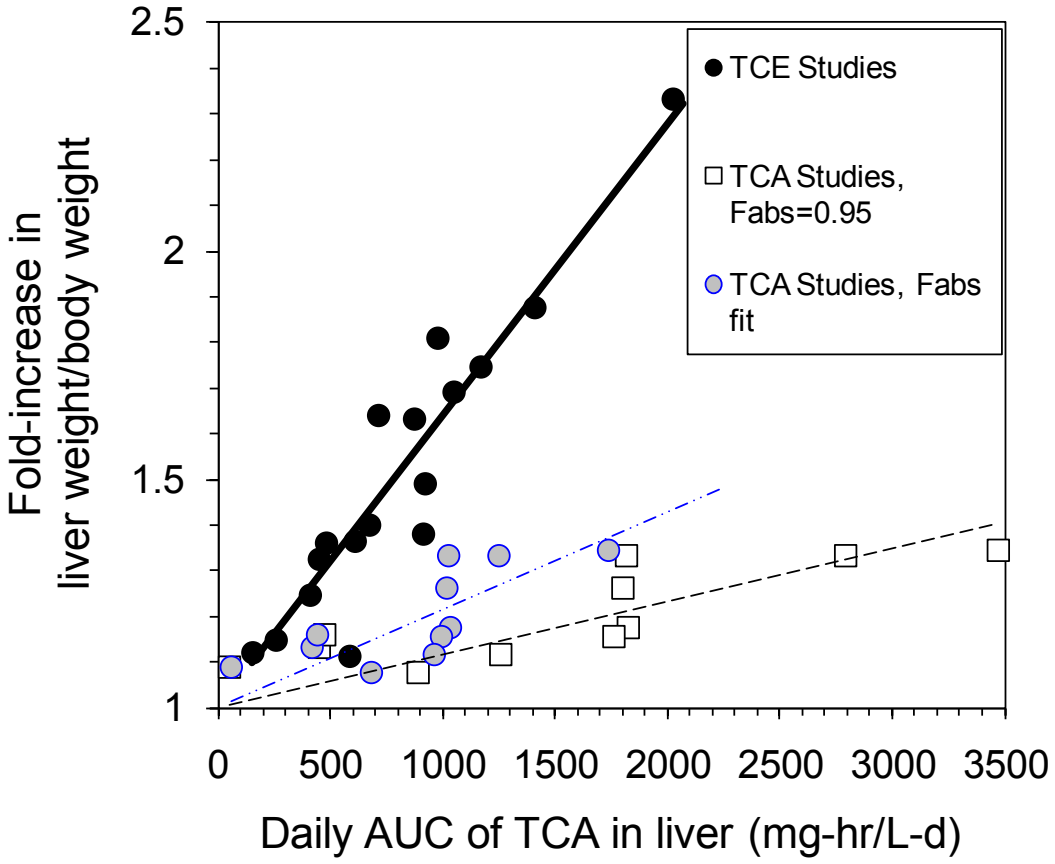
Figure 4-7. Comparison of fold-changes in relative liver weight for data sets in male B6C3F$_1$, Swiss, and NRMI mice between TCE studies [duration 28–42 days]) and studies of direct oral TCA administration to B6C3F$_1$ mice [duration 14–28 days]).

Figure 4-7 clearly shows that for a given amount of TCA produced from TCE, but going through intermediate metabolic pathways, the liver weight increases are substantially greater than, and highly inconsistent with, that expected based on direct TCA administration. In particular, the response from direct TCA administration appears to "saturate" with increasing TCA dose at a level of about 1.4-fold, while the response from TCE administration continues to increase with dose to 1.75-fold at the highest dose administered orally in Buben and O'Flaherty (1985) and over twofold in the inhalation study of Kjellstrand et al. (1983a). Because TCA liver

concentrations are proportional to the dose TCA, and do not depend on whether it is administered in drinking water or internally produced in the liver, the results of the comparison using the TCA liver dose-metric are identical.

Furthermore, while as noted previously, oral studies appear to report a linear relationship between TCE exposure concentration and liver weight induction, the inclusion of inhalation studies on the basis of internal dose led to a highly consistent dose-response curve for among TCE study. Therefore, it is unlikely that differing routes of exposure can explain the inconsistencies in dose-response.

The bioavailability of TCA, which in the above analysis is assumed to be 100%, is another factor that may impact the dose-response. Sweeney et al. (2009), in an analysis of the potential role of TCA in the liver carcinogenesis of tetrachloroethylene, identified a number of previously unpublished TCA kinetic data in mice exposed to TCA via drinking water for 3–14 days. They concluded that fractional absorption of TCA via drinking water exposures is much less than 100%—about 29% at low exposures and decreasing with increasing dose. However, the conclusions of the Sweeney et al. (2009) were based on the Hack et al. (2006) TCE PBPK model, which had a number of deficiencies, as noted in Section 3.5 and Appendix A. Therefore, as discussed in Appendix A, Chiu (2011) reanalyzed those data using the updated TCE PBPK model of Evans et al. (2009) and Chiu et al. (2009) and concluded that while there was evidence of reduced absorption (80–90% at low exposures, and decreasing with increasing dose), it was not as low as that estimated by Sweeney et al. (2009). As discussed in Appendix A, it may be more accurate to characterize the fractional absorption as an empirical parameter reflecting unaccounted-for biological processes as well as experimental variation.
Chiu (2011) also reanalyzed the data on TCE- and TCA-induced hepatomegaly using the central estimates of the fractional absorption of TCA inferred from the analysis described above. Figure 4-8 shows the results, comparing a fixed fractional absorption of 95% with the fitted fractional absorption from Chiu (2011), here plotted using AUC of TCA in the liver as the dose-metric. For reference, the dose-response for administered TCA with an assumption of fixed, nearly complete absorption [analogous to the results from Evans et al. (2009), Figure 4-7] is also included. While the reduced fractional absorption inferred from drinking water data reported by Sweeney et al. (2009) accounts for part of the difference in dose-responses between TCE- and TCA-induced hepatomegaly reported by Evans et al. (2009), it does not appear to be able to account for the entire difference. In particular, the fraction of hepatomegaly contributed by TCA is about 0.20 assuming nearly complete absorption, as compared to about 0.33 assuming the best-fitting fractional absorption inferred from the PBPK model-based analysis. The inability of TCA to account for TCE-induced hepatomegaly is confirmed statistically by analysis of variance (ANOVA), with p-values of $<10^{-4}$. Therefore, assuming a reduced TCA bioavailability does not change the conclusion that the available data are inconsistent with the toxicological hypothesis that TCA can fully account for TCE-induced hepatomegaly.



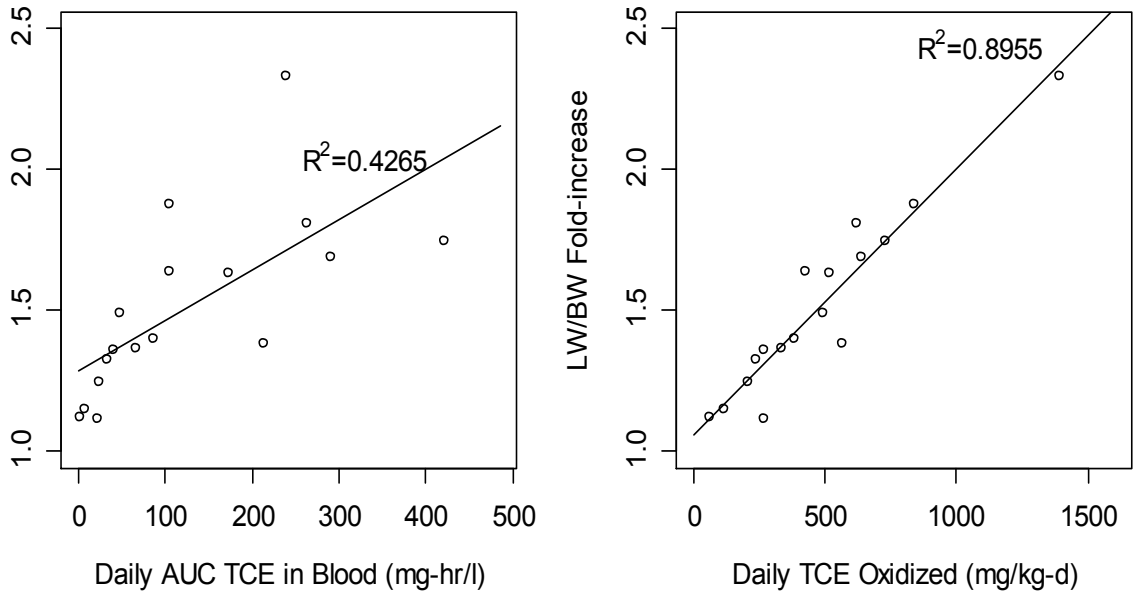
Fold-changes in relative liver weight for data sets in male B6C3F$_1$, Swiss, and NRMI mice between TCE studies (duration 28–42 days) and studies of direct oral TCA administration to B6C3F$_1$ mice (duration 14–28 days). Linear regressions were compared using ANOVA to assess whether the TCE studies were consistent with the TCA studies, using TCA as the dose-metric. For each analysis of drinking water fraction absorption, ANOVA *p*-values were $<10^{-4}$ when comparing the assumption that all of the data had a common slope with the assumption that TCE and TCA data had different slopes.

Sources: Kjellstrand et al. (1983a); Goel et al. (1992); Merrick et al. (1989); Buben and O'Flaherty (1985); DeAngelo et al. (2008; 1989); Kato-Weinstein et al. (2001); Parrish et al. (1996); Green (2003).

Figure 4-8. Comparison of hepatomegaly as a function of AUC of TCA in liver, using values for the TCA drinking water fractional absorption (Fabs).

Additional analyses do, however, support a role for oxidative metabolism in TCE-induced liver weight increases, and that the parent compound TCE is not the likely active moiety [suggested previously by Buben and O'Flaherty (1985)]. In particular, the same studies are shown in Figure 4-9 using PBPK-model based predictions of the AUC of TCE in blood and total

oxidative metabolism, which produces chloral, TCOH, DCA, and other metabolites in addition to TCA. The dose-response relationship between TCE blood levels and liver weight increase, while still having a significant trend, shows substantial scatter and a low R^2 of 0.43. On the other hand, using total oxidative metabolism as the dose-metric leads to substantially more consistency dose-response across studies, and a much tighter linear trend with an R^2 of 0.90 (see Figure 4-9). A similar consistency is observed using liver-only oxidative metabolism as the dose-metric, with R^2 of 0.86 (not shown). Thus, while the slope is similar between liver weight increase and TCE concentration in the blood and liver weight increase and rate of total oxidative metabolism, the data are a much better fit for total oxidative metabolism.



Lines show linear regression. Use of liver oxidative metabolism as a dose-metric gives results qualitatively similar to (B), with $R^2 = 0.86$.

Sources: Kjellstrand et al. (1983a); Goel et al. (1992); Merrick et al. (1989); Buben and O'Flaherty (1985).

Figure 4-9. Fold-changes in relative liver weight for data sets in male $B6C3F_1$, Swiss, and NRMI mice reported by TCE studies of duration 28–42 days using internal dose-metrics predicted by the PBPK model described in Section 3.5: (A) dose-metric is the median estimate of the daily AUC of TCE in blood, (B) dose-metric is the median estimate of the total daily rate of TCE oxidation.

Although the qualitative similarity to the linear dose-response relationship between DCA and liver weight increases is suggestive of DCA being the predominant metabolite responsible for TCE liver weight increases, due to the highly uncertain dosimetry of DCA derived from TCE, this hypothesis cannot be tested on the basis of internal dose. Similarly, another TCE metabolite, CH, has also been reported to induce liver tumors in mice; however, there are no adequate comparative data to assess the nature of liver weight increases induced by this TCE metabolite (see Sections E.2.5 and 4.5.6.3.2). Whether its formation in the liver after TCE exposure correlates with TCE-induced liver weight changes cannot be determined.

4.5.6.2.2. Cytotoxicity

As discussed above, TCE has sometimes been reported to cause minimal/mild focal hepatocellular necrosis or other signs of hepatic injury, albeit of low frequency and mostly at doses ≥1,000 mg/kg-day (Dees and Travis, 1993; Elcombe et al., 1985) or at exposures ≥1,000 ppm in air (Ramdhan et al., 2010; Ramdhan et al., 2008) from 7 to 10 days of exposure. Data from available studies are supportive of a role for oxidative metabolism in TCE-induced cytotoxicity in the liver, though they are not informative as to the actual active moiety(ies). Buben and O'Flaherty (1985) noted a strong correlation (R-squared between glucose-6-phosphatase inhibition and total urinary oxidative metabolites). Ramdhan et al. (2008) conducted parallel experiments at TCE 1,000 and 2,000 ppm (8 hours/day, 7 days) in wild-type and CYP2E1-null mice, the latter of which did not exhibit hepatotoxicity (assessed by serum ALT, AST, and histopathology) and excreted twofold lower amounts of oxidative metabolites TCA and TCOH in urine as compared to wild-type mice. In addition, urinary TCA and TCOH excretion was correlated with serum ALT and AST measures, though the R-squared values (square of the reported correlation coefficients) were relatively low (0.54 and 0.67 for TCOH and TCA, respectively). Ramdhan et al. (2010) reported that TCA and TCOH were detected in the urine of wild type and PPARα-null and humanized mice after TCE exposure with no significant differences between the 1,000 and 2,000 ppm TCE treatments. TCA concentrations were significantly lower and TCOH concentrations higher in exposed PPARα-null mice relative to wild type mice. They stated that urinary TCA levels in wild type mice were incorrectly reported by Ramdhan et al. (2008) but have been corrected in this study. AST and ALT levels were significantly increased in all exposed mice relative to control 41–74 and 36–79% higher for AST and ALT, respectively). Mean levels within each treatment group were higher, though not statistically significantly different, with exposure to 2,000 versus 1,000 ppm TCE. Although increased, such increases were small. Necrosis scores were reported to be significantly higher in TCE-exposed mice relative to controls in all three genotype mice and to be significantly higher with 2,000 vs. 1,000 ppm TCE exposure in wild type mice and hPPARα mice. Inflammation scores were reported to be significantly higher with exposed group than control with 2,000 ppm TCE exposure than controls for each genotype group with a difference between the 2,000 and

1,000 ppm exposure groups in wild type mice. However, necrosis and inflammation score means at the highest TCE exposure levels in any mouse strain were minimal (only occasional necrotic cells in any lobule) for necrosis and mild for inflammation (<2 foci/field).

With respect to CH (166 mg/kg-day) and DCA (~90 mg/kg-day), Daniel et al. (1992) reported that after drinking water treatment, hepatocellular necrosis and chronic active inflammation were reported to be mildly increased in both prevalence and severity in all treated groups after 104 weeks of exposure. The histological findings, from interim sacrifices (n = 5), were considered by the authors to be unremarkable and were not reported. TCA has not been reported to induce necrosis in the liver under the conditions tested. Relatively high doses of DCA (≥1 g/L in drinking water) appear to result in mild focal necrosis with attendant reparative proliferation at lesion sites, but no such effects were reported at lower doses (≤0.5 g/L in drinking water) more relevant for comparison with TCE (DeAngelo et al., 1999; Stauber et al., 1998; Sanchez and Bull, 1990). Enlarged nuclei and changes consistent with increased ploidy, are further discussed below in the context of DNA synthesis.

4.5.6.2.3. DNA synthesis and polyploidization

The effects on DNA synthesis and polyploidization observed with TCE treatment have similarly been observed with TCA and DCA. With respect to CH, George et al. (2000) reported that CH exposure did not alter DNA synthesis in rats and mice at any of the time periods monitored (all well past 2 weeks), with the exception of 0.58 g/L CH at 26 weeks slightly increasing hepatocyte labeling (~two- to threefold of controls) in rats and mice but the percentage labeling still representing ≤3% of hepatocytes.

In terms of whole liver or hepatocyte label incorporation, the most comparable exposure duration between TCE, TCA, and DCA studies is the 10- and 14-day period. Several studies have reported that in this time period, peak label incorporation into individual hepatocytes and whole liver for TCA and DCA have already passed (Pereira, 1996; Carter et al., 1995; Styles et al., 1991; Sanchez and Bull, 1990). A direct time-course comparison is difficult, since data at earlier times for TCE are more limited.

There are conflicting reports of DNA synthesis induction in individual hepatocytes for up to 14 days of DCA or TCA exposure. In particular, Sanchez and Bull (1990) reported tritiated thymidine incorporation in individual hepatocytes up to 2 g/L exposure to DCA or TCA induced little increase in DNA synthesis except in instances and in close proximity to areas of proliferation/necrosis for DCA treatment after 14 days of exposure in male mice. The largest percentage of hepatocytes undergoing DNA synthesis for any treatment group was <1% of hepatocytes. However, they reported treatment- and exposure duration-changes in hepatic DNA incorporation of tritiated thymidine for DCA and TCA. For TCA treatment, the largest increases over control levels for hepatic DNA incorporation (at the highest dose) was a threefold increase after 5 days of treatment and a twofold increase over controls after 14 days of treatment. For

DCA whole-liver tritiated thymidine incorporation was only slightly elevated at necrogenic concentrations and decreased at the 0.3 g/L non-necrogenic level after 14 days of treatment. In contrast to Sanchez and Bull (1990), Stauber and Bull (1997) reported increased tritiated thymidine incorporation for individual hepatocytes after 14 days of treatment with 2 g/L DCA or TCA in male mice. They used a more extended period of tritiated thymidine exposure of 3–5 days and so these results represent aggregate DNA synthesis occurring over a more extended period of time. A "1-day labeling index" was reported as <1% for the highest level of increased incorporation. However, after 14 days, the labeling index was reported to be increased by ~3.5-fold for TCA and ~5.5-fold for DCA over control values. After 28 days, the labeling index was reported to be decreased ~2.3-fold by DCA and increased ~2.5-fold after treatment with TCA. Pereira (1996) reported that for female $B6C3F_1$ mice, 5-day incorporation of BrDU, as a measure of DNA synthesis, was increased at 0.86 and 2.58 g/L DCA treatment for 5 days (~twofold at the highest dose) but that by Day 12 and 33 levels had fallen to those of controls. For TCA exposures, 0.33, 1.10, and 3.27 g/L TCA all gave a similar ~threefold increase in BrdU incorporation by 5 days, but that by 12 and 33 days were not changed from controls. Nonetheless, what is consistent is that these data report that, similar to TCE-exposed mice at 10 days of exposure, cells undergoing DNA synthesis in DCA- or TCA-exposed mice for up to 14 days of exposure to be confined to a very small population of cells in the liver. Thus, these data are consistent with hypertrophy being primarily responsible for liver weight gains as opposed to increases in cell number in mice.

Interestingly, a lack of correlation between whole liver label incorporation and that in individual hepatocytes has been reported by several studies of DCA (Carter et al., 1995; Sanchez and Bull, 1990). For example, Carter et al. (1995) reported no increase in labeling of hepatocytes in comparison to controls for any DCA treatment group from 5 to 30 days of DCA exposure. Rather than increase hepatocyte labeling, DCA induced no change from Days 5 though 15 but significantly decreased levels between days 20 and 30 for 0.5 g/L that were similar to those observed for the 5 g/L exposures. However, for whole-liver DNA tritiated thymidine incorporation, Carter et al. (1995) reported 0.5g/L DCA treatments to show trends of initial inhibition of DNA tritiated thymidine incorporation followed by enhancement of labeling that was not statistically significant from 5 to 30 days of exposure. Examination of individual hepatocytes does not include the contribution of nonparenchymal cell DNA synthesis that would be detected in whole-liver DNA. As noted above, proliferation of the nonparenchymal cell compartment of the liver has been noted in several studies of TCE in rodents, and thus, this is one possible reason for the reported discrepancy.

Another possible reason for this inconsistency with DCA treatment is polyploidization, as was suggested above for TCE. Although this was not examined for DCA or TCA exposure by Sanchez and Bull (1990), Carter et al. (1995) reported that hepatocytes from both 0.5 and 5 g/L DCA treatment groups had enlarged, presumably polyploidy nuclei, with some hepatocyte nuclei

labeled in the mid-zonal area. There were statistically significant changes in cellularity, nuclear size, and multinucleated cells during 30 days of exposure to DCA. The percentage of mononucleated cells hepatocytes was reported to be similar between control and DCA treatment groups at 5- and 10-day exposure. However, at 15 days and beyond, DCA treatments were reported to induce increases in mononucleated hepatocytes, with later time periods also showing DCA-induced increases in nuclear area, consistent with increased polyploidization without mitosis. The consistent reporting of an increasing number of mononucleated cells between 15 and 30 days could be associated with clearance of mature hepatocytes as suggested by the report of DCA-induced loss of cell nuclei. The reported decrease in the numbers of binucleate cells in favor of mononucleate cells is not typical of any stage of normal liver growth (Brodsky and Uryvaeva, 1977). The pattern of consistent increase in percentage liver/body weight induced by 0.5 g/L DCA treatment from days 5 though 30 was not consistent with the increased numbers of mononucleate cells and increase nuclear area reported from day 20 onward. Specifically, the large differences in liver weight induction between the 0.5 g/L and 5 g/L treatment groups at all times studied also did not correlate with changes in nuclear size and percentage of mononucleate cells. Thus, increased liver weight was not a function of cellular proliferation, but probably included both aspects of hypertrophy associated with polyploidization and increased glycogen deposition (see below) induced by DCA. Carter et al. (1995) suggested that although there is evidence of DCA-induced cytotoxicity (e.g., loss of cell membranes and apparent apoptosis), the 0.5 g/L exposure concentration has been shown to increase hepatocellular lesions after 100 weeks of treatment without concurrent peroxisome proliferation or cytotoxicity (DeAngelo et al., 1999).

In sum, the observation of TCE treatment-related changes in DNA content, label incorporation, and mitotic figures are generally consistent with patterns observed for both TCA and DCA. In all cases, hepatocellular proliferation is confined to a very small fraction of hepatocytes, and hepatomegaly observed with all three treatments probably largely reflects cytomegaly rather than cell proliferation. Moreover, label incorporation likely largely reflects polyploidization rather than hepatocellular proliferation, with a possible contribution from nonparenchymal cell proliferation. As with TCE, histological changes in nuclear sizes and number also suggest a significant degree of treatment-related polyploidization, particularly for DCA.

4.5.6.2.4. Apoptosis

Both Elcombe et al. (1985) and Dees and Travis (1993) reported no changes in apoptosis other than increased apoptosis only at a treatment level of 1,000 mg/kg TCE. Dees and Travis (1993) reported that increased apoptoses from TCE exposure "did not appear to be in proportion to the applied TCE dose given to male or female mice." Channel et al. (1998) reported that there was no significant difference in apoptosis between TCE treatment and control groups with data

not shown. However, the extent of apoptosis in any of the treatment groups, or which groups and timepoints were studied for this effect cannot be determined. While these data are quite limited, it is notable that peroxisome proliferators have been suggested inhibit, rather than increase, apoptosis as part of their carcinogenic mode of action (Klaunig et al., 2003).

However, for TCE metabolites, DCA has been most studied, though it is clear that age and species affect background rates of apoptosis. Snyder et al. (1995), in their study of DCA, reported that control mice were reported to exhibit apoptotic frequencies ranging from ~0.04 to 0.085% and that over the 30-day period of their study, the frequency rate of apoptosis declined; it was suggested that this pattern is consistent with reports of the livers of young animals undergoing rapid changes in cell death and proliferation. They reported the rat liver to have a greater than estimated frequency of spontaneous apoptosis (~0.1%) and therefore, greater than that of the mouse. Carter et al. (1995) reported that after 25 days of 0.5 g/L DCA treatment, apoptotic bodies were reported as well as fewer nuclei in the pericentral zone and larger nuclei in central and midzonal areas. This would indicate an increase in the apoptosis associated with potential increases in polyploidization and cell maturation. However, Snyder et al. (1995) reported that mice treated with 0.5 g/L DCA over a 30-day period had a similar trend as control mice of decreasing apoptosis with age. The percentage of apoptotic hepatocytes decreased in DCA-treated mice at the earliest time point studied and remained statistically significantly decreased from controls from 5 to 30 days of exposure. Although the rate of apoptosis was very low in controls, treatment with 0.5 g/L DCA reduced it further (~30–40% reduction) during the 30-day study period. The results of this study not only provide a baseline of apoptosis in the mouse liver, which is very low, but also show the importance of taking into account the effects of age on such determinations. The significance of the DCA-induced reduction in apoptosis reported in this study, from a level that is already inherently low in the mouse, for the mode of action for induction of DCA-induce liver cancer is difficult to discern.

4.5.6.2.5. Glycogen accumulation

As discussed in Sections E.3.2 and E.3.4.2.1, glycogen accumulation has been described to be present in foci in both humans and animals as a result from exposure to a wide variety of carcinogenic agents and predisposing conditions in animals and humans. The data from Elcombe et al. (1985) included reports of TCE-induced pericentral hypertrophy and eosinophilia for both rats and mice but with ~~"~~fewer animals affected at lower doses." In terms of glycogen deposition, Elcombe report ~~"~~somewhat" less glycogen pericentrally in the livers of rats treated with TCE at 1,500 mg/kg than controls with less marked changes at lower doses restricted to fewer animals. They do not comment on changes in glycogen in mice. Dees and Travis (1993) reported TCE-induced changes to ~~"~~include an increase in eosinophilic cytoplasmic staining of hepatocytes located near central veins, accompanied by loss of cytoplasmic vacuolization." Since glycogen is removed using conventional tissue processing and staining techniques, an

increase in glycogen deposition would be expected to increase vacuolization and thus, the report from Dees and Travis (1993) is consistent with less, not more, glycogen deposition. Neither study produced a quantitative analysis of glycogen deposition changes from TCE exposure. Although not explicitly discussing liver glycogen content or examining it quantitatively in mice, these studies suggest that TCE-induced liver weight increases did not appear to be due to glycogen deposition after 10 days of exposure and any decreases in glycogen were not necessarily correlated with the magnitude of liver weight gain either.

For TCE and TCA 500 mg/kg treatments in mice for 10 days, changes in glycogen were not reported in the general descriptions of histopathological changes (Dees and Travis, 1993; Styles et al., 1991; Elcombe et al., 1985) or were specifically described by the authors as being similar to controls (Nelson et al., 1989). However, for DCA, glycogen deposition was specifically noted to be increased with treatment, although no quantitative analyses was presented that could give information as to the nature of the dose-response (Nelson et al., 1989).

In regard to cell size, although increased glycogen deposition with DCA exposure was noted by Sanchez and Bull (1990) to occur to a similar extent in B6C3F$_1$ and Swiss Webster male mice despite differences in DCA-induced liver weight gain. Lack of quantitative analyses of that accumulation in this study precludes comparison with DCA-induced liver weight gain. Carter et al. (1995) reported that in control mice, there was a large variation in apparent glycogen content, but did not perform a quantitative analysis of glycogen deposition. The variability of this parameter in untreated animals and the extraction of glycogen during normal tissue processing for light microscopy make quantitative analyses for dose-response difficult unless specific methodologies are employed to quantitatively assess liver glycogen levels as was done by Kato-Weinstein et al. (2001) and Pereira et al. (2004a).

Bull et al. (1990) reported that glycogen deposition was uniformly increased from 2 g/L DCA exposure with photographs of TCA exposure showing slightly less glycogen staining than controls. However, the abstract and statements in the paper suggest that there was increased PAS-positive material from TCA treatment that has caused confusion in the literature in this regard. Kato-Weinstein et al. (2001) reported that in male B6C3F$_1$ mice exposed to DCA and TCA, the DCA treatment increased glycogen and TCA decreased glycogen content of the liver by using both chemical measurement of glycogen in liver homogenates and by using ethanol-fixed sections stained with PAS, a procedure designed to minimize glycogen loss.

Kato-Weinstein et al. (2001) reported that glycogen-rich and -poor cells were scattered without zonal distribution in male B6C3F$_1$ mice exposed to 2 g/L DCA for 8 weeks. For TCA treatments, they reported centrilobular decreases in glycogen and ~25% decreases in whole liver by 3 g/L TCA. Kato-Weinstein et al. (2001) reported whole-liver glycogen to be increased ~1.50-fold of control (90 vs. 60 mg glycogen/g liver) by 2 g/L DCA after 8 weeks of exposure to male B6C3F$_1$ mice, with a maximal level of glycogen accumulation occurring after 4 weeks of DCA exposure. Pereira et al. (2004a) reported that after 8 weeks of exposure to 3.2 g/L DCA,

liver glycogen content was 2.2-fold of control levels (155.7 vs. 52.4 mg glycogen/g liver) in female B6C3F$_1$ mice. Thus, the baseline level of glycogen content reported by (~60 mg/g) and the increase in glycogen after DCA exposure was consistent between Kato-Weinstein et al. (2001) and Pereira et al. (2004a). However, the increase in liver weight reported by Kato-Weinstein et al. (2001) of 1.60-fold of control percentage liver/body weight cannot be accounted for by the 1.50-fold of control glycogen content. Glycogen content only accounts for 5% of liver mass so that 50% increase in glycogen cannot account for the 60% increase liver mass induced by 2 g/L DCA exposure for 8 weeks reported by Kato-Weinstein (2001). Thus, DCA-induced increases in liver weight are occurring from other processes as well. Carter et al. (2003) and DeAngelo et al. (1999) reported increased glycogen after DCA treatment at much lower doses after longer periods of exposure (100 weeks). Carter reported increased glycogen at 0.5 g/L DCA and DeAngelo et al. (1999) at 0.03 g/L DCA in mice. However, there is no quantitation of that increase.

4.5.6.2.6. Peroxisome proliferation and related effects

TCA and DCA have both been reported to induce peroxisome proliferation or increases in related enzyme markers in rodent hepatocytes (Parrish et al., 1996; Mather et al., 1990; DeAngelo et al., 1989, 1997). Between TCA and DCA, both induce peroxisome proliferation in various strains of mice, but it clear that TCA and DCA are weak PPARα agonists and that DCA is weaker than TCA in this regard (Nelson et al., 1989) using a similar paradigm.

George et al. (2000) reported that CH exposure did not hepatic PCO activity in rats and mice at any of the time periods monitored. It is notable that the only time at which DNA synthesis index was (slightly) increased, at 26 weeks, there remained a lack of induction of PCO. A number of measures that may be related to peroxisome proliferation were investigated in Leakey et al. (2003b). Of the enzymes associated with PPARα agonism (total CYP, CYP2B isoform, CYP4A, or lauric acid β-hydroxylase activity), only CYP4A and lauric acid β-hydroxylase activity were significantly increased at 15 months of exposure in the dietary-restricted group administered the highest dose (100 mg/kg CH) with no other groups showing a statistically significant increased response (n = 12/group). There is an issue of interpretation of peroxisomal enzyme activities and other enzymes associated with PPARα receptor activation to be a relevant event in liver cancer induction at a time period in which tumors or foci are already present. Although not statistically significant, the 100 mg/kg CH exposure group of ad-libitum-fed mice also had an increase in CH-induced increases of CYP4A and lauric acid β-hydroxylase activity. Seng et al. (2003) described CH toxicokinetics and peroxisome proliferation-associated enzymes in mice at doses up to 1,000 mg/kg-day for 2 weeks with dietary control or caloric restriction. Lauric acid β-hydroxylase and PCO activities were reported to be induced only at doses >100 mg/kg in all groups, with dietary-restricted mice showing the greatest induction.

Differences in serum levels of TCA, the major metabolite remaining 24 hours after dosing, were reported not to correlate with hepatic lauric acid β-hydroxylase activities across groups.

Direct quantitative inferences regarding the magnitude of response in these studies in comparison to TCE, however, are limited by possible variability and confounding. In particular, many studies used cyanide-insensitive PCO as a surrogate for peroxisome proliferation, but the utility of this marker may be limited for a number of reasons. First, several studies have shown that this activity is not well correlated with the volume or number of peroxisomes that are increased as a result of exposure to TCE or it metabolites (Nakajima et al., 2000; Nelson et al., 1989; Elcombe et al., 1985). In addition, this activity appears to be highly variable both as a baseline measure and in response to chemical exposures. Laughter et al. (2004) presented data showing WY-14,643 induced increases in PCO activity that varied up to sixfold between different experiments in wild-type mice. They also showed that, in some instances, PCO activity in untreated PPARα-null mice was up to sixfold greater than that in wild-type mice. Parrish et al. (1996) noted that control values between experiments varied as much as a factor of twofold for PCO activity and thus, their data were presented as percentage of concurrent controls. Furthermore, Melnick et al. (1987) reported that corn oil administration alone can elevate PCO (as well as catalase) activity, and corn oil has also been reported to potentiate the induction of PCO activity of TCA in male mice (DeAngelo et al., 1989). Thus, quantitative inferences regarding the magnitude of response in these studies are limited by a number of factors. For example, in the studies reported in DeAngelo et al. (2008), a small number of animals was studied for PCO activity at interim sacrifices (n = 5). PCO activity varied 2.7-fold as baseline controls. Although there was a 10-fold difference in TCA exposure concentration, the increases in PCO activity at 4 weeks were 1.3-, 2.4-, and 5.3-fold of control. More information on the relationship of PCO enzyme activity and its relationship to carcinogenicity is discussed in Section E.3.4 and below.

4.5.6.2.7. Oxidative stress

Very limited data are available as to oxidative stress and related markers induced by the oxidative metabolites of TCE. As discussed in Appendix E, there are limited data that do not indicate significant oxidative stress and associated DNA damage associated with acute and subacute TCE treatment. In regard to DCA and TCA, Larson and Bull (1992b) exposed male B6C3F$_1$ mice or F344 rats to single doses TCA or DCA in distilled water by gavage (n = 4). In the first experiment, TBARS was measured from liver homogenates and assumed to be malondialdehyde. The authors stated that a preliminary experiment had shown that maximal TBARS was increased 6 hours after a dose of DCA and 9 hours after a dose of TCA in mice and that by 24 hours, TBARS concentrations had declined to control values. Time-course information in rats was not presented. A dose of 100 mg/kg DCA (rats or mice) or TCA (mice) did not elevate TBARS concentrations over that of control liver with this concentration of TCA

not examined in rats. For TCA, there was a slight dose-related increase in TBARS over control values starting at 300 mg/kg in mice with the increase in TBARS increasing at a rate that was lower than the magnitude of increase in dose. Of note, is the report that the induction of TBARS in mice is transient and subsided within 24 hours of a single dose of DCA or TCA, that the response in mice appeared to be slightly greater with DCA than TCA at similar doses, and that for DCA, there was similar TBARS induction between rats and mice at similar dose levels.

Austin et al. (1996) is a follow-up publication of the preliminary experiment cited in Larson and Bull (1992b). Male B6C3F$_1$ mice were treated with single doses of DCA or TCA via gavage with liver examined for 8-OHdG. The authors stated that in order to conserve animals, controls were not employed at each time point. There was a statistically significant increase over controls in 8-OHdG for the 4- and 6-hour time points for DCA (~1.4- and 1.5-fold of control, respectively) but not at 8 hours in mice. For TCA, there was a statistically significant increase in 8-OHdG at 8 and 10 hours for TCA (~1.4- and 1.3-fold of control, respectively).

Consistent results as to low, transient increases in markers of "oxidative stress" were also reported by Parrish et al. (1996), who in addition to examining oxidative stress alone, attempted to examine its possible relationship to PCO and liver weight in male B6C3F$_1$ mice exposed to TCA or DCA for 3 or 10 weeks (n = 6). The dose-related increase in PCO activity at 21 days for TCA was not increased similarly for DCA. Only the 2.0 g/L dose of DCA was reported to induce a statistically significant increase at 21-days of exposure of PCO activity over control (~1.8-fold of control). After 71 days of treatment, TCA induced dose-related increases in PCO activities that were approximately twice the magnitude as that reported at 21 days. Treatments with DCA at the 0.1 and 0.5 g/L exposure levels produced statistically significant increases in PCO activity of ~1.5- and 2.5-fold of control, respectively. The administration of 1.25 g/L clofibric acid in drinking water, used as a positive control, gave ~six–sevenfold of control PCO activity at 21 and 71 days of exposure. Parrish et al. (1996) reported that laurate hydroxylase activity was elevated significantly only by TCA at 21 days and to approximately the same extent (~1.4–1.6-fold of control) at all doses tested and at 71 days, both the 0.5 and 2.0 g/L TCA exposures resulting in a statistically significant increase in laurate hydroxylase activity (i.e., 1.6- and 2.5-fold of control, respectively). No change was reported after DCA exposure. Laurate hydroxylase activity was within the control values, varying 1.7-fold between 21 and 71 days experiments. Levels of 8-OHdG in isolated liver nuclei were reported to not be altered from 0.1, 0.5, or 2.0 g/L TCA or DCA after 21 days of exposure and this negative result was reported to remain even when treatments were extended to 71 days of treatment. The authors noted that the level of 8-OHdG increased in control mice with age (i.e., ~twofold increase between 71- and 21-day-old control mice). Thus, the increases in PCO activity noted for DCA and TCA were not associated with 8-OHdG levels (which were unchanged) and also not with changes in laurate hydrolase activity observed after either DCA or TCA exposure. Of note, is that the authors report taking steps to minimize artifactual responses for their 8-OHdG

determinations. The authors concluded that their data suggest that peroxisome proliferative properties of TCA were not linked to oxidative stress or carcinogenic response.

4.5.6.3. Comparisons of TCE-Induced Carcinogenic Responses with TCA, DCA, and CH Studies

4.5.6.3.1. Studies in rats

As discussed above, data on TCE carcinogenicity in rats, while not reporting statistically significantly increased risks, are not entirely adequate due to low numbers of animals, increased systemic toxicity, and/or increased treatment-related or accidental mortality. Notably, several studies in rats noted a few very rare types of liver or biliary tumors (cystic cholangioma, cholangiocarcinoma, or angiosarcomas) in treated animals. For TCA, DCA, and CH, there are even fewer studies in rats, so there is a very limited ability to assess the consistency or lack thereof in rat carcinogenicity among these compounds.

For TCA, the only available study in rats (DeAngelo et al., 1997) has been frequently cited in the literature to indicate a lack of response in this species for TCA-induced liver tumors. However, this study does report an apparent dose-related increase in multiplicity of adenomas and an increase in carcinomas over control at the highest dose. The use by DeAngelo et al. (1997) of a relatively low number of animals per treatment group (n = 20–24) limits this study's ability to determine a statistically significant increase in tumor response. Its ability to determine an absence of treatment-related effects is similarly limited. In particular, a power calculation of the study shows that for most endpoints (incidence and multiplicity of all tumors at all exposure DCA concentrations), the Type II error, which should be >50%, was <8%. The only exception was for the incidence of adenomas and of adenomas and carcinomas for the 0.5 g/L treatment group (58%), at which, notably, there was a reported increase in reported adenomas or adenomas and carcinomas combined over control (15 vs. 4%). Therefore, the likelihood of a false null hypothesis was not negligible. Thus, while suggesting a lower response than for mice for liver tumor induction, this study is inconclusive for determining whether TCA induces a carcinogenic response in the liver of rats.

For DCA, there are two long-term studies in rats (DeAngelo et al., 1996; Richmond et al., 1995) that appear to have reported the majority of their results from the same data set and that were consequently subject to similar design limitations and DCA-induced neurotoxicity in this species. DeAngelo et al. (1996) reported increased hepatocellular adenomas and carcinomas in male F344 rats exposed to DCA for 2 years. However, the data from exposure concentrations at the 5 g/L dose had to be discarded and the 2.5 g/L DCA dose had to be continuously lowered during the study due to neurotoxicity. There was a DCA-induced increased in adenomas and carcinomas combined reported for the 0.5 g/L DCA (24.1 vs. 4.4% adenomas and carcinomas combined in treated vs. controls) and an increase at a variable dose started at 2.5 g/L DCA and continuously lowered (28.6 vs. 3.0% adenomas and carcinomas combined in treated vs.

controls). Only combined incidences of adenomas and carcinomas for the 0.5 g/L DCA exposure group was reported to be statistically significant by the authors although the incidence of adenomas was 17.2 vs. 4% in treated vs. control rats. Hepatocellular tumor multiplicity was reported to be increased in the 0.5 g/L DCA group (0.31 adenomas and carcinomas/animal in treated vs. 0.04 in control rats) but was reported by the authors to not be statistically significant. At the starting dose of 2.5 g/L that was continuously lowered due to neurotoxicity, the increased multiplicity of HCCs was reported by the authors to be to be statistically significant (0.25 carcinomas/animals vs. 0.03 in control) as well as the multiplicity of combined adenomas and carcinomas (0.36 adenomas and carcinomas/animals vs. 0.03 in control rats). Issues that affect the ability to determine the nature of the dose-response for this study include: (1) the use of a small number of animals (n = 23, n = 21, and n = 23 at final sacrifice for the 2.0 g/L sodium chloride control, 0.05 g/L and 0.5 g/L treatment groups) that limit the power of the study to both determine statistically significant responses and to determine that there are not treatment-related effects (i.e., power); (2) apparent addition of animals for tumor analysis not present at final sacrifice (i.e., 0.05 and 0.5 g/L treatment groups); and (3) most of all, the lack of a consistent dose for the 2.5 g/L DCA exposed animals.

Similar issues are present for the study of Richmond et al. (1995), which was conducted by the same authors as DeAngelo et al. (1996) and appeared to be the same data set. There was a small difference in reports of the results between the two studies for the same data for the 0.5 g/L DCA group in which Richmond et al. (1995) reported a 21% incidence of adenomas and DeAngelo et al. (1996) reported a 17.2% incidence. The authors did not report any of the results of DCA-induced increases of adenomas and carcinomas to be statistically significant. The same issues discussed above for DeAngelo et al. (1996) apply to this study. Similar to the DeAngelo et al. (1997) study of TCA in rats, the use in these DCA studies (DeAngelo et al., 1996; Richmond et al., 1995) of relatively small numbers of rats limits the detection of treatment-related effects and the ability to determine whether there were treatment-related effects (Type II error), especially at the low concentrations of DCA exposure.

For CH, George et al. (2000) exposed male F344/N rats to CH in drinking water for 2 years. Groups of animals were sacrificed at 13, 26, 52, and 78 weeks following the initiation of dosing, with terminal sacrifices at week 104. Only a few animals received a complete pathological examination. The number of animals surviving >78 weeks and the number examined for hepatocellular proliferative appeared to differ (42–44 animals examined, but 32–35 surviving until the end of the experiment). Only the lowest treatment group had increased liver tumors that were marginally significantly increased.

Leuschner and Beuscher (1998) examined the carcinogenic effects of CH in male and female Sprague-Dawley rats (69–79 g, 25–29 days old at initiation of the experiment) administered 0, 15, 45, and 135 mg/kg CH in unbuffered drinking water 7 days/week (n = 50/group) for 124 weeks in males and 128 weeks in females. Two control groups were

noted in the methods section without explanation as to why they were conducted as two groups. The authors reported no substance-related influence on organ weights and no macroscopic evidence of tumors or lesions in male or female rats treated with CH for 124 or 128 weeks. However, no data were presented on the incidence of tumors in either treatment or control groups. The authors did report a statistically significant increase in the incidence of hepatocellular hypertrophy in male rats at the 135 mg/kg dose (14/50 animals vs. 4/50 and 7/50 in Controls I and II). For female rats, the incidence of hepatocellular hypertrophy was reported to be 10/50 rats (Control I) and 16/50 (Control II) rats with 18/50, 13/50 and 12/50 female rats having hepatocellular hypertrophy after 15, 45, and 135 mg/kg CH, respectively. The lack of reporting in regard to final body weights, histology, and especially background and treatment group data for tumor incidences, limit the interpretation of this study. Whether this paradigm was sensitive for induction of liver cancer cannot be determined.

Therefore, given the limitations in the available studies, a comparison of rat liver carcinogenicity induced by TCE, TCA, DCA, and CH reveals no strong inconsistencies, but nor does it provide much insight into the relative importance of different TCE metabolites in liver tumor induction.

4.5.6.3.2. Studies in mice

Similar to TCE, the bioassay data in mice for DCA, TCA, and CH are much more extensive and have shown that all three compounds induce liver tumors in mice. Several 2-year bioassays have been reported for CH (Leakey et al., 2003b; George et al., 2000; Daniel et al., 1992). For many of the DCA and TCA studies, the focus was not carcinogenic dose-response, but rather investigation of the nature of the tumors and potential modes of action in relation to TCE. As a result, studies often employed relatively high concentrations of DCA or TCA and/or were conducted for ≤1 year. As shown previously in Section 4.5.6.2.1, the dose-response curves for increased liver weight for TCE administration in male mice are more similar to those for DCA administration and TCE oxidative metabolism than for direct TCA administration (inadequate data were available for CH). An analogous comparison for DCA-, TCA-, and CH-induced tumors would be informative, ideally using data from 2-year studies.

4.5.6.3.2.1. TCE carcinogenicity dose-response data

Unfortunately, the database for TCE, while consistently showing an induction of liver tumors in mice, is very limited for making inferences regarding the shape of the dose-response curve. For many of these experiments, only liver tumor incidence, not multiplicity, was provided. NTP (1990), Bull et al. (2002), and Anna et al. (1994) conducted gavage experiments in which they only tested one dose of ~1,000 mg/kg-day TCE. NCI (1976) tested two doses that were adjusted during exposure to an average of 1,169 and 2,339 mg/kg-day in male mice with only twofold dose spacing in only two doses tested. Maltoni et al. (1988; 1986) conducted

inhalation experiments in two sets of B6C3F$_1$ mice and one set of Swiss mice at three exposure concentrations that were threefold apart in magnitude between the low and mid-dose and twofold apart in magnitude between the mid- and high dose. However, for one experiment in male B6C3F$_1$ mice (BT306), the mice fought and suffered premature mortality and for two the experiments in B6C3F$_1$ mice, although using the same strain, the mice were obtained from differing sources with very different background liver tumor levels. For the Maltoni et al. (1988; 1986) study, a general descriptor of "hepatoma" was used for liver neoplasia rather than describing hepatocellular adenomas and carcinomas so that comparison of that data with those from other experiments is difficult. More importantly, while the number of adenomas and carcinomas may be the same between treatments or durations of exposure, the number of adenomas may decrease as the number of carcinomas increase during the course of tumor progression. Such information is lost by using only a hepatoma descriptor.

Given the limited database, it would be useful if different studies could be combined to yield a more comprehensive dose-response curve, as was done for liver weight, above. However, this is probably not appropriate for several reasons. First, only the NTP (1990) study was performed with dosing duration and time of sacrifice both being the "standard" 104 weeks. NCI (1976), Maltoni et al. (1988; 1986), Anna et al. (1994), and Bull et al. (2002) all had shorter dosing periods and either longer (Maltoni et al., 1988; Maltoni et al., 1986) or shorter (the other three studies) observation times. Therefore, because of potential dose-rate effects and differences in the degree of expression of TCE-induced tumors, it is difficult to even come up with a comparable administered dose-metric across studies. Moreover, the background tumor incidences are substantially different across experiments, even controlling for mouse strain and sex. For example, across gavage studies in male B6C3F$_1$ mice, the incidence of HCCs ranged from 1.2 to 16.7% (Anna et al., 1994; NTP, 1990; NCI, 1976) and the incidence of adenomas ranged from 1.2 to 14.6% (Anna et al., 1994; NTP, 1990) in control B6C3F$_1$ mice. After ~1,000 mg/kg-day TCE treatment, the incidence of carcinomas ranged from 19.4 to 62% (Bull et al., 2002; Anna et al., 1994; NTP, 1990; NCI, 1976), with three of the studies (Anna et al., 1994; NTP, 1990; NCI, 1976) reporting a range of incidences between 42.8 and 62.0%. The incidence of adenomas ranged from 28 to 66.7% (Bull et al., 2002; Anna et al., 1994; NTP, 1990). In the Maltoni et al. (1988; 1986) inhalation study as well, male B6C3F$_1$ mice from two different sources had very different control incidences of hepatomas (~2 vs. ~20%).

Therefore, only data from the same experiment in which more than a single exposed dose group was used provide reliable data on the dose-response relationship for TCE hepatocarcinogenicity, and incidences from these experiments are shown in Figures 4-10 and 4-11. Except for one of the two Maltoni et al. (1988; 1986) inhalation experiments in male B6C3F$_1$ mice, all of these data sets show relatively proportional increases with dose, albeit with somewhat different slopes as may be expected across strains and sexes. Direct comparison is difficult, since the "hepatomas" reported by Maltoni et al. (1988; 1986) are much more

heterogeneous, including neoplastic nodules, adenomas, and carcinomas, than the carcinomas reported by NCI (1976). Nonetheless, although the data limitations preclude a conclusive statement, these data are generally consistent with the linear relationship observed with TCE-induced liver weight changes.

4.5.6.3.2.2. DCA carcinogenicity dose-response data

Pereira (1996) reported that for 82-week exposures to DCA in female B6C3F$_1$ mice, DCA exposure concentrations of 0, 2, 6.67, and 20 mmol/L (0, 0.26, 0.86, and 2.6 g/L) led to close, proportionally increasing adenoma prevalences of 2.2, 6, 25, and 84.2%, though adenoma multiplicity increased more than linearly between the highest two doses. Unfortunately, too few carcinomas were observed at these doses and duration to meaningfully inform the shape of the dose-response relationship. More useful is DeAngelo et al. (1999), which reported on a study of DCA hepatocarcinogenicity in male B6C3F$_1$ mice over a lifetime exposure. DeAngelo et al. (1999) used 0.05, 0.5, 1.0, 2.0, and 3.5 g/L exposure concentrations of DCA in their 100-week drinking water study. The number of animals at final sacrifice was generally low in the DCA treatment groups and variable. The multiplicity or number of HCCs/animals was significantly increased over controls in a dose-related manner at all DCA treatments including 0.05 g/L DCA, and a no-observed-effect level (NOEL) was not identified. Between the 0.5 and 3.5 g/L exposure concentrations of DCA, the magnitude of increase in multiplicity was similar to the increases in magnitude in dose. The incidence of HCCs was increased at all doses as well, but was not statistically significant at 0.05 g/L. However, given that the number of mice examined for this response (n = 33), the power of the experiment at this dose was only 16.9% to be able to determine that there was not a treatment-related effect. Indeed, Figure 4-12 replots the data from DeAngelo et al. (1999) with an abscissa drawn to scale (unlike the figure in the original paper, which was not to scale), suggests even a slightly greater-than-linear effect at the lowest dose (0.05 g/L, or 8 mg/kg-day) as compared to the next lowest dose (0.5 g/L, or 84 mg/kg-day), though of course the power of such a determination is limited. The authors did not report the incidence or multiplicity of adenomas for the 0.05 g/L exposure group in the study or the incidence or multiplicity of adenomas and carcinomas in combination. For the animals surviving from 79 to 100 weeks of exposure, the incidence and multiplicity of adenomas peaked at 1 g/L, while HCCs continued to increase at the higher doses. This would be expected where some portion of the adenomas would either regress or progress to carcinomas at the higher doses.

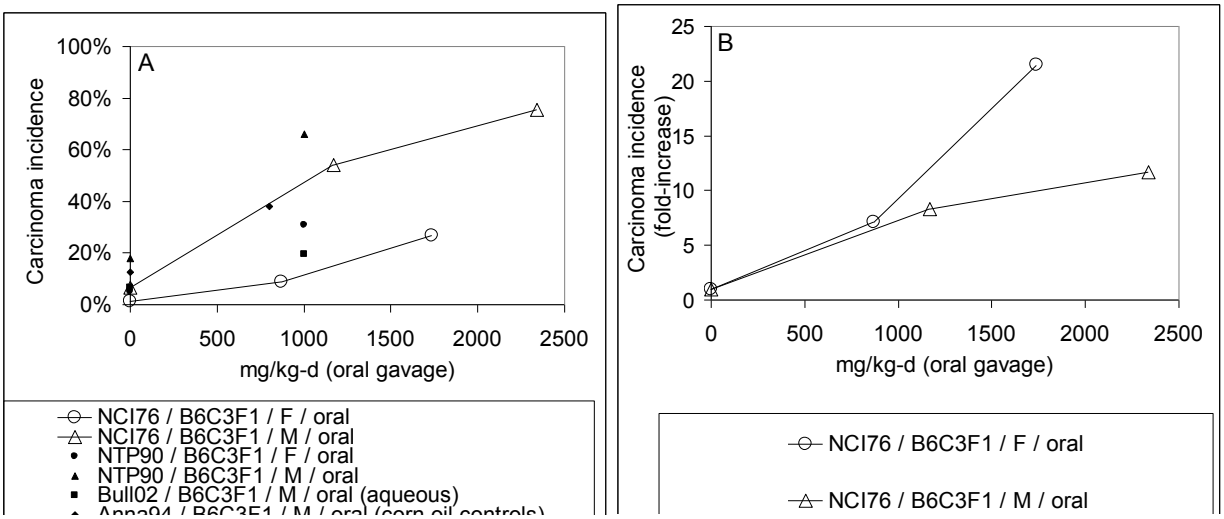
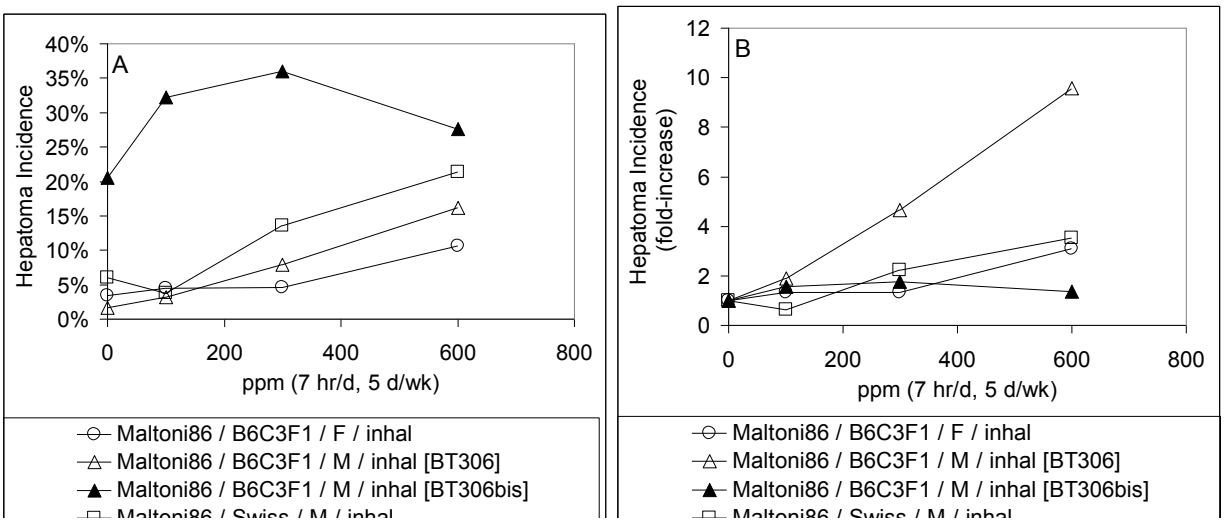
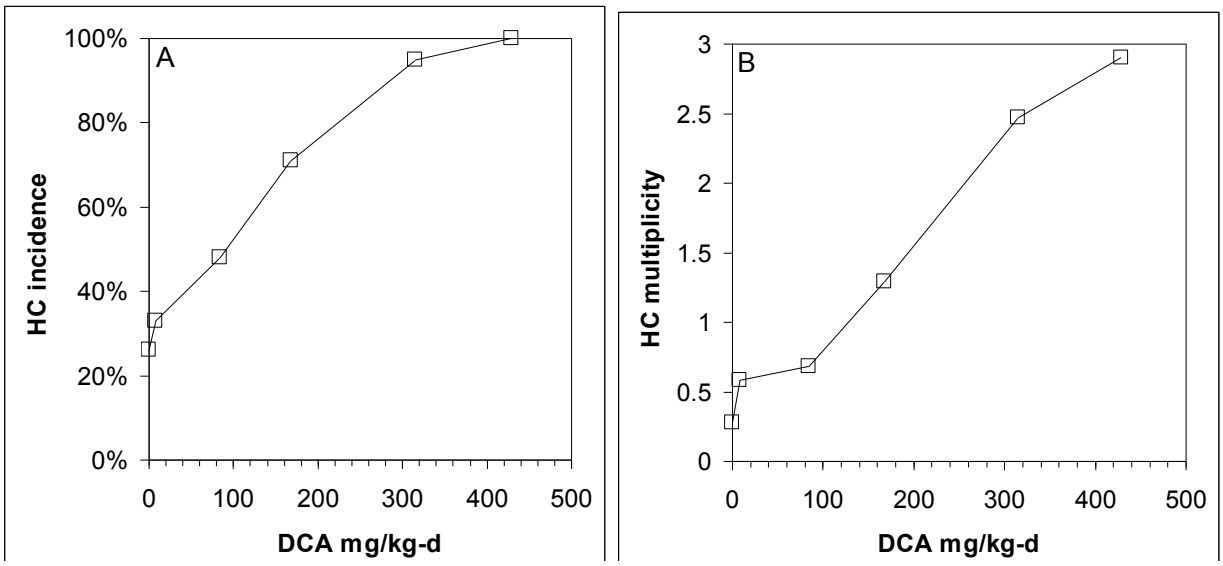


Figure 4-10. Dose-response relationship, expressed as (A) percentage incidence and (B) fold-increase over controls, for TCE hepatocarcinogenicity in NCI (1976). For comparison, incidences of carcinomas for NTP (1990), Anna et al. (1994), and Bull et al. (2002) are included, but without connecting lines since they are not appropriate for assessing the shape of the dose-response relationship.



Note that the BT306 experiment reported excessive mortality due to fighting, and so the paradigm was repeated in experiment BT306bis using mice from a different source.

Figure 4-11. Dose-response relationship, expressed as (A) incidence and (B) fold-increase over controls, for TCE hepatocarcinogenicity in Maltoni et al. (1988; 1986).



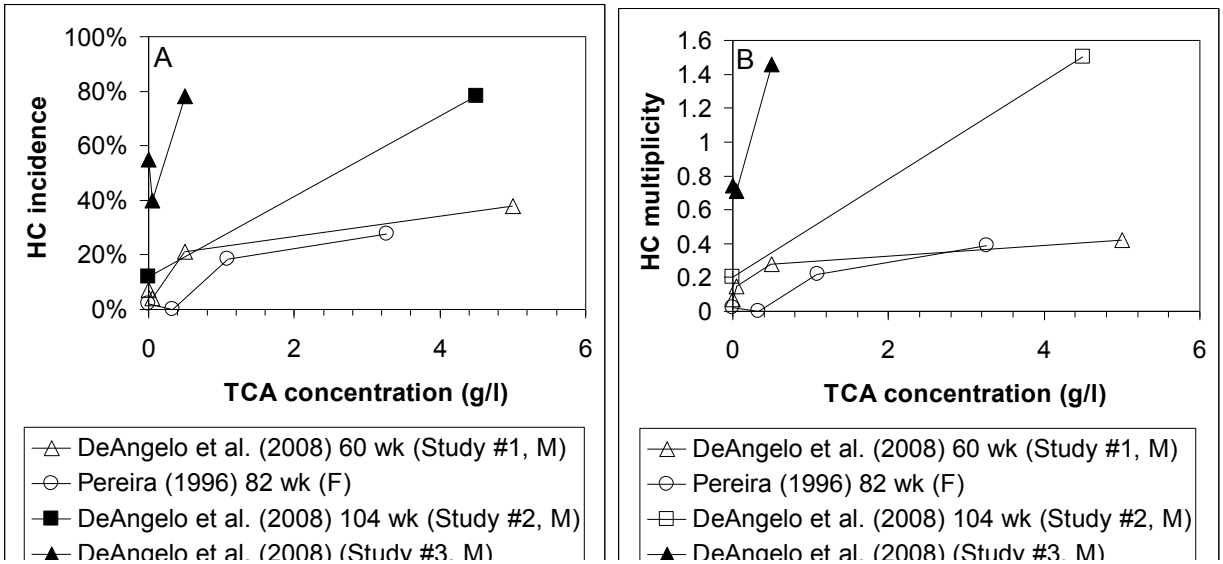
Drinking water concentrations were 0, 0.05, 0.5, 1, 2, and 3.5 g/L, from which daily average doses were calculated using observed water consumption in the study.

Figure 4-12. Dose-response data for HCCs (A) incidence and (B) multiplicity, induced by DCA from DeAngelo et al. (1999).

Associations of DCA carcinogenicity with various noncancer, possibly precursor, effects was also investigated. Importantly, the doses that induced tumors in DeAngelo et al. (1999) were reported to not induce widespread cytotoxicity. An attempt was also made to relate differing exposure levels to subchronic changes and peroxisomal enzyme induction. Interestingly, DeAngelo et al. (1999) reported that peroxisome proliferation was significantly increased at 3.5 g/L DCA only at 26 weeks, not correlated with tumor response, and not increased at either 0.05 or 0.5 g/L treatments. The authors concluded that DCA-induced carcinogenesis was not dependent on peroxisome proliferation or chemically sustained proliferation, as measured by DNA synthesis. Slight hepatomegaly was present by 26 weeks in the 0.5 g/L group and decreased with time. By contrast, increases in both percentage liver/body weight and the multiplicity of HCCs increased proportionally with DCA exposure concentration after 79–100 weeks of exposure. DeAngelo et al. (1999) presented a figure comparing the number of HCCs/animal at 100 weeks compared with the percentage liver/body weight at 26 weeks that showed a linear correlation ($r^2 = 0.9977$), while peroxisome proliferation and DNA synthesis did not correlate with tumor induction profiles. The proportional increase in liver weight with DCA exposure was also reported for shorter durations of exposure as noted previously. Therefore, for DCA, both tumor incidence and liver weight appear to increase proportionally with dose.

4.5.6.3.2.3. TCA carcinogenicity dose-response data

Pereira (1996) reported that for 82-week exposures to TCA in female B6C3F$_1$ mice, TCA exposure concentrations of 0, 2, 6.67, and 20 mmol/L (0, 0.33, 1.1, and 3.3 g/L) led to increasing incidences and multiplicity of adenomas and of carcinomas (see Figure 4-13). DeAngelo et al. (2008) reported the results of three experiments exposing male B6C3F$_1$ mice to neutralized TCA in drinking water (incidences also in Figure 4-13). Rather than using five exposure levels that were generally twofold apart, as was done in DeAngelo et al. (1999) for DCA, DeAngelo et al. (2008) studied only three doses of TCA that were an order of magnitude apart, which limits the elucidation of the shape of the dose-response curve. In addition, for the 104-week data, DeAngelo et al. (2008) contained two studies, each conducted in a separate laboratories—the two lower doses were studied in one study and the highest dose in another. The first 104-week study was conducted using 2 g/L sodium chloride, or 0.05, 0.5, or 5 g/L TCA in drinking water for 60 weeks (Study #1) while the other two were conducted for a period of 104 weeks (Study #2 with 2.5 g/L neutralized acetic acid or 4.5 g/L TCA exposure groups and Study #3 with deionized water, 0.05 g/L TCA and 0.5 g/L TCA exposure groups). In addition, a relatively small number of animals were used for the determination of a tumor response (n ~ 30 at final necropsy).



Combined HCA + HCC were not reported in Pereira (1996).

Sources: (DeAngelo et al., 2008; Pereira, 1996)).

Figure 4-13. Reported incidences of HCCs and hepatocellular adenomas plus carcinomas (HCA + HCC) in various studies in B6C3F$_1$ mice.

In Study #1, the incidence data for adenomas observed at 60 weeks at 0.05, 0.5, and 5.0 g/L TCA were 2.1-, 3.0- and 5.4-fold of control values, with similar fold increases in multiplicity. As shown by Pereira (1996), 60 weeks does not allow for full tumor expression, so whether the dose-response relationship is the same at 104 weeks is not certain. For instance, Pereira (1996) examined the tumor induction in female B6C3F$_1$ mice and demonstrated that foci, adenoma, and carcinoma development in mice are dependent on duration of exposure (period of observation in controls). In control female mice a 360- vs. 576-day observation period showed that at 360 days, no foci or carcinomas and only 2.5% of animals had adenomas, whereas by 576 days of observation, 11% had foci, 2% adenomas, and 2% had carcinomas. For DCA and TCA treatments, foci, adenomas, and carcinoma incidence and multiplicity did not reach full expression until 82 weeks at the three doses employed. Although the numbers of animals were relatively low and variable at the two highest doses (18–28 mice), there were 50–53 mice studied at the lowest dose level and 90 animals studied in the control group.

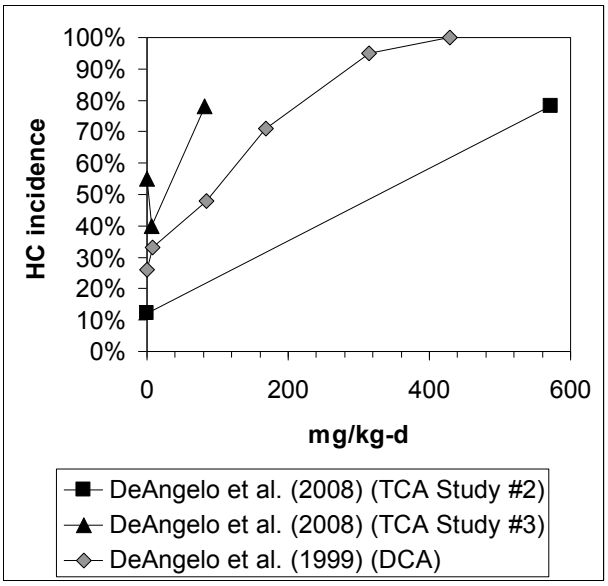
Therefore, the 104-week DeAngelo et al. (2008) data from Studies #2 and #3 would generally be preferred for elucidating the TCA dose-response relationship. However, Study #2 was only conducted at one dose, and although Study #3 used lower doses, it exhibited extraordinarily high control incidences of liver tumors. In particular, while the incidence of adenomas and carcinomas was 12% in Study #2, it was reported to be 64% in Study #3. The mice in Study #3 were of very large size (weighing ~50 g at 45 weeks) as compared to Study #1, Study #2, or most other bioassays in general, and the large background rate of tumors reported is consistent with the body-weight-dependence observed by Leakey et al. (2003a).

To put into context the 64% incidence data for carcinomas and adenomas reported in DeAngelo et al. (2008) for the control group of Study #3, other studies cited in this review for male B6C3F$_1$ mice show a much lower incidence in liver tumors with: (1) NCI (1976) reporting a colony control level of 6.5% for vehicle and 7.1% incidence of HCCs for untreated male B6C3F$_1$ mice (n = 70–77) at 78 weeks; (2) Herren-Freund et al. (1987) reporting a 9% incidence of adenomas in control male B6C3F$_1$ mice with a multiplicity of 0.09 ± 0.06 and no carcinomas (n = 22) at 61 weeks; (3) NTP (1990) reporting an incidence of 14.6% adenomas and 16.6% carcinomas in male B6C3F$_1$ mice after 103 weeks (n = 48); and (4) Maltoni et al. (1988; 1986) reporting that B6C3F$_1$ male mice from the "NCI source" had a 1.1% incidence of "hepatoma" (carcinomas and adenomas) and those from "Charles River Co." had a 18.9% incidence of "hepatoma" during the entire lifetime of the mice (n = 90 per group). The importance of examining an adequate number of control or treated animals before confidence can be placed in those results in illustrated by Anna et al. (1994) in which at 76 weeks, 3/10 control male B6C3F$_1$ mice that were untreated and 2/10 control animals given corn oil were reported to have adenomas, but from 76 to 134 weeks, 4/32 mice were reported to have adenomas (multiplicity of 0.13 ± 0.06) and 4/32 mice were reported to have carcinomas (multiplicity of 0.12 ± 0.06). Thus, the reported combined incidence of carcinomas and adenomas of 64% reported by

DeAngelo et al. (2008) for the control mice of Study #3 not only is inconsistent and much higher than those reported in Studies #1 and #2, but also is much higher than reported in a number of other studies of TCE.

Therefore, this large background rate and the increased mortality for these mice limit their use for determining the nature of the dose-response for TCA liver carcinogenicity. At the two lowest doses of 0.05 and 0.5 g/L TCA from Study #3, the differences in the incidences and multiplicities for all tumors were twofold at 104 weeks. However, there was no difference in any of the tumor results (i.e., adenoma, carcinoma, and combinations of adenoma and carcinoma incidence and multiplicity) between the 4.5 g/L dose group in Study #2 and the 0.5 g/L dose group in Study #3 at 104 weeks. By contrast, at 60 weeks of exposure, but within the same study (Study #1), there was a twofold increase in multiplicity for adenomas, and for adenomas and carcinomas combined between the 0.5 and 5.0 g/L TCA exposure groups. These results are consistent with the two highest exposure levels reaching a plateau of response after a long enough duration of exposure for full expression of the tumors (i.e., ~90% of animals having liver tumors at the 0.5 and 5 g/L exposures). However, whether such a plateau would have been observed in mice with a more "normal" body weight, and hence a lower background tumor burden, cannot be determined.

Because of the limitations of different studies, it is difficult to discern whether the liver tumor dose-response curves of TCA and DCA are different in a way analogous to that for liver weight (see Figure 4-14). Certainly, it is clear that at the same concentration in drinking water or estimated applied dose, DCA is more potent than TCA, as DCA induces nearly 100% incidence of carcinomas at a lower dose than TCA. Therefore, like with liver weight gains, DCA has a steeper dose-response function than TCA. However, the evidence for a "plateau" in tumor response at high doses with TCA, as was observed for liver weight, is equivocal, as it is confounded by the highly varying background tumor rates and the limitations of the available study paradigms.



Only carcinomas were reported in DeAngelo et al. (1999), so combined adenomas and carcinomas could not be compared.

Sources: (DeAngelo et al., 2008; DeAngelo et al., 1999).

Figure 4-14. Reported incidence of HCCs induced by DCA and TCA in 104-week studies.

DeAngelo et al. (2008) attempted to identify a NOEL for tumorigenicity using tumor multiplicity data and estimated TCA dose. However, it is not an appropriate descriptor for these data, especially given that "statistical significance" of the tumor response is the determinant used by the authors to support the conclusions regarding a dose in which there is no TCA-induced effect. Due to issues related to the appropriateness of use of the concurrent control in Study #3, only the 60-week experiment (i.e., Study #1) is useful for the determination of tumor dose-response. Not only is there not allowance for full expression of a tumor response at the 60-week time point, but a power calculation of the 60-week study shows that the Type II error, which should be >50% and thus, greater than the chances of "flipping a coin," was 41 and 71% for incidence and 7 and 15% for multiplicity of adenomas for the 0.05 and 0.5 g/L TCA exposure groups. For the combination of adenomas and carcinomas, the power calculation was 8 and 92% for incidence and 6 and 56% for multiplicity at 0.05 and 0.5 g/L TCA exposure. Therefore, the designed experiment could accept a false null hypothesis, especially in terms of tumor multiplicity, at the lower exposure doses and erroneously conclude that there is no response due to TCA treatment.

In terms of correlations with other noncancer, possibly precursor effects, DeAngelo et al. (2008) also reported that PCO activity, which varied 2.7-fold as baseline controls, was 1.3-, 2.4-, and 5.3-fold of control for the 0.05, 0.5, and 5 g/L TCA exposure groups in Study #1 at 4 weeks

and adenomas incidence was 2.1-, 3.0-, and 5.4-fold of control and not similar at the lowest dose level at 60 weeks. However, it is not clear whether the similarly between PCO and carcinogenicity at 60 weeks would persist for tumor incidence at 104 weeks. DeAngelo et al. (2008) provided regression analyses to compare "percent of hepatocellular neoplasia," as indicated by tumor multiplicity, with TCA dose, represented by estimations of the TCA dose in mg/kg-day, and with PCO activity for the 60- and 104-week data. Whether adenomas and carcinomas combined or individual tumor type were used in these analyse was not reported by the authors. However, it would be preferable to compare "precursor" levels of PCO at earlier time points, rather than at a time when there was already a significant tumor response. In addition, linear regression analyses of these data are difficult to interpret because of the wide dose spacing of these experiments. In such a situation, for a linear regression, control and 5 g/L exposure levels will basically determine the shape of the dose-response curve since the 0.05 and 0.5 g/L exposure levels are so close to the control (zero) value. Thus, dose-response appears to be linear between control and the 5.0 g/L value with the two lowest doses not affectively changing the slope of the line (i.e., "leveraging" the regression). Moreover, at the 5 g/L dose level, there is potential for effects due to palatability, as reported in one study in which drinking water consumption declined at this concentration (DeAngelo et al., 2008). Thus, the value of these analyses is limited by: (1) the use of data from Study #3 in a tumor prone mouse that is not comparable to those used in Studies #1 and #2; (2) the appropriateness of using PCO values from later time points and the variability in PCO control values; (3) the uncertainty of the effects of palatability on the 5 g/L TCA results, which were reported in one study to reduce drinking water consumption; and (4) the dose-spacing of the experiment.

4.5.6.3.2.4. CH carcinogenic dose-response

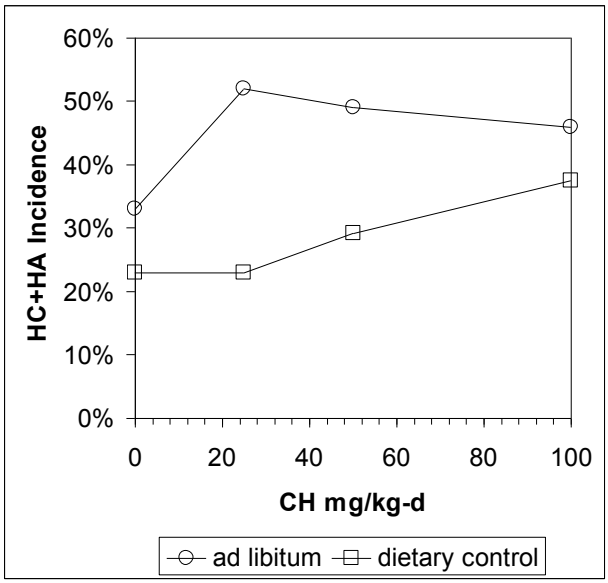
Although a much more limited database in rodents than for TCA or DCA, there is evidence that CH is also a rodent liver hepatocarcinogen [see also Section E.2.5 and Caldwell and Keshava (2006)].

Daniel et al. (1992) exposed adult male B6C3F$_1$ 28-day-old mice to 1 g/L CH in drinking water for 30 and 60 weeks (n = 5 for interim sacrifice) and for 104 weeks (n = 40). The concentration of CH was 1 g/L and estimated to provide a 166-mg/kg-day dose. It is not clear from the report what control group better matched the CH group, as the mean initial body weights of the groups as well as the number of animals varied considerably in each group (i.e., ~40% difference in mean body weights at the beginning of the study). Liver tumors were increased by CH treatment. The percentage incidence of liver carcinomas and adenomas in the surviving animals was 15% in control and 71% in CH-treated mice and the incidence of HCC was reported to be 46% in the CH-treated group. The number of tumors/animals was also significantly increased with CH treatment. However, because this was a single-dose study, a comparison with the dose-response relationship with TCE, TCA, or DCA is not feasible.

George et al. (2000) exposed male B6C3F$_1$ mice to CH in drinking water for 2 years. Groups of animals were sacrificed at 26, 52, and 78 weeks following the initiation of dosing, with terminal sacrifices at week 104. Only a few animals received a complete pathological examination. Preneoplastic foci and adenomas were increased in the livers of all CH treatment groups at 104 weeks. The percentage incidences of hepatocellular adenomas were reported to be 21.4, 43.5, 51.3, and 50% in control, 13.5, 65.0 and 146.6 mg/kg-day CH treatment groups, respectively. The percentage incidences of HCCs were reported to be 54.8, 54.3, 59.0 and 84.4% in these same groups. The resulting percentage incidence of hepatocellular adenomas and carcinomas were reported to be 64.3, 78.3, 79.5 and 90.6%. Of concern is the reporting of a 64% incidence of HCCs and adenomas in the control group of mice for this experiment, which is the same as that for another study published by this same laboratory (DeAngelo et al., 2008). DeAngelo et al. (2008) did not identify them as being contemporaneous studies or sharing controls, but a comparison of the control data published by DeAngelo et al. (2008) for TCA and that published by George et al. (2000) for the CH studies shows them to be the same data set. Therefore, as discussed above, this data set was derived from B6C3F$_1$ mice that were large (~50 g) and resultantly tumor prone, making determinations of the dose-response of CH from this experiment difficult. Therefore, for the purposes of comparison of dose-response relationships, this study has the same limitations as the DeAngelo et al. (2008) study, discussed above.

Leakey et al. (2003b)studied the effects of CH exposure (0, 25, 50, and 100 mg/kg-day, 5 days/week, 104–105 weeks via gavage) in male B6C3F$_1$ mice with dietary control used to manipulate body growth (n = 48 for 2-year study and n = 12 for the 15-month interim study). Dietary control was reported to decrease background liver tumor rates (decreased by 15–20%) and was reported to be associated with decreased variation in liver-to-body weight ratios, thereby potentially increasing assay sensitivity. In dietary-controlled groups and groups fed ad libitum, liver adenomas and carcinomas (combined) were reported to be increased with CH treatment. With dietary restriction, there was a more discernable CH tumor-response with overall tumor incidence reduced, and time-to-tumor increased by dietary control in comparison to ad-libitum-fed mice. Incidences of hepatocellular adenoma and carcinoma overall rates were reported to be 33, 52, 49, and 46% for control, 25, 50, and 100 mg/kg ad-libitum-fed mice, respectively. For dietary controlled mice the incidence rates were reported to be 22.9, 22.9, 29.2, and 37.5% for controls, 25, 50, and 100 mg/kg CH, respectively. Body weights were matched and carefully controlled in this study. These data are shown in Figure 4-15, relative to control incidences. It is evident from these data that dietary control significantly changes the apparent shape of the dose-response curve, presumably by reducing variability between animals. While the ad libitum dose groups had an apparent ~~"s~~aturation" of response, this was not evident with the dietary controlled group. Of note is that all of the other bioassays for TCE, TCA, DCA, and CH were in ad-libitum-fed mice. Therefore, it is difficult to compare the dose-response curves for CH-treated

mice on dietary restriction to those fed ad libitum. However, the rationale for dietary restriction in the B6C3F$_1$ mouse is to prevent the types of weight gain and corresponding high background tumor levels observed in DeAngelo et al. (2008) and George et al. (2000). As stated previously, most other studies of TCA, DCA, and TCE had background levels that, while varied, were lower than the ad-libitum-fed mice studied in Leakey et al. (2003b)



Source: Leakey et al. (2003b)

Figure 4-15. Effects of dietary control on the dose-response curves for changes in liver tumor incidences induced by

Of note is that incidences of adenomas and carcinomas combined do not show differences in tumor progression as carcinomas may increase and adenomas may regress. Liver weight increases at 15 months did not correlate with 2-year tumor incidences in the ad libitum group, but a consistent dose-response shape between these two measures is evident in the dietary controlled group. However, of note is the reporting of liver weight at 15 months is for a time period in which foci and liver tumors have been reported to have already occurred in other studies, so hepatomegaly in the absence of these changes is hard to detect.

In terms of other noncancer effects that may be associated with tumor induction, it is notable that while dietary restriction reduced the overall level of CH-mediated tumor induction, it led to greater CH-mediated induction of peroxisome proliferation-associated enzymes. Moreover, between control groups, dietary restricted mice appeared to have higher levels of lauric acid ω-hydrolase activity than ad-libitum-fed mice. Seng et al. (2003) report that lauric acid β-hydroxylase and PCO were induced only at exposure levels >100 mg/kg CH, again with dietary-restricted groups showing the greatest induction. Such data argue against the role of peroxisome proliferation in CH liver tumor induction in mice.

Leakey et al. (2003b) gave no descriptions of liver pathology, other than incidence of mice with fatty liver changes. Hepatic malondialdehyde concentration in ad-libitum-fed and dietary-controlled mice did not change with CH exposure at 15 months, but the dietary-controlled groups were all approximately half that of the ad-libitum-fed mice. Thus, while overall increased tumors observed in the ad libitum diet correlated with increased malondialdehyde concentration, there was no association between CH dose and malondialdehyde induction for either diet.

Overall, from the CH studies in mice, there is an apparent increase in liver adenomas and carcinomas induced by CH treatment by either drinking water or gavage with all available studies performed in male B6C3F$_1$ mice. However, the background levels of hepatocellular adenomas and carcinomas in these mice in George et al. (2000) and body-weight data from this study are high, consistent with the association between large body weight and background tumor susceptibility shown with dietary control (Leakey et al., 2003b). With dietary control, Leakey et al. (2003b) report a dose-response relationship between exposure and tumor incidence that is proportional to dose.

4.5.6.3.2.5. Degree of concordance among TCE, TCA, DCA, and CH dose-response relationships

A quantitative comparison of the carcinogenicity dose-response relationships among TCE, TCA, DCA, and CH—analogous to the quantitative comparison between TCE and TCA hepatomegaly—was considered. This first step in such a comparison would an examination of the dose-response data for TCE alone to see if they are consistent with a single dose-response relationship. As shown in Figures 4-10 and 4-11, there is substantial variability among the available liver tumor dose-response data that was not observed for hepatomegaly. The strain of mice used in the bioassays had a difference in not only TCE liver tumor response, but also background liver tumor incidence. Differences in exposure paradigms in the bioassays also leads to difference in tumor incidence and reporting. In addition, unlike the case with TCE hepatomegaly data in mice, the TCE dose-response data for liver tumors in mice exposed via inhalation and gavage are not consistent with a common dose-response curve even on an internal dose basis (e.g., Rhomberg, 2000) (Section 5.2). This heterogeneity is also evident for the TCA dose-response data, as shown in Figure 4-13, which may in part be due to the differences in study duration. Furthermore, among all of the available cancer bioassay data for TCE, TCA, DCA, and CH, the control incidences for background liver tumors vary from about 1% to over 50%, and difference of >50-fold that adds substantial uncertainty to any joint analysis. Therefore, differences within and across the databases of these compounds, such as the comparability of study durations, control tumor incidences, and carcinogenic potency, preclude either a quantitative analysis or a definitive conclusion. This question may be better addressed

experimentally where similar animals are exposed to different compounds in the same experimental setting.

4.5.6.3.3. Inferences from liver tumor phenotype and genotype

A number of studies have investigation tumor phenotypes, such as c-Jun staining, tincture, and dysplacity, or genotypes, such as H-ras mutations, to inform both the identification of the active agents of TCE liver tumor induction as well as what mode(s) of action may be involved.

4.5.6.3.3.1. Tumor phenotype—staining and appearance

The descriptions of tumors in mice reported by the NCI (1976), NTP (1990), and Maltoni et al. (1988; 1986) studies are also consistent with phenotypic heterogeneity as well as spontaneous tumor morphology (see Section E.3.4.1.5). As noted in Section E.3.1, HCCs observed in humans are also heterogeneous. For mice, Maltoni et al. (1986) described malignant tumors of hepatic cells to be of different subhistotypes, of various degrees of malignancy, and unique or multiple, and have different sizes (usually detected grossly at necropsy) from TCE exposure. In regard to phenotype, tumors were described as usual type observed in Swiss and $B6C3F_1$ mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens and to frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. For the NCI (1976) study, the mouse liver tumors were described in detail and to be heterogeneous ~~–as~~ described in the literature" and similar in appearance to tumors generated by carbon tetrachloride. The description of liver tumors in this study and tendency to metastasize to the lung are similar to descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation exposure. The NTP (1990) study reported that TCE exposure is associated with increased incidence of HCC (tumors with markedly abnormal cytology and architecture) in male and female mice. Hepatocellular adenomas were described as circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal-appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent, but the tumors lacked typical lobular organization. HCCs were reported to have markedly abnormal cytology and architecture with abnormalities in cytology cited as including increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies, and variations in nuclear appearance. Furthermore, in many instances, several or all of the abnormalities were reported to be present in different areas of the tumor and variations in architecture, with some of the HCCs having areas of trabecular organization. Mitosis was variable in amount and location. Therefore, the phenotype of tumors reported from TCE exposure was heterogeneous in appearance between and within tumors from all three of these studies.

Caldwell and Keshava (2006) reported that Bannasch (2001) and Bannasch et al. (2001) describe the early phenotypes of preneoplastic foci induced by many oncogenic agents (DNA-reactive chemicals, radiation, viruses, transgenic oncogenes and local hyperinsulinism) as insulinomimetic. These foci and tumors have been described by tincture (after hematoxylin and eosin staining of structural contents) as primarily eosinophilic (pink, reflecting eosin staining, e.g., staining of intracellular and extracellular protein), basophilic (blue, reflecting hematoxylin staining, e.g., staining of ribosomes and arginine rich basic nucleorprotein such as histones), and to be heterogeneous. Primary eosin staining is associated with a less malignant state of the hepatocyte with increased ribosomal content, decreased glycogen content, and increased basophilia of the cytoplasm by hematoxylin staining to be indicative of a more malignant state or tumor progression (Carter et al., 2003; Bannasch, 2001). Several studies do identify foci and tumors as primarily eosinophilic or basophilic, but do not give specific criteria for how a foci or tumor (which can be and usually is made up of a mixture of phenotypically heterogenous cells) are assigned to be one category or another. Caldwell and Keshava (2006) noted that the tumors observed after TCE exposure are consistent with the description for the main tumor lines of development described by Bannasch et al. (2001). Thus, the response of liver to DCA (glycogenesis with emergence of glycogen poor tumors) is similar to the progression of preneoplastic foci to tumors induced from a variety of agents and conditions associated with increased cancer risk. Furthermore Caldwell and Keshava (2006) note that Bull et al. (2002) report expression of insulin receptor to be elevated in tumors of control mice or mice treated with TCE, TCA, and DCA but not in nontumor areas, suggesting that this effect is not specific to DCA.

There is a body of literature that has focused on the effects of TCE and its metabolites after rats or mice were exposed to "mutagenic" agents to "initiate" hepatocarcinogenesis and this is discussed in Section E.4.2. TCE and its metabolites were reported to affect tumor incidence, multiplicity, and phenotype when given to mice as a co-exposure with a variety of "initiating" agents and with other carcinogens. Pereira and Phelps (1996) reported that methyl nitrosourea (MNU) alone induced basophilic foci and adenomas. MNU and low concentrations of DCA or TCA in female mice were reported to induce heterogeneous for foci and tumor with a higher concentration of DCA inducing more eosinophilic and a higher concentration of TCA inducing more tumors that were basophilic. Pereira et al. (2001) reported that not only dose, but also gender affected phenotype in mice that had already been exposed to MNU and were then exposed to DCA. As for other phenotypic markers, Lantendresse and Pereira (1997) reported that exposure to MNU and TCA or DCA induced tumors that had some commonalities (i.e., were hererogeneous), but differences were noted for female mice exposed to DCA or TCA as co-exposures with MNU.

With regard to the phenotype of TCA and DCA-induced tumors, Stauber and Bull (1997) reported the for male $B6C3F_1$ mice, DCA-induced "lesions" contained a number of smaller

lesions that were heterogeneous and more eosinophilic with larger "lesions" tending to be less numerous and more basophilic. For TCA results using this paradigm, the "lesions" were reported to be less numerous, more basophilic, and larger than those induced by DCA. Carter et al. (2003) used tissues from the DeAngelo et al. (1999) study and examined the heterogeneity of the DCA-induced lesions and the type and phenotype of preneoplastic and neoplastic lesions pooled across all time points. Carter et al. (2003) examined the phenotype of liver tumors induced by DCA in male B6C3F_1 mice and the shape of the dose-response curve for insight into its mode of action. They reported a dose-response of histopathologic changes (all classes of premalignant lesions and carcinomas) occurring in the livers of mice at doses of 0.05−3.5 g/L DCA for 26–100 weeks and suggested that foci and adenomas demonstrated neoplastic progression with time at lower doses than observed DCA genotoxicity. Preneoplastic lesions were identified as eosinophilic, basophilic and/or clear cell (grouped with clear cell and mixed cell), and dysplastic. Altered foci were 50% eosinophilic with about 30% basophilic. As foci became larger and evolved into carcinomas, they became increasingly basophilic. The pattern held true throughout the exposure range. There was also a dose and length of exposure related increase in atypical nuclei in "noninvolved" liver. Glycogen deposition was also reported to be dose-dependent with periportal accumulation at the 0.5 g/L exposure level. Carter et al. (2003) suggested that size and evolution into a more malignant state are associated with increasing basophilia, a conclusion consistent with those of Bannasch (1996) and that there is a greater periportal location of lesions suggestive as the location from which they arose. Consistent with the results of DeAngelo et al. (1999), Carter et al. (2003) reported that DCA (0.05–3.5 g/L) increased the number of lesions per animal relative to animals receiving distilled water, that DCA shortened the time to development of all classes of hepatic lesions, and that the phenotype of the lesions were similar to those spontaneously arising in controls. Along with basophilic and eosinophilic lesions or foci, Carter et al. (2003) concluded that DCA-induced tumors also arose from isolated, highly dysplastic hepatocytes in male B6C3F_1 mice chronically exposed to DCA suggesting another direct neoplastic conversion pathway other than through eosinophilic or basophilic foci.

Rather than male B6C3F_1 mice, Pereira (1996) studied the dose-response relationship for the carcinogenic activity of DCA and TCA and characterized their lesions (foci, adenomas, and carcinomas) by tincture in females (the generally less sensitive gender). Like the studies of TCE by Maltoni et al. (1988; 1986), female mice were also reported to have increased liver tumors after TCA and DCA exposures. Pereira (1996) pool lesions were pooled for phenotype analysis so the effect of duration of exposure could not be determined and adenomas could not be separated from carcinomas for "tumors." However, Pereira (1996) reported that a decrease in the concentration of DCA resulted in a decrease in the number of foci and a shift in the phenotype from primarily eosinophilic foci (i.e., ~95% eosinophilic at 2.58 g/L DCA) to basophilic foci (~57% eosinophilic at 0.26 g/L). For TCA, the number of foci was reported to ~40 basophilic

and ~60 eosinophilic, regardless of dose. Spontaneously occurring foci were more basophilic by a ratio of 7/3. Pereira (1996) described the foci of altered hepatocytes and tumors induced by DCA in female B6C3F$_1$ mice to be eosinophilic at higher exposure levels, but at lower or intermittent exposures, they were half eosinophilic and half basophilic. Regardless of exposure level, half of the TCA-induced foci were reported to be half eosinophilic and half basophilic with tumors 75% basophilic. In control female mice, the limited numbers of lesions were mostly basophilic, with most of the rest being eosinophilic with the exception of a few mixed tumors. The limitations of descriptions of tincture and especially for inferences regarding peroxisome proliferator from the description of "basophilia" is discussed in Section E.3.4.1.5.

Thus, the results appear to differ between male and female B6C3F$_1$ mice in regard to tincture for DCA and TCA at differing doses. What is apparent is that the tincture of the lesions is dependent on the stage of tumor progression, agent (DCA or TCA), gender, and dose. Also what is apparent from these studies is that both DCA and TCA are heterogeneous in their tinctoral characteristics.

Overall, tumors induced by TCA, DCA, CH, and TCE are all heterogeneous in their physical and tinctural characteristics in a manner this not markedly distinguishable from spontaneous lesions or those induced by a wide variety of chemical carcinogens. For instance, Daniel et al. (1992), which studied DCA and CH carcinogenicity (discussed above), noted that morphologically, there did not appear to be any discernable differences in the visual appearance of the DCA- and CH-induced tumors. Therefore, these data do not provide strong insights into elucidating the active agent(s) for TCE hepatocarcinogenicity or their mode(s) of action.

4.5.6.3.3.2. C-Jun staining

Stauber and Bull (1997) reported that in male B6C3F$_1$ mice, the oncoproteins, c-Jun and c-Fos, were expressed in liver tumors induced by DCA but not those induced by TCA. Although Bull et al. (2004) suggested that the negative expression of c-Jun in TCA-induced tumors may be consistent with a characteristic phenotype shown in general by peroxisome proliferators as a class, as pointed out by Caldwell and Keshava (2006), there is no supporting evidence of this. Nonetheless, the observation that TCA and DCA have different levels of oncogene expression led to a number of follow-up studies by this group. No data on oncoprotein immunostaining are available for CH.

Stauber et al. (1998) studied induction of "transformed" hepatocytes by DCA and TCE treatment in vitro, including an examination of c-Jun staining. Stauber et al. (1998) isolated primary hepatocytes from 5 to 8-week-old male B6C3F$_1$ mice (n = 3) and subsequently cultured them in the presence of DCA or TCA. In a separate experiment, 0.5 g/L DCA was given to mice as pretreatment for 2 weeks prior to isolation. The authors assumed that the anchorage-independent growth of these hepatocytes was an indication of an "initiated cell." After 10 days in culture with DCA or TCA (0, 0.2, 0.5, and 2.0 mM), concentrations of ≥0.5 mM DCA and

TCA both induced an increase in the number of colonies that was statistically significant, with DCA showing dose-dependence as well as slightly greater overall increases than TCA. In a time-course experiment, the number of colonies from DCA treatment in vitro peaked by 10 days and did not change through days 15–25 at the highest dose and, at lower concentrations of DCA, increased time in culture induced similar peak levels of colony formation by days 20–25 as that reached by 10 days at the higher dose. Therefore, the number of colonies formed was independent of dose if the cells were treated long enough in vitro. However, not only did treatment with DCA or TCA induce anchorage independent growth but untreated hepatocytes also formed larger numbers of colonies with time, although at a lower rate than those treated with DCA. The level reached by untreated cells in tissue culture at 20 days was similar to the level induced by 10 days of exposure to 0.5 mM DCA. The time course of TCA exposure was not tested to see if it had a similar effect with time as did DCA. The colonies observed at 10 days were tested for c-Jun expression with the authors noting that "colonies promoted by DCA were primarily c-Jun positive in contrast to TCA promoted colonies that were predominantly c-Jun negative." Of the colonies that arose spontaneously from tissue culture conditions, 10/13 (76.9%) were reported to be c-Jun+, those treated with DCA 28/34 (82.3%) were c-Jun+, and those treated with TCA 5/22 (22.7%) were c-Jun+. Thus, these data show heterogeneity in cell in colonies but with more that were c-Jun+ colonies occurring by tissue culture conditions alone than in the presence of DCA, rather than in the presence of TCA.

Bull et al. (2002) administered TCE, TCA, DCA, and combinations of TCA and DCA to male B6C3F$_1$ mice by daily gavage (TCE) or drinking water (TCA, DCA, and TCA + DCA) for 52–79 weeks, in order to compare a number of tumor characteristics, including c-Jun expression, across these different exposures. Bull et al. (2002) reported lesion reactivity to c-Jun antibody to be dependent on the proportion of the DCA and TCA administered after 52 weeks of exposure. Given alone, DCA was reported to produce lesions in mouse liver for which approximately half displayed a diffuse immunoreactivity to a c-Jun antibody, half did not, and none exhibited a mixture of the two. After TCA exposure alone, no lesions were reported to be stained with this antibody. When given in various combinations, DCA and TCA co-exposure induced a few lesions that were only c-Jun+, many that were only c-Jun–, and a number with a mixed phenotype whose frequency increased with the dose of DCA. For TCE exposure of 79 weeks, TCE-induced lesions were reported to also have a mixture of phenotypes (42% c-Jun+, 34% c-Jun–, and 24% mixed) and to be most consistent with those resulting from DCA and TCA co-exposure but not either metabolite alone.

A number of the limitations of the experiment are discussed in Caldwell et al. (2008b) Specifically, for the DCA- and TCA-exposed animals, the experiment was limited by low statistical power, a relatively short duration of exposure, and uncertainty in reports of lesion prevalence and multiplicity due to inappropriate lesions grouping (i.e., grouping of hyperplastic nodules, adenomas, and carcinomas together as "tumors"), and incomplete histopathology

determinations (i.e., random selection of gross lesions for histopathology examination). For determinations of immunoreactivity to c-Jun, Bull et al. (2002) combined hyperplastic nodules, adenomas, and carcinomas in most of their treatment groups, so differences in c-Jun expression across differing types of lesions were not discernable.

Nonetheless, these data collectively strongly suggest that TCA is not the sole agent of TCE-induced mouse liver tumors. In particular, TCE-induced tumors that were, in order of frequency, c-Jun+, c-Jun–, and of mixed phenotype, while c-Jun+ tumors have never been observed with TCA treatment. Nor do these data support DCA as the sole contributor, since mixed phenotypes were not observed with DCA treatment.

4.5.6.3.3.3. Tumor genotype: H-ras mutation frequency and spectrum

An approach to determine the potential modes of action of DCA and TCA through examination of the types of tumors each "induced" or "selected" was to examine H-ras activation (Bull et al., 2002; Ferreira-Gonzalez et al., 1995; Anna et al., 1994; Nelson et al., 1989). No data of this type were available for CH. This approach has also been used to try to establish an H-ras activation pattern for "genotoxic" and "nongenotoxic" liver carcinogens compounds and to make inferences concerning peroxisome proliferator-induced liver tumors. However, as noted by Stanley et al. (1994), the genetic background of the mice used and the dose of carcinogen may affect the number of activated H-ras containing tumors which develop. In addition, the stage of progression of "lesions" (i.e., foci vs. adenomas vs. carcinomas) also has been linked the observance of H-ras mutations. Fox et al. (1990) note that tumors induced by phenobarbital (0.05% drinking water [H_2O], 1 year), chloroform (200 mg/kg corn oil gavage, 2 times weekly for 1 year) or ciprofibrate (0.0125% diet, 2 years) had a much lower frequency of H-ras gene activation than those that arose spontaneously (2-year bioassays of control animals) or induced with the "genotoxic" carcinogen benzidine-2 hydrochloric acid (HCl) (120 ppm, drinking H_2O, 1 year) in mice. In that study, the term "tumor" was not specifically defined, but a correlation between the incidence of H-ras gene activation and the development of either a hepatocellular adenoma or HCC was reported to be made with no statistically significant difference between the frequency of H-ras gene activation in the hepatocellular adenomas and carcinomas. Histopathological examination of the spontaneous tumors, tumors induced with benzidine-2 HCl, phenobarbital, and chloroform was not reported to reveal any significant changes in morphology or staining characteristics. Spontaneous tumors were reported to have 64% point mutation in codon 61 (n = 50 tumors examined) with a similar response for benzidine of 59% (n = 22 tumors examined), whereas the mutation rates were 7% (n = 15 tumors examined) for phenobarbital, 21% (n = 24 tumors examined) for chloroform, and 21% (n = 39 tumors examined) for ciprofibrate. The ciprofibrate-induced tumors were reported to be more eosinophilic as were the surrounding normal hepatocytes.

Hegi et al. (1993) tested ciprofibrate-induced tumors in the NIH3T3 cotransfection-nude mouse tumorigenicity assay, which the authors stated was capable of detecting a variety of activated protooncogenes. The tumors examined (ciprofibrate-induced or spontaneously arising) were taken from the Fox et al. (1990) study, screened previously, and found to be negative for H-ras activation. With the limited number of samples examined, Hegi et al. (1993) concluded that ras protooncogene activation or activation of other protooncogenes using the nude mouse assay were not frequent events in ciprofibrate-induced tumors and that spontaneous tumors were not promoted with it. Using the more sensitive methods, the H-ras activation rate was reported to be raised from 21 to 31% for ciprofibrate-induced tumors and from 64 to 66% for spontaneous tumors. Stanley et al. (1994) studied the effect of methylclofenapate (MCP) (25 mg/kg for up to 2 years), a peroxisome proliferator, in $B6C3F_1$ (relatively sensitive) and C57BL/10J (relatively resistant) mice for H-ras codon 61 point mutations in MCP-induced liver tumors (hepatocellular adenomas and carcinomas). In the $B6C3F_1$ mice, the number of tumors with codon 61 mutations was 11/46 and for C57BL/10J mice 4/31. Unlike the findings of Fox et al. (1990), Stanley et al. (1994) reported an increase in the frequency of mutation in carcinomas, which was reported to be twice that of adenomas in both strains of mice, indicating that stage of progression was related to the number of mutations in those tumors, although most tumors induced by MCP did not have this mutation.

Anna et al. (1994) reported that the H-ras codon 61 mutation frequency was not statistically different in liver tumors from DCA- and TCE-treated mice from a highly variable number of tumors examined. From their concurrent controls, they reported that H-ras codon 61 mutations in 17% (n = 6) of adenomas and 100% (n = 5) of carcinomas. For historical controls (published and unpublished), they reported mutations in 73% (n = 33) of adenomas and mutations in 70% (n = 30) of carcinomas. For tumors from TCE-treated animals, they reported mutations in 35% (n = 40) of adenomas and 69% (n = 36) of carcinomas, while for DCA-treated animals, they reported mutations in 54% (n = 24) of adenomas and in 68% (n = 40) of carcinomas. Anna et al. (1994) reported more mutations in TCE-induced carcinomas than adenomas. In regard to mutation spectra in H-ras oncogenes in control or spontaneous tumors, the patterns were slightly different but those from TCE treatment were mostly similar to that of DCA-induced tumors (0.5% in drinking water).

The study of Ferreira -Gonzalez (1995) in male $B6C3F_1$ mice has the advantage of comparison of tumor phenotype at the same stage of progression (HCC), for allowance of the full expression of a tumor response (i.e., 104 weeks), and an adequate number of spontaneous control lesions for comparison with DCA or TCA treatments. However, tumor phenotype at an end stage of tumor progression may not be indicative of earlier stages of the disease process. In spontaneous liver carcinomas, 58% were reported to show mutations in H-61 as compared with 50% of tumor from 3.5 g/L DCA-treated mice and 45% of tumors from 4.5 g/L TCA-treated mice. A number of peroxisome proliferators have been reported to have a much smaller

mutation frequency that spontaneous tumors (e.g., 13–24% H-ras codon 61 mutations after methylclofenopate depending on mouser strain, Stanely et al. [1994]: 21–31% for ciprofibrate-induced tumors and 64−66% for spontaneous tumors, Fox et al. [1990] and Hegi et al. [1993]). Thus, the heterogeneous response for H-ras mutations was similar for spontaneous and DCA-, and TCA-induced HCCs and differed from the reduced H-ras mutation frequencies reported for a number of peroxisome proliferators.

In his review, Bull (2000) suggested that ―the report by Anna et al. (1994) indicated that TCE-induced tumors possessed a different mutation spectra in codon 61 of the H-ras oncogene than those observed in spontaneous tumors of control mice." Bull (2000) stated that ―results of this type have been interpreted as suggesting that a chemical is acting by a mutagenic mechanism" but went on to suggest that it is not possible to a priori rule out a role for selection in this process and that differences in mutation frequency and spectra in this gene provide some insight into the relative contribution of different metabolites to TCE-induced liver tumors. Bull (2000) noted that data from Anna et al. (1994), Ferreira-Gonzalez et al. (1995), and Maronpot et al. (1995b) indicated that mutation frequency in DCA-induced tumors did not differ significantly from that observed in spontaneous tumors. Bull (2000) also noted that the mutation spectra found in DCA-induced tumors has a striking similarity to that observed in TCE-induced tumors, and DCA-induced tumors were significantly different than that of TCA-induced liver tumors.

Bull et al. (2002) reported that mutation frequency spectra for the H-ras codon 61 in mouse liver ―tumors" induced by TCE (n = 37 tumors examined) were significantly different than that for TCA (n = 41 tumors examined), with DCA-treated mice tumors giving an intermediate result (n = 64 tumors examined). In this experiment, TCA-induced ―tumors" were reported to have more mutations in codon 61 (44%) than those from TCE (21%) and DCA (33%). This frequency of mutation in the H-ras codon 61 for TCA is the opposite pattern as that observed for a number of peroxisome proliferators in which the number of mutations at H-ras codon 61 in tumors has been reported to be much lower than spontaneously arising tumors (see above). Bull et al. (2002) noted that the mutation frequency for all TCE, TCA, or DCA tumors was lower in this experiment than for spontaneous tumors reported in other studies (they had too few spontaneous tumors to analyze in this study), but that this study utilized lower doses and was of shorter duration than that of Ferreira-Gonzalez (1995). Furthermore, the disparities from previous studies may also be impacted by lesion grouping, mentioned above, in which lower stages of progression are grouped with more advanced stages.

Overall, in terms of H-ras mutation, TCE-induced tumors appears to be more like DCA-induced tumors (which are consistent with spontaneous tumors), or those resulting from a co-exposure to both DCA and TCA (Bull et al., 2002), than from those induced by TCA. As noted above, Bull et al. (2002) reported the mutation frequency spectra for the H-ras codon 61 in mouse liver tumors induced by TCE to be significantly different than that for TCA, with

DCA-treated mice tumors giving an intermediate result and for TCA-induced tumors to have a H-ras profile that is the opposite than those of a number of other peroxisome proliferators. More importantly, however, these data, along with the measures discussed above, show that mouse liver tumors induced by TCE are heterogeneous in phenotype and genotype in a manner similar to that observed in spontaneous tumors.

4.5.6.3.4. "Stop" experiments

Several stop experiments, in which treatment is terminated early in some dose groups, have attempted to ascertain the whether progression differences exist between TCA and DCA. After 37 weeks of treatment and then a cessation of exposure for 15 weeks, Bull et al. (1990) reported that after a combined 52-week period, liver weight and percentage of liver/body weight were reported to still be statistically significantly elevated after DCA or TCA treatment. The authors partially attribute the remaining increases in liver weight to the continued presence of hyperplastic nodules in the liver. In terms of liver tumor induction, the authors stated that "statistical analysis of tumor incidence employed a general linear model ANOVA with contrasts for linearity and deviations from linearity to determine if results from groups in which treatments were discontinued after 37 weeks were lower than would have been predicted by the total dose consumed." The multiplicity of tumors (incidence was not used) observed in male mice exposed to DCA or TCA at 37 weeks and then sacrificed at 52 weeks were compared with those exposed for a full 52 weeks. The response in animals that received the shorter duration of DCA exposure was very close to that which would be predicted from the total dose consumed by these animals. By contrast, the response to TCA exposure for the shorter duration was reported by the authors to deviate significantly ($p = 0.022$) from the linear model predicted by the total dose consumed. However, in the prediction of "dose-response," foci, adenomas, and carcinomas were combined into one measure. Therefore, foci, a certain percentage of which have been commonly shown to spontaneously regress with time, were included in the calculation of total "lesions." Moreover, only a sample of lesions were selected for histological examination, and as is evident in the sample, some lesions appeared "normal" upon microscopic examination (see below). Therefore, while suggesting that cessation of exposure diminished the number of "lesions," methodological limitations temper any conclusions regarding the identity and progression of lesion with continuous vs. noncontinuous DCA and TCA treatment.

Additionally, Bull et al. (1990) noted that after stopping treatment, DCA lesions appeared to arrest their progression in contrast to TCA lesions, which appeared to progress. In particular, those in the stop treatment group (at 2 g/L) with 0/19 lesions examined histologically were carcinomas, while in the continuous treatment groups, a significant fraction of lesions examined were carcinomas at the higher exposure (6/23 at 2 g/L). By contrast, at terminal sacrifice, a larger fraction of the lesions examined were carcinomas in the stop treatment groups (3/5 at 2 g/L) than in the continuous treatment group (2/7 and 4/16 at 1 g/L and 2 g/L, respectively).

However, as mentioned above, these inferences are based on examination of only a subset of lesions. Specifically, for TCA treatment, the number of animals examined for determination of which "lesions" were foci, adenomas, and carcinomas was 11/19 mice with "lesions" at 52 weeks, while all 4 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. For DCA treatment, the number of animals examined was only 10/23 mice with "lesions" at 52 weeks, while all 7 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. Most importantly, when lesions were examined microscopically, some did not turn out to be preneoplastic or neoplastic—for example, two lesions appeared "to be histologically normal" and one necrotic.

While limited, the conclusions of Bull et al. (1990) are consistent with later experiments performed by Pereira and Phelps (1996). They noted that in MNU-treated mice that were then treated with DCA, the yield of altered hepatocytes decreases as the tumor yields increase between 31 and 51 weeks of exposure, suggesting progression of foci to adenomas, but that adenomas did not appear to progress to carcinomas. For TCA, Pereira and Phelps (1996) reported that "MNU-initiated" adenomas promoted with TCA continued to progress. However, the use of MNU initiation complicates direct comparisons with treatment with TCA or DCA alone.

No similar data comparing stop and continued treatment of TCE are available to assess the consistency or lack-thereof with TCA or DCA. Moreover, the informative of such a comparison would be limited by designs of the available TCA and DCA studies, which have used higher concentrations in conjunction with the much lower durations of exposure. While higher doses allow for responses to be more easily detected, it introduces uncertainty as to the effects of the higher doses alone. In addition, because the overall duration of the experiments is also generally much less than 104 weeks, it is not possible to discern whether the differences in results between those animals in which treatment was suspended in comparison to those in which had not had been conducted would persist with longer durations.

4.5.6.4. Conclusions Regarding the Role of TCA, DCA, and CH in TCE-Induced Effects in the Liver

In summary, it is likely that oxidative metabolism is necessary for TCE-induced effects in the liver. However, the specific metabolite or metabolites responsible for both noncancer and cancer effects is less clear. TCE, TCA, and DCA exposures have all been associated with induction of peroxisomal enzymes but are all weak PPARα agonists. The available data strongly support TCA ***not*** being the sole or predominant active moiety for TCE-induced liver effects. With respect to hepatomegaly, TCE and TCA dose-response relationships are quantitatively inconsistent, for TCE leads to greater increases in liver/body weight ratios that expected from predicted rates of TCA production. In fact, above a certain dose of TCE, liver/body weight ratios are greater than that observed under any conditions studied so far for TCA. Histological

changes and effects on DNA synthesis are generally consistent with contributions from either TCA or DCA, with a degree of polyploidization, rather than cell proliferation, likely to be significant for TCE, TCA, and DCA. With respect to liver tumor induction, TCE leads to a heterogeneous population of tumors, not unlike those that occur spontaneously or that are observed following TCA-, DCA-, or CH-treatment. Moreover, some liver phenotype experiments, particularly those utilizing immunostaining for c-Jun, support a role for both DCA and TCA in TCE-induced tumors, with strong evidence that TCA cannot solely account for the characteristics of TCE-induced tumors. In addition, H-ras mutation frequency and spectrum of TCE-induced tumors more closely resembles that of spontaneous tumors or of those induced by DCA, and were less similar in comparison to that of TCA-induced tumors. The heterogeneity of TCE-induced tumors is similar to that observed to be induced by a broad category of carcinogens, and to that observed in human liver cancer. Overall, then, it is likely that multiple TCE metabolites, and therefore, multiple pathways, contribute to TCE-induced liver tumors.

4.5.7. Mode of Action for TCE Liver Carcinogenicity

This section will discuss the evidentiary support for several hypothesized modes of action for liver carcinogenicity (including mutagenicity and peroxisome proliferation, as well as several additional proposed hypotheses and key events with limited evidence or inadequate experimental support), following the framework outlined in the *Cancer Guidelines* (U.S. EPA, 2005e, b).[9]

4.5.7.1. Mutagenicity

The hypothesis is that TCE acts by a mutagenic mode of action in TCE-induced hepatocarcinogenesis. According to this hypothesis, the key events leading to TCE-induced liver tumor formation constitute the following: TCE oxidative metabolite CH, after being produced in the liver, cause direct alterations to DNA (e.g., mutation, DNA damage, and/or micronuclei induction). Mutagenicity is a well-established cause of carcinogenicity.

4.5.7.1.1. Experimental support for the hypothesized mode of action

The genotoxicity, as described by the ability of TCE, CH, TCA, and DCA to induce mutations, was discussed previously in Section 4.2. The strongest data for mutagenic potential are for CH, thought to be a relatively short-lived intermediate in the metabolism of TCE that is rapidly converted to TCA and TCOH in the liver (see Section 3.3). CH causes a variety of

[9]As recently reviewed (Guyton et al., 2008), the approach to evaluating mode of action information described in EPA's *Cancer Guidelines* (U.S. EPA, 2005e, 2005b) considers the issue of human relevance of a hypothesized mode of action in the context of hazard evaluation. This excludes, for example, consideration of toxicokinetic differences across species; specifically, the Cancer Guidelines state, "the toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action." In addition, information suggesting quantitative differences in the occurrence of a key event between test species and humans are noted for consideration in the dose-response assessment, but is not considered in human relevance determination.

genotoxic effects in available in vitro and in vivo assays, with particularly strong data as to its ability to induce aneuploidy. It has been argued that CH mutagenicity is unlikely to be the cause of TCE carcinogenicity because the concentrations required to elicit these responses are generally quite high, several orders of magnitude higher that achieved in vivo (Moore and Harrington-Brock, 2000). For example, peak concentrations of CH in the liver of around 2–3 mg/kg have been reported after TCE administration at doses that are hepatocarcinogenic in chronic bioassays (Greenberg et al., 1999; Abbas and Fisher, 1997). Assuming a liver density of about 1 kg/L, these concentrations are orders of magnitude less than the minimum concentrations reported to elicit genotoxic responses in the Ames test and various in vitro measures of micronucleus, aneuploidy, and chromosome aberrations, which are in the 100–1,000 mg/L range. However, it is not clear how much of a correspondence is to be expected from concentrations in genotoxicity assays in vitro and concentrations in vivo, as reported in vivo CH concentrations are in whole-liver homogenate while in vitro concentrations are in culture media. In addition, a few in vitro studies have reported positive results at concentrations as low as 1 or 10 mg/L, including Furnus et al. (1990) for aneuploidy in Chinese hamster CHED cells (10 mg/L), Eichenlaub-Ritter et al. (1996) for bivalent chromosomes in meiosis I in MF1 mouse oocytes (10 mg/L), and Gibson et al. (1995) for cell transformation in Syrian hamster embryo cells after a 7-day treatment. Moreover, some in vivo genotoxicity assays of CH reported positive results at doses similar to those eliciting a carcinogenic response in chronic bioassays. For example, Nelson and Bull (1988) reported increased DNA SSBs at 100 CH mg/kg (oral) in male B6C3F$_1$ mice, although the result was not replicated by Chang et al. (1992). In another example, four of six in vivo mouse genotoxicity studies reported that CH induced micronuclei in mouse bone-marrow erythrocytes, with the lowest effective doses in positive studies ranging from 83 to 500 mg/kg [positive: Russo and Levis (1992a); Russo et al. (1992); Marrazzini et al. (1994); Beland et al. (1999); and negative: Leuschner and Leuschner (1991); Leopardi et al. (1993)]. However, the use of i.p. administration in these and many other in vivo genotoxicity assays complicates the comparison with carcinogenicity data. Also, it is difficult with the available data to assess the contributions from the genotoxic effects of CH along with those from the genotoxic and nongenotoxic effects of other oxidative metabolites (discussed in Sections 4.5.5.2 and 4.5.5.3).

Furthermore, altered DNA methylation, another heritable mechanism by which gene expression may be altered, is discussed in Section 4.5.7.3.7. As discussed previously, the differential patterns of H-ras mutations observed in liver tumors induced by TCE, TCA, and DCA may be more indicative of tumor selection and tumor progression resulting from exposure to these agents rather than a particular mechanism of tumor induction. The state of the science of cancer and the role of epigenetic changes, in addition to genetic changes, in the initiation and progression of cancer and specifically liver cancer, are discussed in Section E.3.1.

Therefore, while data are insufficient to conclude that a mutagenic mode of action mediated by CH is operant, a mutagenic mode of action, mediated either by CH or by some other oxidative metabolite of TCE, cannot be ruled out.

4.5.7.2. PPARα Receptor Activation

The hypothesis is that TCE acts by a PPARα agonism mode of action in TCE-induced hepatocarcinogenesis. According to this hypothesis, the key events leading to TCE-induced liver tumor formation constitute the following: the TCE oxidative metabolite TCA, after being produced in the liver, activates the PPARα receptor, which then causes alterations in cell proliferation and apoptosis and clonal expansion of initiated cells. This mode of action is assumed to apply only to the liver.

4.5.7.2.1. Experimental support for the hypothesized mode of action

Proliferation of peroxisomes and increased activity of a number of related marker enzymes has been observed in rodents treated with TCE, TCA, and DCA. The peroxisome-related effects of TCE are most likely mediated primarily through TCA based on TCE metabolism producing more TCA than DCA and the lower doses of TCA required to elicit a response relative to DCA. However, Bull (2004a) and Bull et al. (2004) have recently suggested that peroxisome proliferation occurs at higher exposure levels than those that induce liver tumors for TCE and its metabolites. They report that a direct comparison in the no-effect level or low-effect level for induction of liver tumors in the mouse and several other endpoints shows that, for TCA, liver tumors occur at lower concentrations than peroxisome proliferation in vivo but that PPARα activation occurs at a lower dose than either tumor formation or peroxisome proliferation. A similar comparison for DCA shows that liver tumor formation occurs at a much lower exposure level than peroxisome proliferation or PPARα activation. In vitro transactivation studies have shown that human and murine versions of PPARα are activated by TCA and DCA, while TCE itself is relatively inactive in the in vitro system, at least with mouse PPARα (Maloney and Waxman, 1999; Zhou and Waxman, 1998). In addition, Laughter et al. (2004) reported that the responses of acyl CoA oxidase (ACO), PCO, and CYP4A induction by TCE, TCA, and DCA were substantially diminished in PPARα-null mice. Therefore, evidence suggests that TCE, through its metabolites TCA and DCA, activate PPARα, and that at doses relevant to TCE-induced hepatocarcinogenesis, the role of TCA in PPARα agonism is likely to predominate.

It has been suggested that PPARα receptor activation is both the mode of action for TCA liver tumor induction as well as the mode of action for TCE liver tumor induction, as a result of the metabolism of TCE to TCA (Corton, 2008; NRC, 2006). Section E.3.4 addressed the status of the PPARα mode-of-action hypothesis for liver tumor induction and provides a more detailed discussion. However, as discussed previously and in Section E.2.1.10, TCE-induced increases in

liver weight have been reported in male and female mice that do not have a functional PPARα receptor (Nakajima et al., 2000). The dose-response for TCE-induced liver weight increases differs from that of TCA (see Section E.2.4.2). The phenotype of the tumors induced by TCE have been described to differ from those by TCA and to be more like those occurring spontaneously in mice, those induced by DCA, or those resulting from a combination of exposures to both DCA and TCA (see Section E.2.4.4). As to whether TCA induces tumors through activation of the PPARα receptor, the tumor phenotype of TCA-induced mouse liver tumors has been reported to have a different pattern of H-ras mutation frequency from other peroxisome proliferators (see Section E.2.4.4) (Bull et al., 2002; Stanley et al., 1994; Hegi et al., 1993; Fox et al., 1990). While TCE, DCA, and TCA are weak peroxisome proliferators, liver weight induction from exposure to these agents has not correlated with increases in peroxisomal enzyme activity (e.g., PCO activity) or changes in peroxisomal number or volume. By contrast, as discussed above, liver weight induction from subchronic exposures appears to be a more accurate predictor of carcinogenic response for DCA, TCA, and TCE in mice (see also Section E.2.4.4). The database for cancer induction in rats is much more limited than that of mice for determination of a carcinogenic response to these chemicals in the liver and the nature of such a response.

While many compounds known to cause rodent liver tumors with long-term treatment also activate the nuclear receptor PPARα, the mechanisms by which PPARα activation contributes to tumorigenesis are not completely known (Yang et al., 2007; NRC, 2006; Klaunig et al., 2003). As reviewed by Keshava and Caldwell (2006), PPARα activation leads to a highly pleiotropic response and may play a role in toxicity in multiple organs as well as in multiple chronic conditions besides cancer (obesity, atherosclerosis, diabetes, inflammation). Klaunig et al. (2003) and NRC (2006) proposed that the key causal events for PPARα agonist-induced liver carcinogenesis, after PPARα activation, are perturbation of cell proliferation and/or apoptosis, mediated by gene expression changes, and selective clonal expansion. It has also been proposed that sufficient evidence for this mode of action consists of evidence of PPARα agonism (i.e., in a receptor assay) in combination with either light- or electron-microscopic evidence for peroxisome proliferation or both increased liver weight and one more of the in vivo markers of peroxisome proliferation (Klaunig et al., 2003). However, it should be noted that peroxisome proliferation and in vivo markers such as PCO are not considered causal events (NRC, 2006; Klaunig et al., 2003), and that their correlation with carcinogenic potency is poor (Marsman et al., 1988). Therefore, for the purposes of this discussion, peroxisome proliferation and its markers are considered indicators of PPARα activation, as it is well established that these highly specific effects are mediated through PPARα (Klaunig et al., 2003; Peters et al., 1997).

As recently reviewed by Guyton et al. (2009), recent data suggest that PPARα activation along with these hypothesized causal events may not be sufficient for carcinogenesis. In particular, Yang et al. (2007) reported comparisons between mice treated with Wy-14643 and

transgenic mice in which PPARα was constitutively activated in hepatocytes without the presence of ligand. Yang et al. (2007) reported that, in contrast to Wy-14643-treatment, the transgene did not induce liver tumors at 11 months, despite inducing PPARα-mediated effects of a similar type and magnitude seen in response to tumorigenic doses of Wy-14643 in wild-type mice (decreased serum fatty acids, induction of PPARα target genes, altered expression of cell-cycle control genes, and a sustained increase in cellular proliferation). Nonetheless, it is important to discuss the extent to which PPARα activation mediates the effects proposed by Klaunig et al. (2003) and NRC (2006), even if the hypothesized sequence of key events may not be sufficient for carcinogenesis. Investigation continues into additional events that may also contribute, such as nonparenchymal cell activation and micro-RNA-based regulation of protooncogenes (Shah et al., 2007; Yang et al., 2007). Specifically addressed below are gene expression changes, proliferation, clonal expansion, and mutation frequency or spectrum.

With respect to gene expression changes due to TCE, Laughter et al. (2004) evaluated transcript profiles induced by TCE in wild-type and PPARα-null mice. As noted in Sections E.3.4.1.3 and E.3.1.2, there are limitations to the interpretation of such studies, some of which are discussed below. Also noted in Appendix E are discussions of how studies of peroxisome proliferators, indicate of the need for phenotypic anchoring, especially since gene expression is highly variable between studies and within studies using the same experimental paradigm. Section E.3.4 also provides detailed discussions of the status of the PPARα hypothesis. Of note, all null mice at the highest TCE dose (1,500 mg/kg-day) were moribund prior to the end of the planned 3-week experiment (Laughter et al., 2004), and it was proposed that this may reflect a greater sensitivity in PPARα-null mice to hepatotoxins due to defects in tissue repair abilities. Laughter et al. (2004) also noted that four genes known to be regulated by other peroxisome proliferators also had altered expression with TCE treatment in wild-type, but not null mice. Ramdhan et al. (2010) report that not only do PPARα-null mice, but also humanized mice (PPARα-null mice with inserted human PPARα) have underlying dysregulation of lipid metabolism and gene expression. However, in a comparative analysis, Bartosiewicz et al. (2001) concluded that TCE induced a different pattern of transcription than two other peroxisome proliferators, di(2-ethylhexyl) phthalate (DEHP) and clofibrate. In addition, Keshava and Caldwell (2006) compared gene expression data from Wy-14643, dibutyl phthalate (DBP), gemifibrozil, and DEHP, and noted a lack of consistent results across PPARα agonists. Thus, available data are insufficient to conclude that TCE gene expression changes are similar to other PPAR agonists, or even that there are consistent changes (beyond the in vivo markers of peroxisome proliferation, such as ACO, PCO, CYP4A, etc.) among different agonists. It should also be noted that Laughter et al. (2004) did not compare baseline (i.e., control levels of) gene expression between null and wild-type control mice, hindering interpretation of these results (Keshava and Caldwell, 2006). The possible relationship between PPARα activation and hypomethylation are discussed in Section 4.5.7.3.7.

In terms of proliferation, mitosis itself has not been examined in PPARα-null mice, but BrdU incorporation, a measure of DNA synthesis that may reflect cell division, polyploidization, or DNA repair, was observed to be diminished in null mice as compared to wild-type mice at 500 and 1,000 mg/kg-day TCE (Laughter et al., 2004). However, BrdU incorporation in null mice was still about threefold higher than controls, although it was not statistically significantly different due to the small number of animals, high variability, and the two- to threefold higher baseline levels of BrdU incorporation in control null mice as compared to control wild-type mice. Therefore, while PPARα appears to contribute to the short-term increase in DNA synthesis observed with TCE treatment, these results cannot rule out other contributing mechanisms. However, since it is likely that both cellular proliferation and increased ploidy contribute to the observed TCE-induced increases in DNA synthesis, it is not clear as to whether the observed decrease in BrdU incorporation is due to reduced proliferation, reduced polyploidization, or both.

With respect to clonal expansion, it has been suggested that tumor characteristics such as tincture (i.e., the staining characteristics light microscopy sections of tumor using H&E stains) and oncogene mutation status can be used to associate chemical carcinogens with a particular mode of action such as PPARα agonism (NRC, 2006; Klaunig et al., 2003). This approach is problematic primarily because of the lack of specificity of these measures. For example, with respect to tincture, it has been suggested that TCA-induced foci and tumors resemble those of other peroxisome proliferators in basophilia and lack of expression of GGT and GST-pi. However, as discussed in Caldwell and Keshava (2006), the term "basophilic" in describing foci and tumors can be misleading, because, for example, multiple lineages of foci and tumors exhibit basophilia, including those not associated with peroxisome proliferators (Carter et al., 2003; Bannasch et al., 2001; Bannasch, 1996). Moreover, a number of studies indicate that foci and tumors induced by other "classic" peroxisome proliferators may have different phenotypic characteristics from that attributed to the class through studies of WY-14643, including DEHP (Voss et al., 2005) and clofibric acid (Michel et al., 2007). Furthermore, even the combination of GGT and GST-pi negative, basophilic foci are nonspecific to peroxisome proliferators, as they have been observed in rats treated with AfB1 and AfB1 plus phenobarbital, none of which are peroxisome proliferators (Grasl-Kraupp et al., 1993; Kraupp-Grasl et al., 1990). Finally, while Bull et al. (2004) suggested that negative expression of c-Jun in TCA-induced tumors may be consistent with a characteristic phenotype of peroxisome proliferators, no data could be located to support this statement. Therefore, of phenotypic information does not appear to be reliable for associating a chemical with a PPARα agonism mode of action.

Mutation frequency or spectrum in oncogenes has also been suggested to be an indicator of a PPARα agonism mode of action being active (NRC, 2006), with the idea being that specific genotypes are being promoted by PPARα agonists. Although not a highly specific marker, H-ras codon 61 mutation frequency and spectra data do not support a similarity between mutations in

TCE-, TCA-, or DCA-induced tumors and those due to other peroxisome proliferators. For example, while ciprofibrate and methylclofenopate had lower mutation frequencies than historical controls (Stanley et al., 1994; Hegi et al., 1993), TCA-induced tumors had mutation frequencies similar to or higher than historical controls (Bull et al., 2002; Ferreira-Gonzalez et al., 1995). Anna et al. (1994) and Ferreira-Gonzalez et al. (1995) also reported TCE and DCA-induced tumors to have mutation frequencies similar to historical controls, although Bull et al. (2002) reported lower frequencies for these chemicals. However, the data reported by Bull et al. (2002) consist of mixed lesions at different stages of progression, and such differing stages, in addition to differences in genetic background and dose, can influence the frequency of H-ras mutations (Stanley et al., 1994). In addition, a greater frequency of mutations was reported in carcinomas than adenomas, and Bull et al. (2002) stated that this suggested that H-ras mutations were a late event. Moreover, Fox et al. (1990) noted that tumors induced by phenobarbital, chloroform, and ciprofibrate all had a much lower frequency of H-ras gene activation than those that arose spontaneously, so this marker does not have good specificity. Mutation spectrum is similarly of low utility for supporting a PPARα agonism mode of action. First, because many peroxisome proliferators been reported to have low frequency of mutations, the comparison of mutation spectrum would be limited to a small fraction tumors. In addition to the low power due to small numbers, the mutation spectrum is relatively nonspecific, as Fox et al. (1990) reported that of the tumors with mutations, the spectra of the peroxisome proliferator ciprofibrate, historical controls, and the genotoxic carcinogen benzidine-2 HCl were similar.

In summary, TCE clearly activates PPARα, and some of the effects contributing to tumorigenesis that Klaunig et al. (2003) and NRC (2006) propose to be the result of PPARα agonism are observed with TCE, TCA, or DCA treatment. While this consistency is supportive a role for PPARα, all of the proposed key causal effects with the exception of PPARα agonism itself are nonspecific, and may be caused by multiple mechanisms. There is more direct evidence that several of these effects, including alterations in gene expression and changes in DNA synthesis, are mediated by multiple mechanisms in the case of TCE, and a causal linkage to PPARα specifically is lacking. Therefore, because, as discussed further in the mode of action discussion below, there are multiple lines of evidence supporting the role of multiple pathways of TCE-induced tumorigenesis, the hypothesis that PPARα agonism and the key causal events proposed by Klaunig et al. (2003) and NRC (2006) constitute the sole or predominant mode of action for TCE-induced carcinogenesis is considered unlikely.

Furthermore, as reviewed by Guyton et al. (2009), recent data strongly suggest that PPARα and key events hypothesized by Klaunig et al. (2003) are not sufficient for carcinogenesis induced by the purported prototypical agonist, Wy-14643. Therefore, the proposed PPARα mode of action is likely "incomplete" in the sense that the sequence of key

events[10] necessary for cancer induction has not been identified. A recent 2-year bioassay of the peroxisome proliferator DEHP showed that it can induce a liver tumor response in mice lacking PPARα similar to that in wild-type mice (Ito et al., 2007). Klaunig et al. (2003) previously concluded that PPARα agonism was the sole mode of action for DEHP-induced liver tumorigenesis based on the lack of tumors in PPARα-null mice after 11 months treatment with Wy-14643 (Peters et al., 1997). They also assumed that due to the lack of markers of PPARα agonism in PPARα-null mice after short-term treatment with DEHP (Ward et al., 1998), a long-term study of DEHP in PPARα-null mice would yield the same results as for Wy-14643. However, due the finding by Ito et al. (2007) that PPARα-null mice exposed to DEHP do develop liver tumors, they concluded that DEHP can induce liver tumors by multiple mechanisms (Takashima et al., 2008; Ito et al., 2007). Hence, since there is no 2-year bioassay in PPARα-null mice exposed to TCE or its metabolites, it is not justifiable to use a similar argument based on Peters et al. (1997) and short-term experiments to suggest that the PPARα mode of action is operative. Therefore, the conclusion is supported that the hypothesized PPARα mode of action is inadequately specified because the data do not adequately show the proposed key events individually being required for hepatocarcinogenesis, nor do they show the sequence of key events collectively to be sufficient for hepatocarcinogenesis.

4.5.7.2.2. Quantitative relationships between key events and tumor induction

The issues of whether there is a quantitative relationship between hypothesized key events and tumor induction were recently examined in Guyton et al. (2009) and are discussed below. Furthermore, IARC has recently concluded that additional mechanistic information has become available, including studies with DEHP in PPARα-null mice, studies with several transgenic mouse strains, carrying human PPARα or with hepatocyte-specific constitutively activated PPARα and a study in humans exposed to DEHP from the environment that has changed its conclusions regarding the relevance of rodent tumor data to human risk (Grosse et al., 2011). Data from these new studies suggest that many molecular signals and pathways in several cell types in the liver, rather than a single molecular event, contribute to cancer development in rodents with IARC concluding that the human relevance of the molecular events leading to DEHP induced cancer in several target tissues (e.g., liver and testis) in rats or mice could not be ruled out, resulting in the evaluation of DEHP as a Group-2B agent, rather than Group 3.

This following discussion is from Guyton et al. (2009):

[10]As defined by the EPA *Cancer Guidelines* (2005b) a "~~k~~ey event" is "~~a~~n empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element," and the term "~~m~~ode of action" (MOA) is defined as "~~a~~ sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation." Therefore, a single key event alone is necessary, but not necessarily sufficient for carcinogenesis; however, the *sequence* of key events constituting a MOA needs to be sufficient for carcinogenesis.

Are key or associative events in the PPAR-α activation MOA quantitatively predictive of hepatocarcinogenicity?

Another question to consider is whether potency for PPAR-α activation or its attendant sequelae is quantitatively associated with carcinogenic activity or potency. If so, differences in sensitivity for carcinogenesis (such as may occur across species) could be predicted using quantitative information about the key events alone. If robust correlations were established, then they could potentially be used either to quantitatively account for pharmacodynamic differences that impact carcinogenic potency or as precursor events in nonlinear dose response assessment. However, there are limitations in the dose-response data available for analyses of quantitative relationships between potencies for precursor events in the proposed PPAR-α activation MOA and for liver tumor induction. Most tumor data, including for the best characterized PPAR-α agonists, are for exposure concentrations inducing well above 50% tumor incidence with less-than-lifetime administration. Precursor events have typically been studied at a single dose, often eliciting a near maximal response, thus precluding benchmark-based comparisons across studies. This is especially true for Wy-14,643, which has been administered most often at only one exposure concentration (1,000 ppm) that elicits a 100% tumor incidence after 1 year or less (Peters et al., 1997) and that also appears to be necrogenic (Woods et al., 2007a). On the other hand, hypothesized precursor events such as hepatomegaly, peroxisome proliferation, and increased DNA synthesis appear to have reached their maximal responses at 50 ppm Wy-14,643, with some statistically significant responses as low as 5 ppm (Marsman et al., 1992; Wada et al., 1992). Potencies across compounds have rarely been compared in a single study using the same experimental paradigm. These deficits in the database notwithstanding, provided below is an assessment of the quantitative predictive power of the potency for four proposed data elements for establishing the hypothesized MOA for hepatocarcinogenesis: PPAR-α activation in mice; and hepatomegaly, DNA synthesis, and increased peroxisome proliferation in rats.

PPAR- α activation in mice

Table 2 [reproduced as Table 4-66] presents data for four peroxisome proliferators in order of decreasing potency for inducing mouse liver tumors. These compounds were selected because of their importance to environmental human health risk assessments and because data to derive receptor activation potency indicators were available from a single study (Maloney and Waxman, 1999). The transactivation potencies of MEHP, Wy-14,643, dichloroacetic acid (DCA), and TCA for the mouse PPAR-α were monitored using a luciferase reporter gene containing multiple PPAR response elements derived from the rat hydratase/dehyrogenase promoter in transiently transfected COS-1 monkey kidney cells. The derived potency indicators were compared to the TD_{50} (i.e., the daily dose inducing tumors in half of the mice that would otherwise have remained tumor-free) from the Carcinogenic Potency Database (CPDB) of Gold et al. (2005). Note that for Wy-14,643, the dose listed yielded a maximal response and thus represents an upper limit to the TD_{50} (indicated by "<"). Two estimates of PPAR-α transactivation potency are given, the first based on 50% of the maximal response (i.e., EC_{50}) and the second based on the effective

concentration required for a 2-fold increase in activity (i.e., EC2-fold) (Maloney and Waxman, 1999). Orally administered DEHP undergoes presystemic hydrolysis catalyzed by lipase to MEHP in the gut, with mice exhibiting higher lipase activities in the small intestine compared to rats and marmosets (Ito et al., 2005; Kessler et al., 2004; Pollack et al., 1985). Therefore, because the mouse liver is likely exposed predominantly to MEHP rather than DEHP and unmetabolized ----------be explained by pharmacokinetics, i.e., hepatic conversion of DEHP to its mono-ester MEHP, since studies in rats demonstrate that orally administered DEHP undergoes presystemic hydrolysis to MEHP in the gut (Kessler et al., 2004; Pollack et al., 1985). Possible explanations for these results include one or more of the following: (1) the transactivation assay is not an accurate quantitative indicator of in vivo receptor activation; (2) the rate and nature of effects downstream of PPAR-α activation depends on the ligand; or (3) there are rate-limiting events independent of PPAR-α activation that contribute to mouse hepatocarcinogenesis by the agonists examined.

Hepatomegaly, DNA synthesis, and peroxisome proliferation in rats

Table 1 [reproduced as 4-67] compares potency indicators for various precursor effects at the TD_{50} for four PPAR-α agonists and rat hepatocarcinogens. Our analysis of whether there are consistent levels of in vivo precursor effect induction across peroxisome proliferators at the TD_{50} does not include all of the data from a similar, prior analysis by Ashby et al. (1994) for several reasons. First, unlike the CPDB, Ashby et al. (1994) did not adjust carcinogenicity data for less-than-lifetime dosing, which is relevant for most compounds. Second, for those mouse carcinogens reported in the CPDB, only acute data are available regarding DNA synthesis effects from Ashby et al.. Therefore, our analysis was restricted to rat precursor and potency data for the four compounds Wy-14,643, nafenopin, clofibrate, and DEHP and included both 1-week and 13-week data to separately address transient and sustained changes in DNA synthesis. Even for this small set of compounds, several limitations in the rat database were apparent. Because no single study provided comparative data for the precursor endpoints of interest, four separate reports were used. In the Wada et al. (1992) and Tanaka et al. (1992) studies of Wy-14,643 and clofibrate, respectively, administered doses were within 10% of the TD_{50}. However, nafenopin data were only available at a single dose of 500 ppm (Lake et al., 1993), which was linearly interpolated to the TD_{50}. The highest administered dose of DEHP was 12,500 ppm (David et al., 1999), a dose notably below the TD_{50}, and thus a lower limit based on the assumption of monotonicity with dose is shown. A further data limitation is that in the CPDB, only the TD_{50} for one of the four compounds, DEHP, incorporates data from studies administering more than one dose for two years.

The results shown in Table 1 [reproduced as Table 4-67] indicate that potency for the occurrence of short-term in vivo markers of PPAR-α activation varies widely in magnitude and lacks any apparent correlation with carcinogenic potency. Such differences have been noted previously. Similar to the results presented in Table 1 [reproduced as Table 4-67], Marsman et al. (1988) noted that although DEHP (12,000 ppm) and Wy-14,643 (1,000 ppm) induced a similar extent of hepatomegaly and peroxisome proliferation (measured either morphologically or biochemically) after 1 year, the frequency of hepatocelluar lesions was over 100-fold higher in Wy-14,643- relative to DEHP-exposed rats.

In addition, a higher labeling index was reported for 12,500 ppm DEHP than the maximal level attained after 50–1,000 ppm Wy-14,643 (David et al., 1999; Tanaka et al., 1992; Wada et al., 1992). We did not examine such differences in maximal responses in our analysis. We also do not present differences in response with dose and time seen among PPAR-α agonists, which are prominent enough to prevent displaying dose-response data on a common scale. For instance, labeling index is increased in a dose-dependent manner at 1 week by clofibrate (1.500, 4.500 and 9.000 ppm) but is decreased compared with controls at 13 weeks at the two higher doses (Tanaka et al., 1992). Together, these findings underscore the significant chemical-specific quantitative differences in these markers that limit their utility for predicting carcinogenic dose-response relationships.

Table 4-66. Potency indicators for mouse hepatocarcinogenicity and in vitro transactivation of mouse PPARα for four PPARα agonists

	Carcinogenic potency indicators (mg/kg-d)	**Transactivation potency indicators (μM)**	
Chemical	TD_{50}	EC_{50}	$EC_{twofold}$
Hepatocarcinogens			
Wy-14,643	<10.8	0.63	~0.4
DCA	119	~300	~300
TCA	584	~300	~300
DEHP/MEHP	700	~0.7	~0.7

Note: TD_{50} = the daily dose inducing tumors in half of the mice that would otherwise have remained tumor-free, estimated from the Carcinogenic Potency Database (Gold et al., 2005). EC_{50} = the effective concentration yielding 50% of the maximal response; $EC_{twofold}$ = the effective concentration required for a twofold increase in activity. Transactivation potencies were estimated from Maloney and Waxman (1999). The "<" symbol denotes an upper limit due to maximal response. A "~" symbol indicates that the transactivation potency was approximated from figures in Maloney and Waxman (1999).

MEHP = monoethylhexyl phthalate

Source: reproduced from Table 2 of Guyton et al. (2009).

Table 4-67. Potency indicators for rat hepatocarcinogenicity and common short-term markers of PPARα activation for four PPARα agonists

Chemical	Tumor TD_{50} (ppm in diet)	Fold-increase over control at tumor TD_{50}					
		1 wk			13 wks		
		RLW	LI	PCO	RLW	LI	PCO
Wy-14,643	109	1.8	12	13	2.6	6.8	39
Nafenopin	275	1.4	3.6	7.6	1.5	1.12	6.7
Clofibrate	4.225	1.4	4.4	4.2	1.4	0.95	3.7
DEHP	17.900	≥1.4	≥19	≥3.6	≥1.9	≥1.25	≥4.9

Note: For ease of comparison with precursor effect studies, administered doses for the tumor TD_{50}s in the Carcinogenic Potency Database were back-converted to equivalent ppm in diet using the formula of Gold et al. (2005), *i.e.*, TD_{50} (mg/kg-day) = TD_{50}(ppm in diet) × 0.04 (for male rats). Administered doses for precursor data on Wy-14,643 (Wada et al., 1992) and clofibrate (Tanaka et al., 1992) were within 10% of the TD_{50}. Because nafenopin precursor data were only available at 0 and 500 ppm (Lake et al., 1993), these doses were linearly interpolated to the TD_{50}. Because the highest administered dose of DEHP in precursor effect studies was 12,500 ppm (David et al., 1999), a lower limit is shown, based on the assumption of monotonicity with dose.

RLW = relative liver weight, LI = labeling index, PCO = cyanide insensitive palmitoyl CoA oxidation

Source: reproduced from Table 1 of Guyton et al. (2009).

4.5.7.3. Additional Proposed Hypotheses and Key Events with Limited Evidence or Inadequate Experimental Support

Several effects that been hypothesized to be associated with liver cancer induction are discussed in more detail below, including increased liver weight, DNA hypomethylation, and pathways involved in glycogen accumulation such as insulin signaling proteins. As discussed above, TCE and its metabolites reportedly increase nuclear size and ploidy in hepatocytes, and these effects likely account for much of the increases in labeling index and DNA synthesis caused by TCE. Importantly, these changes appear to persist with cessation of treatment, with liver weights, but not nuclear sizes, returning to control levels (Kjellstrand et al., 1983a). In addition, glycogen deposition, DNA synthesis, increases in mitosis, or peroxisomal enzyme activity do not appear correlated with TCE-induced liver weight changes.

4.5.7.3.1. Increased liver weight

Increased liver weight or liver/body weight ratios (hepatomegaly) is associated with increased risk of liver tumors in rodents, but it is relatively nonspecific (Allen et al., 2004). The evidence presented above for TCE and its metabolites suggest a similarity in dose-response between liver weight increases at short-term durations of exposure and liver tumor induction observed from chronic exposure. Liver weight increases may results from several concurrent processes that have been associated with increase cancer risk (e.g., hyperplasia, increased ploidy, and glycogen accumulation) and when observed after chronic exposure may result from the

increased presence of foci and tumors themselves. Therefore, there are inadequate data to adequately define a mode of action hypothesis for hepatocarcinogenesis based on liver weight increases.

4.5.7.3.2. "Negative selection"

As discussed above, TCE, TCA, and DCA all cause transient increases in DNA synthesis. This DNA synthesis has been assumed to result from proliferation of hepatocytes. However, the dose-related TCA- and DCA-induced increases in liver weight not correlate with patterns of DNA synthesis; moreover, there have been reports that DNA synthesis in individual hepatocytes does not correlate with whole liver DNA synthesis measures (Carter et al., 1995; Sanchez and Bull, 1990). With continued treatment, decreases in DNA synthesis have been reported for DCA (Carter et al., 1995). More importantly, several studies show that transient DNA synthesis is confined to a very small population of cells in the liver in mice exposed to TCE for 10 days or to DCA or TCA for up to 14 days of exposure. Therefore, generalized mitogenic stimulation is not likely to play a role in TCE-induced liver carcinogenesis.

Bull (2000) has proposed that the TCE metabolites TCA and DCA may contribute to liver tumor induction through so-called "negative selection" by way of several possible processes. First, it is hypothesized that the mitogenic stimulation by continued TCA and DCA exposure is downregulated in normal hepatocytes, conferring a growth advantage to initiated cells that either do not exhibit the downregulation of response or are resistant to the downregulating signals. This is implausible as both the normal rates of cell division in the liver and the TCE-stimulated increases are very low. Polyploidization has been reported to decrease the normal rates of cell division even further. That the transient and relatively low level of DNA synthesis reported for TCE, DCA, and TCA is reflective of proliferation rather than polyploidization is not supported by data on mitosis. A mechanism for such "downregulation" has not been identified experimentally.

A second proposed contributor to "negative-selection" is direct enhancement by TCA and DCA in the growth of certain populations of initiated cells. While differences in phenotype of end stage tumors have been reported between DCA and TCA, the role of selection and emergence of potentially different foci has not been elucidated. Neither have pathway perturbations been identified that are common to liver cancer in human and rodent for TCE, DCA, and TCA. The selective growth of clones of hepatocytes that may progress fully to cancer is a general feature of cancer and not specific to at TCE, TCA, or DCA mode of action.

A third proposed mechanism by which TCE may enhance liver carcinogenesis within this "negative selection" paradigm is through changing apoptosis. However, as stated above, TCE has been reported to either not change apoptosis or to cause a slight increase at high doses. Rather than increases in apoptosis, peroxisome proliferators have been suggested to inhibit apoptosis as part of their carcinogenic mode of action. However, the age and species studied

appear to greatly affect background rates of apoptosis (Snyder et al., 1995) with the rat having a greater rate of apoptosis than the mouse. DCA has been reported to induce decreases in apoptosis in the mouse (Carter et al., 1995; Snyder et al., 1995). However, the significance of the DCA-induced reduction in apoptosis, from a level that is already inherently low in the mouse, for the mode of action for induction of DCA-induce liver cancer is difficult to discern.

Therefore, for a mode of action for hepatocarcinogenesis based on —negative selection," there are inadequate data to adequately define the mode-of-action hypothesis, or the available data do not support such a mode of action being operative.

4.5.7.3.3. Polyploidization

Polyploidization may be an important key event in tumor induction. For example, in addition to TCE, partial hepatectomy, nafenopin, methylclofenopate, DEHP, diethylnitrosamine, *N*-nitrosomorpholine, and various other exposures that contribute to liver tumor induction also shift the hepatocyte ploidy distribution to be increasingly diploid or polypoid (Hasmall and Roberts, 2000; Miller et al., 1996; Vickers and Lucier, 1996; Melchiorri et al., 1993; Styles et al., 1988). As discussed by Gupta (2000), —[w]orking models indicate that extensive polyploidy could lead to organ failure, as well as to oncogenesis with activation of precancerous cell clones." However, the mechanism(s) by which increased polypoidy enhances carcinogenesis is not currently understood. Due to increased DNA content, polypoid cells will generally have increased gene expression. However, polyploid cells are considered more highly differentiated and generally divide more slowly and are more likely to undergo apoptosis, perhaps thereby indirectly conferring a growth advantage to initiated cells (see Section E.1). Of note is that changes in ploidy have been observed in transgenic mouse models that are also prone to develop liver cancer (see Section E.3.3.1). It is likely that polyploidization occurs with TCE exposure and it is biologically plausible that polyploidization can contribute to liver carcinogenesis, although the mechanism(s) is (are) not known. However, whether polyploidization is necessary for TCE-induced carcinogenesis is not known, as no experiment in which polyploidization specifically is blocked or diminished has been performed and the extent of polyploidization has not been quantified. Therefore, there are inadequate data to adequately define a mode-of-action hypothesis for hepatocarcinogenesis based on polyploidization.

4.5.7.3.4. Glycogen storage

As discussed above, several studies have reported that DCA causes accumulation of glycogen in mouse hepatocytes. Such glycogen accumulation has been suggested to be pathogenic, as it is resistant to mobilization by fasting (Kato-Weinstein et al., 1998). In humans, glycogenesis due to glycogen storage disease or poorly controlled diabetes has been associated with increased risk of liver cancer (Rake et al., 2002; Wideroff et al., 1997; Adami et al., 1996;

La Vecchia et al., 1994). Glycogen accumulation has also been reported to occur in rats exposed to DCA.

For TCE exposure in mice or rats, glycogen content of hepatocytes has been reported to be somewhat less than or the same as controls, or not remarked upon in the studies. TCA exposure has been reported to decrease glycogen content in rodent hepatocytes while DCA has been reported to increase it (Kato-Weinstein et al., 2001). There is also evidence that DCA-induced increases in glycogen accumulation are not proportional to liver weight increases and only account for a relatively small portion of increases in liver mass. DCA-induced increases in liver weight are not a function of cellular proliferation but probably include hypertrophy associated with polyploidization, increased glycogen deposition, and other factors.

While not accounting for increases in liver weight, excess glycogen can still not only be pathogenic, but also a predisposing condition for hepatocarcinogenesis. Some hypotheses regarding the possible relationship between glycogenesis and carcinogenesis have been posed that lend them biological plausibility. Evert et al. (2003), using an animal model of hepatocyte exposure to a local hyperinsulinemia from transplanted islets of Langerhans with remaining tissue is hypoinsulinemic, reported that insulin induces alterations resembling preneoplastic foci of altered hepatocytes that develop into hepatocellular tumors in later stages of carcinogenesis. Lingohr et al. (2001) suggested that normal hepatocytes downregulate insulin-signaling proteins in response to the accumulation of liver glycogen caused by DCA and that the initiated cell population, which does not accumulate glycogen and is promoted by DCA treatment, responds differently from normal hepatocytes to the insulin-like effects of DCA. Bull et al. (2002) reported increased insulin receptor protein expression in tumor tissues regardless of whether they were induced by TCE, TCA, or DCA. Given the greater activity of DCA relative to TCA on carbohydrate metabolism, it is unclear whether changes in these pathways are causes or simply reflect the effects of tumor progression. Therefore, it is biologically plausible that changes in glycogen status may occur from the opposing actions of TCE metabolites, but changes in glycogen content due to TCE exposure has not been quantitatively studied. The possible contribution of these effects to TCE-induced hepatocarcinogenesis is unclear. Therefore, there are inadequate data to adequately define a mode-of-action hypothesis for TCE-induced hepatocarcinogenesis based on changes in glycogen storage or even data to support increased glycogen storage to result from TCE exposure.

4.5.7.3.5. Inactivation of GST-zeta

DCA has been shown to inhibit its own metabolism in that pretreatment in rodents prior to a subsequent challenge dose leads to a longer biological half-life (Schultz et al., 2002). This self-inhibition is hypothesized to occur through inactivation of GST-zeta (Schultz et al., 2002). In addition, TCE has been shown to cause the same prolongation of DCA half-life in rodents, suggesting that TCE inhibits GST-zeta, probably through the formation of DCA (Schultz et al.,

2002). DCA-induced inhibition of GST-zeta has also been reported in humans, with GST-zeta polymorphisms reported to influence the degree of inactivation (Blackburn et al., 2001; Blackburn et al., 2000; Tzeng et al., 2000). Board et al. (2001) reported one variant to have significantly higher activity with DCA as a substrate than other GST-zeta isoforms, which could affect DCA susceptibility.

GST-zeta, which is identical to maleylacetoacetate isomerase, is part of the tyrosine catabolism pathway, which is disrupted in Type 1 hereditary tyrosinemia, a disease associated with the development of HCC at a young age (Tanguay et al., 1996). In particular, GST-zeta metabolizes maleylacetoacetate (MAA) to fumarylacetoacetate (FAA) and maleylacetone (MA) to fumarylacetone (Cornett et al., 1999; Tanguay et al., 1996). It has been suggested that the increased cancer risk with this disease, as well as through DCA exposure, results from accumulation of MAA and MA, both alkylating agents, or FAA, which displays apoptogenic, mutagenic, aneugenic, and mitogenic activities (Bergeron et al., 2003; Jorquera and Tanguay, 2001; Kim et al., 2000; Cornett et al., 1999; Tanguay et al., 1996). However, the possible effects of DCA through this pathway will depend on whether MAA, MA, or FAA is the greater risk factor, since inhibition of GST-zeta will lead to greater concentrations of MAA and MA and lower concentrations of FAA. Therefore, if MAA is the more active agent, then DCA may increase carcinogenic risk, while if FAA is the more active, then DCA may decrease carcinogenic risk. Tzeng et al. (2000) proposed the latter based on the greater genotoxicity of FAA, and in fact suggested that DCA may ―merit consideration for trial in the clinical management of hereditary tyrosinemia type 1."

Therefore, TCE-induced inactivation GST-zeta, probably through formation of DCA, may play a role in TCE-induced hepatocarcinogenesis. However, this mode of action is not sufficiently delineated at this point for further evaluation, as even the question of whether its actions through this pathway may increase or decrease cancer risk has yet to be experimentally tested.

4.5.7.3.6. Oxidative stress

Several studies have attempted to study the possible effects of ―oxidative stress" and DNA damage resulting from TCE exposures. The effects of induction of metabolism by TCE, as well as through co-exposure to ethanol, have been hypothesized to increase levels of ―oxidative stress" as a common effect for both exposures (see Section E.4.3.4). In terms of contributing to a carcinogenic mode of action, the term ―oxidative stress" is a somewhat nonspecific term, as it is implicated as part of the pathophysiologic events in a multitude of disease processes and is part of the normal physiologic function of the cell and cell signaling. Commonly, it appears to refer to the formation of reactive oxygen species leading to cellular or DNA damage. As discussed above, however, measures of oxidative stress induced by TCE, TCA, and DCA appear to be either not apparent, or at the very most, transient and nonpersistent with continued treatment

(Toraason et al., 1999; Channel et al., 1998; Parrish et al., 1996; Larson and Bull, 1992b). Therefore, while the available data are limited, there is insufficient evidence to support a role for such effects in TCE-induced liver carcinogenesis.

Oxidative stress has been hypothesized to be part of the mode of action for peroxisome proliferators, but has been found to be correlated with neither cell proliferation nor carcinogenic potency of peroxisome proliferators (see Section E.3.4.1.1). For instance, Parrish et al. (1996) reported that increases in PCO activity noted for DCA and TCA were not associated with 8-OHdG levels (which were unchanged) and also not with changes laurate hydrolase activity observed after either DCA or TCA exposure. The authors concluded that their data do not support an increase in steady-state oxidative damage to be associated with TCA initiation of cancer and that extension of treatment to time periods sufficient to insure peroxisome proliferation failed to elevate 8-OHdG in hepatic DNA. The authors thus, suggested that peroxisome proliferative properties of TCA were not linked to oxidative stress or carcinogenic response.

4.5.7.3.7. Changes in gene expression (e.g., hypomethylation)

Studies of gene expression as well as considerations for interpretation of studies of using the emerging technologies of DNA, siRNA, and miRNA microarrays for mode-of-action analyses are included in Sections E.3.1.2 and E.3.4.2.2. Caldwell and Keshava (2006) and Keshava and Caldwell (2006) report on both genetic expression studies and studies of changes in methylation status induced by TCE and its metabolites as well as differences and difficulties in the patterns of gene expression between differing PPARα agonists. In particular are concerns for the interpretation of studies that employ pooling of data as well as interpretation of ―snapshotsǁ in time of multiple gene changes." For instance, in the Laughter et al. (2004) study, it is not clear whether transcription arrays were performed on pooled data as well as the issue of phenotypic anchoring as data on percentage liver/body weight indicates significant variability within TCE treatment groups, especially in PPARα-null mice. For studies of gene expression using microarrays Bartosiewicz et al. (2001) used a screening analysis of 148 genes for xenobiotic-metabolizing enzymes, DNA repair enzymes, heat shock proteins, cytokines, and housekeeping gene expression patterns in the liver in response TCE. The TCE-induced gene induction was reported to be highly selective; only Hsp 25 and 86 and CYP were upregulated at the highest dose tested. Collier et al. (2003) reported differentially expressed mRNA transcripts in embryonic hearts from Sprague-Dawley rats exposed to TCE with sequences downregulated with TCE exposure appearing to be those associated with cellular housekeeping, cell adhesion, and developmental processes. TCE was reported to induce upregulated expression of numerous stress-response and homeostatic genes.

For the Laughter et al. (2004) study, transcription profiles using macroarrays containing approximately 1,200 genes were reported in response to TCE exposure with 43 genes reported to

be significantly altered in the TCE-treated wild-type mice and 67 genes significantly altered in the TCE-treated PPARα knockout mice. However, the interpretation of this information is difficult because in general, PPARα knockout mice have been reported to be more sensitive to a number of hepatotoxins partly because of defects in the ability to effectively repair tissue damage in the liver (Shankar et al., 2003; Mehendale, 2000) and because a comparison of gene expression profiles between controls (wild-type and PPARα knockout) were not reported. As reported by Voss et al. (2006), dose-, time course-, species-, and strain-related differences should be considered in interpreting gene array data. The comparison of differing PPARα agonists presented in Keshava and Caldwell (2006) illustrates the pleiotropic and varying liver responses of the PPARα receptor to various agonists, but did not imply that these responses were responsible for carcinogenesis.

As discussed in Section E.3.3.5, aberrant DNA methylation is a common hallmark of all types of cancers, with hypermethylation of the promoter region of specific tumor suppressor genes and DNA repair genes leading to their silencing (an effect similar to their mutation) and genome-wide hypomethylation (Pereira et al., 2004b; Ballestar and Esteller, 2002; Berger and Daxenbichler, 2002; Rhee et al., 2002; Herman et al., 1998). Whether DNA methylation is a consequence or cause of cancer is a long-standing issue (Ballestar and Esteller, 2002). Fraga et al. (2005; 2004) reported global loss of monoacetylation and trimethylation of histone H4 as a common hallmark of human tumor cells; they suggested, however, that genomewide loss of 5-methylcytosine (associated with the acquisition of a transformed phenotype) exists not as a static predefined value throughout the process of carcinogenesis but rather as a dynamic parameter (i.e., decreases are seen early and become more marked in later stages).

DNA methylation is a naturally occurring epigenetic mechanism for modulating gene expression, and disruption of this mechanism is known to be relevant to human carcinogenesis. As reviewed by Calvisi et al. (2007),

> [a]berrant DNA methylation occurs commonly in human cancers in the forms of genome-wide hypomethylation and regional hypermethylation. Global DNA hypomethylation (also known as demethylation) is associated with activation of protooncogenes, such as c-Jun, c-Myc, and c-HA-Ras, and generation of genomic instability. Hypermethylation on CpG islands located in the promoter regions of tumor suppressor genes results in transcriptional silencing and genomic instability.

While clearly associated with cancer, it has not been conclusively established whether these epigenetic changes play a causative role or are merely a consequence of transformation (Tryndyak et al., 2006). However, as Calvisi et al. (2007) note, "Current evidence suggests that hypomethylation might promote malignant transformation via multiple mechanisms, including chromosome instability, activation of protooncogenes, reactivation of transposable elements, and loss of imprinting."

Although little is known about how it occurs, a hypothesis has also been proposed that that the toxicity of TCE and its metabolites may arise from its effects on DNA methylation status. In regard to methylation studies, many are co-exposure studies as they have been conducted in initiated animals with some studies being very limited in their reporting and conduct. Caldwell and Keshava (2006) reviewed the body of work regarding TCE, DCA, and TCA. Methionine status has been noted to affect the emergence of liver tumors (Counts et al., 1996). Tao et al. (2000) and Pereira et al. (2004a) have studied the effects of excess methionine in the diet to see if it has the opposite effects as a deficiency (i.e., a reduction in a carcinogenic response rather than enhancement). However, Tao et al. (2000) reported that the administration of excess methionine in the diet is not without effect and can result in percentage liver/body weight ratios. Pereira et al. (2004a) reported that methionine treatment alone at the 8 g/kg level increased liver weight, decreased lauryl-CoA activity, and increased DNA methylation.

Pereira et al. (2004a) reported that very high levels of methionine supplementation to an AIN-760A diet affected the number of foci and adenomas after 44 weeks of co-exposure to 3.2 g/L DCA. However, while the highest concentration of methionine (8.0 g/kg) was reported to decrease both the number of DCA-induce foci and adenomas, the lower level of methionine co-exposure (4.0 g/kg) increased the incidence of foci. Co-exposure of methionine (4.0 or 8.0 g/kg) with 3.2 g/L DCA was reported to decrease by ~25% DCA-induced glycogen accumulation, increase mortality, but not to have much of an effect on peroxisome enzyme activity (which was not elevated by >33% over control for DCA exposure alone). The authors suggested that their data indicate that methioninine treatment slowed the progression of foci to tumors. Given that increasing hypomethylation is associated with tumor progression, decreased hypomethylation from large doses of methionine are consistent with a slowing of progression. Whether these results would be similar for lower concentrations of DCA and lower concentrations of methionine that were administered to mice for longer durations of exposure cannot be ascertained from these data. It is possible that in a longer-term study, the number of tumors would be similar. Finally, a decrease in tumor progression by methionine supplementation is not shown to be a specific event for the mode of action for DCA-induced liver carcinogenicity.

Tao et al. (2000) reported that 7 days of gavage dosing of TCE (1,000 mg/kg in corn oil), TCA (500 mg/kg, neutralized aqueous solution), and DCA (500 mg/kg, neutralized aqueous solution) in 8-week-old female B6C3F$_1$ mice resulted in not only increased liver weight, but also increased hypomethylation of the promoter regions of c-Jun and c-Myc genes in whole-liver DNA. However, data were shown for 1–2 mice per treatment. Treatment with methionine was reported to abrogate this response only at a 300 mg/kg i.p dose, with 0–100 mg/kg doses of methionine having no effect. Ge et al. (2001a) reported DCA- and TCA-induced DNA hypomethylation and cell proliferation in the liver of female mice at 500 mg/kg and decreased methylation of the c-Myc promoter region in liver, kidney, and urinary bladder. However,

increased cell proliferation preceded hypomethylation. Ge et al. (2002) also reported hypomethylation of the c-Myc gene in the liver after exposure to the peroxisome proliferators 2,4-dichlorophenoxyacetic acid (1,680 ppm), DBP (20,000 ppm), gemfibrozil (8,000 ppm), and Wy-14,643 (50–500 ppm, with no effect at 5 or 10 ppm) after 6 days in the diet. Caldwell and Keshava (2006) concluded that hypomethylation did not appear to be a chemical-specific effect at these concentrations. As noted Section E.3.3.5, chemical exposure to a number of differing carcinogens have been reported to lead to progressive loss of DNA methylation.

After initiation by *N*-methyl-*N*-nitrosourea (25 mg/kg) and exposure to 20 mmol/L DCA or TCA (46 weeks), Tao et al. (2004a) report similar hypomethylation of total mouse liver DNA by DCA and TCA with tumor DNA showing greater hypomethylation. A similar effect was noted for the differentially methylated region-2 of the insulin-like growth factor-II (IGF-II) gene. The authors suggest that hypomethylation of total liver DNA and the IGF-II gene found in nontumorous liver tissue would appear to be the result of a more prolonged activity and not cell proliferation, while hypomethylation of tumors could be an intrinsic property of the tumors. As pointed out by Caldwell and Keshava (2006), overexpression of IGF-II gene in liver tumors and preneoplastic foci has been shown in both animal models of hepatocarcinogenesis and humans, and may enhance tumor growth, acting via the overexpressed IGF-I receptor (Scharf et al., 2001; Werner and Le Roith, 2000).

Diminished hypomethylation was observed in Wy-14643-treated PPARα-null mice as compared to wild-type mice, suggestive of involvement of PPARα in mediating hypomethylation (Pogribny et al., 2007), but it is unclear how relevant these results are to TCE and its metabolites. First, the doses of Wy-14643 administered are associated with substantial liver necrosis and mortality with long-term treatment (Woods et al., 2007a), adding confounding factors to the interpretation of their results. Hypomethylation by Wy-14643 progressively increased with time up to 5 months (Pogribny et al., 2007), consistent with the sustained DNA synthesis caused by Wy-14643 and a role for proliferation in causing hypomethylation. Regardless, as discussed above, it is unlikely that PPARα is the mediator of the observed transient increase in DNA synthesis by DCA, so even if it is important for hypomethylation by TCA, there may be more than one pathway for this effect.

To summarize, aberrant DNA methylation status, including hypomethylation, is clearly associated with both human and rodent carcinogenesis. Hypomethylation itself appears to be sufficient for carcinogenesis, as diets deficient in choline and methionine that induce hypomethylation have been shown to cause liver tumors in both rats and mice (Henning and Swendseid, 1996; Wainfan and Poirier, 1992; Ghoshal and Farber, 1984; Mikol et al., 1983). However, it is not known to what extent hypomethylation is necessary for TCE-induced carcinogenesis. However, as noted by Bull (2004a) and Bull et al. (2004), the doses of TCA and DCA that have been tested for induction of hypomethylation are quite high compared to doses at which tumor induction occurs—at least 500 mg/kg-day. Whether these effects are still manifest

at lower doses relevant to TCE carcinogenicity, particularly with respect to DCA, has not been investigated. Finally, the role of PPARα in modulating hypomethylation, possibly through increased DNA synthesis as suggested by experiments with Wy-14643, are unknown for TCE and its metabolites.

4.5.7.3.8. Cytotoxicity

Cytotoxicity and subsequent induction of reparative hyperplasia have been proposed as key events for a number of chlorinated solvents, such as chloroform and carbon tetrachloride. However, as discussed above and discussed by Bull (2004a) and Bull et al. (2004), TCE treatment at doses relevant to liver carcinogenicity results in relatively low cytotoxicity. While a number of histological changes with TCE exposure are observed, in most cases necrosis is minimal or mild, associated with vehicle effects, and with relatively low prevalence. This is consistent with the low prevalence of necrosis observed with TCA and DCA treatment at doses relevant to TCE exposure. Therefore, it is unlikely that cytotoxicity and reparative hyperplasia play a significant role in TCE carcinogenicity

4.5.7.4. Mode-of-Action Conclusions

The conclusions regarding the mode of action for TCE-induced liver carcinogenesis described in the preceding sections are summarized in Table 4-68. Overall, although a role for many of the proposed key events discussed above cannot be ruled out, there are inadequate data to support the conclusion that any of the particular mode-of-action hypotheses reviewed above are operant. The available data do suggest that the mode of action of liver tumors induced by TCE is complex, as it is likely that key events from several pathways may operate. Nonetheless, because a collection of key events sufficient to induce liver tumors has not been identified, the answer to the first key question ***"1. Is the hypothesized mode of action sufficiently supported in the test animals?"*** is "no" at this time. Consequently, the other key questions of ***"2. Is the hypothesized mode of action relevant to humans?"*** and "***3. Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?***" will not be discussed in a mode-of-action-specific manner. Rather, they are discussed below in more general terms, first qualitatively and then quantitatively, using available relevant data.

4.5.7.4.1. Qualitative human relevance and susceptibility

No data exist that suggest that TCE-induced liver tumorigenesis is caused by processes that are irrelevant in humans. In addition, as discussed above, several of the other effects such as polyploidization, changes in glycogen storage, and inhibition of GST-zeta—are either clearly related to human carcinogenesis or areas of active research as to their potential roles. For example, the effects of DCA on glycogen storage parallel the observation that individuals with conditions that lead to glycogenesis appear to be at an increased risk of liver cancer (Rake et al., 2002; Wideroff et al., 1997; Adami et al., 1996; La Vecchia et al., 1994). In addition, there may be some relationship between the effects of DCA and the mechanism of increased liver tumor risk in childhood in those with Type 1 hereditary tyrosinemia, though the hypotheses needs to be tested experimentally. Similarly, with respect to PPARα activation and downstream events hypothesized to be causally related to liver carcinogenesis, it is generally acknowledged that "a point in the rat/mouse key events cascade where the pathway is biologically precluded in humans cannot be identified, in principle" (NRC, 2006; Klaunig et al., 2003).

In terms of human relevance and susceptibility, it is also useful to briefly review what is known about human HCC. A number of risk factors have been identified for human HCC, including ethanol consumption, hepatitis B and C virus infection, aflatoxin B1 exposure, and, more recently, diabetes and perhaps obesity (El-Serag and Rudolph, 2007). However, it is also estimated that a substantial minority of HCC patients, perhaps 15–50%, have no established risk factors (El-Serag and Rudolph, 2007). In addition, cirrhosis is present in a large proportion of HCC patients, but the prevalence of HCC without underlying cirrhosis, while not precisely known, is still significant, with estimates based on relatively small samples ranging from 7 to 54% (Fattovich et al., 2004).

Table 4-68. Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
Mutagenicity (Section 4.5.7.1)			
One or more oxidative metabolites produced in situ *or delivered systemically to liver.*	*Evidence that TCE or TCE metabolites induces key events:* • Multiple in vitro and in vivo studies demonstrate oxidation of TCE, and availability to the liver (see Section 3.3.2). • CH is a short-lived intermediate that is rapidly converted to TCA and TCOH. *Necessity of key events for carcinogenesis:* • Based on analogy to demonstration that oxidation is necessary for non-cancer effects in the liver. No TCE-specific data.	Yes: demonstrated in humans in vivo and in human cells in vitro.	Known that both human and rodent liver are exposed to the oxidative metabolites. CH is a short-lived intermediate, whereas TCA and TCOH are more stable.
Mutagenicity induced by oxidative metabolites advances acquisition of the multiple critical traits contributing to carcinogenesis.	*Evidence that TCE or TCE metabolites induces key events:* • In rodents, TCE binds to and/or induces damage in DNA and chromosome structure. • TCE has a limited ability to induce mutation in bacterial systems, even with metabolic activation that produce oxidative metabolites. • Oxidative metabolites, particularly CH, can cause a variety of genotoxic effects (including aneuploidy) in available in vitro and in vivo assays (see Section 4.2.1.5). *Necessity of key events for carcinogenesis:* • No TCE-specific data.	Yes: no basis for discounting in vitro or in vivo genotoxicity results.	Evidence for mutagenicity through CH is the strongest, but difficult to assess genotoxic contributions from nongenotoxic contributions from CH and other oxidative metabolites.
Overall Conclusion	*Sufficiency of MOA for carcinogenesis:* • Mutagenicity is assumed to cause cancer, as a sufficient cause.	Yes: well established.	Data are inadequate to support a conclusion that a mutagenic MOA mediated by CH is operant; however, a mutagenic MOA, mediated either by CH or other oxidative metabolites of TCE, cannot be ruled out.

Table 4-68 Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis (continued)

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
Peroxisome proliferation activated receptor alpha activation (Section 4.5.7.2)			
• *TCE oxidative metabolites (e.g., TCA), after being produced in the liver, activate PPARα in the liver.* • *PPARα activation leads to alterations in cell proliferation and apoptosis in the liver.* • *Alterations in cell proliferation and apoptosis causes clonal expansion of initiated cells.* • *Increased number of initiated cells causes cancer.*	*Evidence that TCE or TCE metabolites induces key events:* • Multiple in vitro and in vivo studies demonstrate oxidation of TCE, and availability of metabolites TCA and DCA to the liver (see Section 3.3.2). • TCE, TCA and DCA activate PPARα, induce peroxisome proliferation and hepatocyte proliferation in mice and rats (e.g., DeAngelo et al., 2008; Laughter et al., 2004; Nakajima et al., 2000; Watanabe and Fukui, 2000; Stauber and Bull, 1997; Pereira, 1996; Dees and Travis, 1994; Goel et al., 1992; Sanchez and Bull, 1990; Goldsworthy and Popp, 1987; Elcombe et al., 1985). *Necessity of key events for carcinogenesis:* • No studies of TCE or its metabolites (e.g., cancer bioassays in PPARα-null mice). TCE induces increases in liver weight in male and female mice lacking a functional PPARα receptor (Ramdhan et al., 2010; Nakajima et al., 2000) and in humanized null mice (Ramdhan et al., 2010). Liver tumor response from WY dramatically diminished in PPARα-null mice (Peters et al., 1997); however, liver tumor response from DEHP unchanged in PPARα-null mice (Ito et al., 2007). Thus, inferences regarding TCE are not possible.	Yes. Humans produce oxidative metabolites of TCE, PPARα is present in the human liver.	Highly likely that PPARα is activated in the liver, but it is unlikely that PPARα agonism and its sequelae constitute the sole or predominant MOA for TCE-induced carcinogenesis.
Overall	*Sufficiency of MOA for carcinogenesis:* • No TCE-specific studies; PPARα activation in a transgenic mouse model caused all the key events in the MOA, but not carcinogenesis, suggesting that the MOA is not sufficient for carcinogenesis (Yang et al., 2007). Consistent with hypothesis that TCE liver carcinogenesis involves multiple mechanisms.	Yes. No evidence to suggest that key events are implausible in humans.	

Table 4-68 Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis (continued)

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
Liver weight increases (Section 4.5.7.3.1)			
• *TCE oxidative metabolites, after being produced in the liver, cause liver weight increases.* • *Further key events not specified.*	*Evidence that TCE or TCE metabolites induces key events:* • Multiple in vitro and in vivo studies demonstrate oxidation of TCE, and availability of metabolites TCA and DCA to the liver (see Section 3.3.2). *Necessity of key events for carcinogenesis:* • No studies of TCE or its metabolites. *Sufficiency of MOA for carcinogenesis:* • Hypothesis is inadequately specified for evaluation.	Yes. Humans produce oxidative metabolites of TCE. No evidence that liver weight changes would not occur in humans.	Data are inadequate to define a MOA hypothesis for hepatocarcinogenesis based on liver weight increases.
Negative selection (Section 4.5.7.3.2)			
• *"Negative selection" confers a growth advantage to initiated cells.* • *Increased number of initiated cells causes cancer.*	*Evidence that TCE or TCE metabolites induces key events:* • Transient DNA synthesis is confined to a very small population of cells in mouse liver (e.g., Laughter et al., 2004; Dees and Travis, 1993; Elcombe et al., 1985), but no data on whether this effect is "selective" of initiated cells. *Necessity of key events for carcinogenesis:* • No studies of TCE or its metabolites. *Sufficiency of MOA for carcinogenesis:* • No studies of TCE or its metabolites.	Yes. Humans produce oxidative metabolites of TCE. No evidence that negative selection would not occur in humans.	Data are inadequate to test the MOA hypothesis for hepatocarcinogenesis based on liver weight increases.
Negative selection (Section 4.5.7.3.3)			
• *TCE or its metabolites causes polyploidization of hepatocytes.* • *Increased ploidy is associated with carcinogenesis.*	*Evidence that TCE or TCE metabolites induces key events:* • Polyploidization likely occurs with TCE exposure, although the evidence is limited (Buben and O'Flaherty, 1985). *Necessity of key events for carcinogenesis:* • No studies of TCE or its metabolites. *Sufficiency of MOA for carcinogenesis:* • No studies of TCE or its metabolites.	Yes. Increased ploidy is associated with cancer in humans as well as rodents.	Although it is biologically plausible that polyploidization can contribute to liver carcinogenesis, inadequate data are available to support this hypothesized MOA for TCE.

Table 4-68 Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis (continued)

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
Glycogen storage (Section 4.5.7.3.4)			
• *Increased glycogen storage.* • *Glycogenosis in humans has been associated with increased risk of liver cancer.*	*Evidence that TCE or TCE metabolites induces key events:* • DCA increases glycogen deposition (Nelson et al., 1989) • For TCE and TCA, effects on glycogen were either not reported (Dees and Travis, 1993; Styles et al., 1991; Elcombe et al., 1985) or were described as similar to controls (Nelson et al., 1989). *Necessity of key events for carcinogenesis:* • No studies of TCE or its metabolites. *Sufficiency of MOA for carcinogenesis:* • No studies of TCE or its metabolites.	Yes. No evidence of lack of relevance.	Data are inadequate to define a MOA hypothesis for TCE-induced hepatocarcinogenesis based on changes in glycogen storage, or to support changes in glycogen storage as a result of TCE exposure.
Inactivation of GST-zeta (Section 4.5.7.3.5)			
• *Inactivation of GST-zeta.* • *Hereditary disruption of this pathway in humans has been associated with increased risk of liver cancer, but the active agent has not been identified.*	*Evidence that TCE or TCE metabolites induces key events:* • TCE prolongs DCA half-life in rodents, suggesting that TCE may inhibit GST-zeta, likely through the formation of DCA (Schultz et al., 2002). *Necessity of key events for carcinogenesis:* • No studies of TCE or its metabolites. *Sufficiency of MOA for carcinogenesis:* • No studies of TCE or its metabolites.	Yes. No evidence of lack of relevance.	Data are inadequate to define a MOA hypothesis for TCE-induced hepatocarcinogenesis based on inactivation of GST-zeta.

Table 4-68 Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis (continued)

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
Oxidative stress (Section 4.5.7.3.6)			
• *Oxidative stress.* • *Further key events not specified.*	*Evidence that TCE or TCE metabolites induces key events:* • Measures of oxidative stress induced by TCE, TCA, and DCA either do not occur, or are transient and do not persistent with continued treatment (Channel et al., 1998; Parrish et al., 1996; Larson and Bull, 1992b). *Necessity of key events for carcinogenesis:* • No studies of TCE or its metabolites. *Sufficiency of MOA for carcinogenesis:* • No studies of TCE or its metabolites.	Yes. No evidence of lack of relevance.	Available data are limited to support a role for oxidative stress in TCE-induced liver carcinogenesis.
Epigenetic changes (Section 4.5.7.3.7)			
• *Epigenetic changes, particularly DNA methylation, induced by one or more metabolites (TCA, DCA, and other reactive species) advance acquisition of multiple critical traits contributing to carcinogenesis.*	*Evidence that TCE or TCE metabolites induces key events:* • TCE, TCA and DCA decrease global DNA methylation and promoter hypomethylation (e.g., of c-myc) in mouse liver (Tao et al., 2004a; Ge et al., 2001b; Tao et al., 1998). *Necessity of key events for carcinogenesis:* • No studies of TCE or its metabolites. *Sufficiency of MOA for carcinogenesis:* • No studies of TCE or its metabolites.	Yes. No evidence of lack of relevance.	Although it is biologically plausible that epigenetic changes contribute to liver carcinogenesis, inadequate data are available to support this hypothesized MOA for TCE.

Table 4-68 Summary of mode-of-action conclusions for TCE-induced liver carcinogenesis (continued)

Hypothesized MOA postulated key events	Experimental support	Human relevance	Weight-of-evidence conclusion
Cytotoxicity and reparative hyperplasia(Section 4.5.7.3.8)			
• *One or more reactive intermediates induces hepatotoxicity through cell death.* • *Cell proliferation increases in the liver to repair damage.* • *Increased cell turnover increases the rate of mutations.* • *Increased proliferation cause clonal expansion of initiated (premalignant) cells.* • *Increased number of mutations and/or initiated cells causes cancer.*	*Evidence that TCE or TCE metabolites induces key events:* • TCE treatment at doses relevant to liver carcinogenicity results in relatively low cytotoxicity (Bull, 2004a; Bull et al., 2004). • No evidence that transient increases in DNA synthesis are related to reparative proliferation. *Necessity of key events for carcinogenesis:* • No studies of TCE or its metabolites. *Sufficiency of MOA for carcinogenesis:* • No studies of TCE or its metabolites.	Yes. No evidence of lack of relevance.	It is unlikely that cytotoxicity and reparative hyperplasia play a significant role in TCE carcinogenicity.

However, despite the identification of numerous factors that appear to play a role in the human risk of HCC, the mechanisms are still largely unclear (Yeh et al., 2007). Interestingly, the observation by Leakey et al. (2003a; 2003b) that body weight significantly and strongly impacts background liver tumor rates in B6C3F$_1$ mice parallels the observed epidemiologic associations between liver cancer and obesity (review in El-Serag and Rudolph, 2007). This concordance suggests that similar pathways may be involved in spontaneous liver tumor induction between mice and humans. The extent to which TCE exposure may interact with known risk factors for HCC cannot be determined at this point, but several hypotheses can be posed based on existing data. If TCE affects some of the same pathways involved in human HCC, as suggested in the discussion of several TCE-induced effects above, then TCE exposure may lead a risk that is additive to background.

As discussed above, there are several parallels between the possible key events in TCE-induced liver tumors in mice and what is known about mechanisms of human HCC, though none have been experimentally tested. Altered ploidy distribution and DNA hypomethylation are commonly observed in human HCC (Calvisi et al., 2007; Lin et al., 2003; Zeppa et al., 1998). Interestingly, El-Serag and Rudolph (2007) suggested that the risk of HCC increases with cirrhosis in part because the liver parenchymal cells have decreased proliferative capacity, resulting in an altered milieu that promotes tumor cell proliferation. This description suggests a similarity in mode of action, though via different mechanisms, with the "negative selection" hypothesis proposed by Bull (2000) for TCE and its metabolites although for TCE changes in apoptosis and cell proliferation have not been noted or examined to such an extent to provide evidence of a similar environment. Increased ploidy decreases proliferative capacity, so that may be another mechanism through which the effects of TCE mimic the conditions thought to facilitate the induction of human HCC.

In sum, from the perspective of hazard characterization, the available data support the conclusion that the mode of action for TCE-induced mouse liver tumors is relevant to humans. No data suggest that any of the key events are biologically precluded in humans, and a number of qualitative parallels exist between hypotheses for the mode of action in mice and what is known about the etiology and induction of human HCC. A number of risk factors have been identified that appear to modulate the risk of human HCC, and these may also modulate the susceptibility to the effects from TCE exposure. As noted in Section E.4, TCE exposure in the human population is accompanied not only by external exposures to its metabolites, but brominated analogues of those metabolites that are also rodent carcinogens, a number of chlorinate solvents that are hepatocarcinogenic and alcohol consumption. The types of tumors and the heterogeneity of tumors induced by TCE in rodents parallel those observed in humans (see Section E.3.1.8). The pathways identified for induction of cancer in humans for cancer are similar to those for the induction of liver cancer (see Section E.3.2.1). However, while risk factors have been identified

for human liver cancer that have similarities to TCE-induced effects and those of its metabolites, both the mechanism for human liver cancer induction and that for TCE-induced liver carcinogenesis in rodents are not known.

4.5.7.4.2. Quantitative species differences

As a precursor to the discussion of quantitative differences between humans and rodents and among humans, it should be noted that an adequate explanation for the difference in response for TCE-liver cancer induction between rats and mice has yet to be established or for that difference to be adequately described given the limitations in the rat database. For TCA, there is only one available long-term study in rats that, while suggestive that TCA is less potent in rats than mice, is insufficient to determine if there was a TCA-induced effect or what its magnitude may be. While some have proposed that the lower rate of TCA formation in rats relative to mice would explain the species difference, PBPK modeling suggests that the differences (three–fivefold) may be inadequate to fully explain the differences in carcinogenic potency. Moreover, inferences from comparing the effects of TCE and TCA on liver weight, using PBPK model-based estimates of TCA internal dose-metrics as a result of TCE or TCA administration, indicate that TCA is not likely to play a predominant role in hepatomegaly. Combined with the qualitative correlation between rodent hepatomegaly and hepatocarcinogenesis observed across many chemicals, this suggests that TCA similarly is not a predominant factor in TCE-induced hepatocarcinogenesis. Indeed, there are multiple lines of evidence that TCA is insufficient to account for TCE-induced tumors, including data on tumor phenotype (e.g., c-Jun immunostaining) and genotype (e.g., H-ras mutation frequency and spectrum). For DCA, only a single experiment in rats is available (reported in two publications), and although it suggests lower hepatocarcinogenic potency in rats relative to mice, its relatively low power limits the inferences that can be made as to species differences.

As TCA induces peroxisome proliferation in the mouse and the rat, some have suggested that difference in peroxisomal enzyme induction is responsible for the difference in susceptibility to TCA liver carcinogenesis. The study of DeAngelo et al. (1989) has been cited in the literature as providing evidence of differences between rats and mice for peroxisomal response to TCA. However, data from the most resistant strain of rat (Sprague-Dawley) have been cited in comparisons of peroxisomal enzyme effects but the Osborne-Mendel and F344 rat were not refractory and showed increased PCO activity so it is not correct to state that the rat is refractory to TCA-induction of peroxisome activity (see Section E.2.3.1.5). In addition, as discussed above, inferences based on PCO activity are limited by its high variability, even in control animals, as well as its not necessarily being predictive of the peroxisome number or cytoplasmic volume.

The same assumption of lower species sensitivity by measuring peroxisome proliferation has been applied to humans, as peroxisome proliferation caused by therapeutic PPARα agonists

such as fibrates in humans is generally lower (less than twofold induction) than that observed in rodents (20–50-fold induction). However, as mentioned above, it is known that peroxisome proliferation is not a good predictor of potency (Marsman et al., 1988).

Limited data exist on the relative sensitivity of the occurrence of key events for liver tumor induction between mice and humans and among humans. Pharmacokinetic differences are addressed with PBPK modeling to the extent that data allow, so the discussion here will concentrate on pharmacodynamic differences. Most striking is the difference in ~~–b~~background" rates of liver tumors. Data from NTP indicate that control B6C3F$_1$ mice in 2-year bioassays have a background incidence of HCCs of 26% in males and 10% in females, with higher incidences for combined hepatocellular adenomas and carcinomas (Maronpot, 2007). However, as discussed above, Leakey et al. (2003a; 2003b) report that the background incidence rates are very dependent on the weight of the mice. By contrast, the estimated lifetime risk of liver and biliary tract cancer in the United States (about 75% of which are HCCs) is 0.97% for men and 0.43% for women (Ries et al., 2008). However, regions of the world where additional risk factors (hepatitis infection, alflatoxin exposure) have high prevalence have liver cancer incidences up to more than sixfold greater than the United States (Ferlay et al., 2004). Therefore, one possible quantitative difference that can be flagged for use in dose-response assessment is the background rate of liver tumors between species. Biologically-based dose-response modeling by Chen (2000) suggested that the data were consistent with a purely promotional model in which potency would be proportional to background tumor incidence. However, it is notable that male Swiss mice, which have lower background liver tumor rates than the B6C3F$_1$ strain, were also positive in one long-term bioassay (Maltoni et al., 1988; Maltoni et al., 1986).

Similarly, in terms of intraspecies susceptibility, to the extent that TCE may independently promote pre-existing initiated cells, it can be hypothesized that those with greater risk for developing HCC due to one more of the known risk factors would have a proportional increase in the any contributions from TCE exposure. In addition, in both humans and mice, males appear to be at increased risk of liver cancer, possibly due to sexually dimorphism in inflammatory responses (Lawrence et al., 2007; Naugler et al., 2007; Rakoff-Nahoum and Medzhitov, 2007), suggesting that men may also be more susceptible to TCE-induced liver tumorigenesis than women. It has been observed that human HCC is highly heterogeneous histologically, but within patients and between patients, studies are only beginning to distinguish the different pathways that may be responsible for this heterogeneity (Yeh et al., 2007; Chen et al., 2002b; Feitelson et al., 2002).

Appropriate quantitative data are generally lacking on interspecies differences in the occurrence of most other proposed key events, although many have argued that there are significant quantitative differences between rodents and humans related to PPARα activation (NRC, 2006; Klaunig et al., 2003). For instance, it has been suggested that lower levels of PPARα receptor in human hepatocytes relative to rodent hepatocytes contributes to lower human

sensitivity (Klaunig et al., 2003; Palmer et al., 1998; Tugwood et al., 1996). However, out of a small sample of human livers (n = 6) show similar protein levels to mice (Walgren et al., 2000b). Another proposed species difference has been ligand affinity, but while transactivation assays showed greater affinity of Wy-14643 and perfluorooctanoic acid for rodent relative to human PPARα, they showed TCA and DCA had a similar affinities between species (Maloney and Waxman, 1999). Furthermore, it is not clear that receptor-ligand kinetics (capacity and affinity) are rate-limiting for eliciting hepatocarcinogenic effects, as it is known that maximal receptor occupation is not necessary for a maximal receptor mediated response (Stephenson, 1956) [see also review by Danhof et al. (2007)].

There is also limited in vivo and in vitro data suggesting that increases in cell proliferation mediated by PPARα agonists are diminished in humans and other primates relative to rodents (NRC, 2006; Hoivik et al., 2004; Klaunig et al., 2003). However, Walgren et al. (2000a) reported that TCA and DCA were not mitogenic in either human or rodent hepatocytes in vitro. Furthermore, TCE, TCA, and DCA all induce only transient increases in cell proliferation, so the relevance to TCE of interspecies differences from PPARα agonists that to produce sustained proliferation, such as Wy-14643, is not clear. In addition, comparisons between primate and rodent models should take into account the differences in the ability to respond to any mitogenic stimulation (see Section E.3.2). Primate and human liver respond differently (and much more slowly) to a stimulus such as partial hepatectomy.

Recent studies in "humanized" mice (PPARα-null mice in which a human PPARα gene was subsequently inserted and expressed in the liver) reported that treatment with a PPARα agonist lead to greatly lower incidence of liver tumors as compared to wild-type mice (Morimura et al., 2006). However, these experiments were performed with WY-14643 at a dose causing systemic toxicity (reduced growth and survival), had a duration of <1 year, and involved a limited number of animals. In addition, because liver tumors in mice at <1 year are extremely rare, the finding a one adenoma in WY-14643-treated humanized mice suggests carcinogenic potential that could be further realized with continued treatment (Keshava and Caldwell, 2006). In addition, Yang et al. (2007) recently noted that let-7C, a microRNA involved in cell growth and thought to be a regulatory target of PPARα (Shah et al., 2007), was inhibited by Wy-14643 in wild-type mice, but not in "humanized mice" in which human PPARα was expressed throughout the body on a PPARα-null background. However, these humanized mice had about a 20-fold higher baseline expression of let-7C, as reported in control mice, potentially masking any treatment effects. More generally, it is not known to what extent PPARα-related events are rate-limiting in TCE-induced liver tumorigenesis, for which multiple pathways appear to be operative. So even if quantitative differences mediated by PPARα were well estimated, they would not be directly usable for dose-response assessment in the absence of way to integrate the contributions from the different pathways.

In sum, the only quantitative data and inter- and intraspecies susceptibility suitable for consideration in dose-response assessment are differences background liver tumor risk. These may modulate the effects of TCE if RR, rather than additional risk, is the appropriate common inter- and intraspecies metric. However, the extent to which RR would provide a more accurate estimate of human risk is unknown.

4.6. IMMUNOTOXICITY AND CANCERS OF THE IMMUNE SYSTEM

Chemical exposures may result in a variety of adverse immune-related effects, including immunosuppression (decreased host resistance), autoimmunity, and allergy-hypersensitivity, and may result in specific diseases such as infections, systemic or organ-specific autoimmune diseases, or asthma. Cell-mediated immune response, such as activation of macrophages, natural killer (NK) cells, and cytokine production, can also influence a broader range of diseases, such as cancer. Measures of immune function (e.g., T-cell counts, immunoglobulin [Ig] E levels, specific autoantibodies, cytokine levels) may provide evidence of an altered immune response that precedes the development of clinically expressed diseases. The first section of this section discusses effects relating to immunotoxicity, including risk of autoimmune diseases, allergy and hypersensitivity, measures of altered immune response, and lymphoid cancers. Studies pertaining to effects in humans are presented first, followed by a section discussing relevant studies in animals. The second section of this section discusses evidence pertaining to TCE in relation to lymphoid tissue cancers, including childhood leukemia.

4.6.1. Human Studies

4.6.1.1. Noncancer Immune-Related Effects

4.6.1.1.1. Immunosuppression, asthma, and allergies

In 1982, Lagakos et al. conducted a telephone survey of residents of Woburn, Massachusetts, collecting information on residential history and history of 14 types of medically diagnosed conditions (Lagakos et al., 1986). The survey included 4,978 children born since 1960 who lived in Woburn before age 19. Completed surveys were obtained from approximately 57% of the town residences with listed phone numbers. Two of the wells providing the town's water supply from 1964 to 1979 had been found to be contaminated with a number of solvents, including tetrachloroethylene (21 ppb) and TCE (267 ppb) (as cited in Lagakos et al., 1986). Lagakos et al. (Lagakos et al., 1986) used information from a study by the Massachusetts Department of Environmental Quality and Engineering to estimate the contribution of water from the two contaminated wells to the residence of each participant, based on zones within the town receiving different mixtures of water from various wells, for the period in which the contaminated wells were operating. This exposure information was used to estimate a cumulative exposure based on each child's length of residence in Woburn. A higher cumulative exposure measure was associated with conditions indicative of immunosuppression (e.g., bacterial or viral infections) or hypersensitivity (e.g., asthma). In contrast, a recent study using the National Health and Nutrition Examination Survey data collected from 1999 to 2000 in a representative sample of the U.S. population (n = 550) did not find an association between TCE exposure and self-report of a history of physician-diagnosed asthma (OR: 0.94, 95% CI: 0.77, 1.14) (Arif and Shah, 2007). TCE exposure, as well as exposure to nine other VOCs, was determined through a passive monitor covering a period of 48–72 hours. No clear trend was

seen with self-reported wheeze episodes (OR: 1.29, 95% CI: 0.98, 1.68 for one to two episodes; OR: 0.21, 95% CI: 0.04, 10.05 for three or more episodes in the past 12 months).

Allergy and hypersensitivity, as assessed with measures of immune system parameters or immune function tests (e.g., atopy) in humans, have not been extensively studied with respect to the effects of TCE (see Table 4-69). Lehmann et al. reported data pertaining to immunoglobulin E (IgE) levels and response to specific antigens in relation to indoor levels of VOCs among children (age 36 months) selected from a birth cohort study in Leipzig, Germany (Lehmann et al., 2001). Enrollment into the birth cohort occurred between 1995 and 1996. The children in this allergy study represent a higher-risk group for development of allergic disease, with eligibility criteria that were based on low birth weight (between 1,500 and 2,500 g), or cord blood IgE >0.9 kU/L with double positive family history of atopy. These eligibility criteria were met by 429 children; 200 of these children participated in the allergy study described below, but complete data (IgE and VOC measurements) were available for only 121 of the study participants. Lehmann et al. (2001) measured 26 VOCs via passive indoor sampling in the child‘s bedroom for a period of 4 weeks around the age of 36 months. The median exposure of TCE was 0.42 $\mu g/m^3$ (0.17 and 0.87 $\mu g/m^3$ for the 25^{th} and 75^{th} percentiles, respectively). Blood samples were taken at the 36-month study examination and were used to measure the total IgE and specific IgE antibodies directed to egg white, milk, indoor allergens (house dust mites, cats, and molds), and outdoor allergens (timothy-perennial grass and birch trees). There was no association between TCE exposure and any of the allergens tested in this study, although some of the other VOCs (e.g., toluene, 4-ethyltoluene) were associated with elevated total IgE levels and with sensitization to milk or eggs.

Table 4-69. Studies of immune parameters (IgE antibodies and cytokines) and TCE in humans

Parameter, source of data	Results	Reference, location, diagnosis period, sample size, age
IgE antibodies blood sample, indoor air sampling of 28 volatile organic chemicals in child's bedroom	TCE exposure not associated with sensitization to indoor or outdoor allergens	Lehmann et al. (2001) Germany. 1997–1999. n = 121 36-mo old children
Cytokine secreting CD3+ T-cell populations cord blood, indoor air sampling of 28 volatile organic chemicals in child's bedroom 4 wks after birth	In CD3+ cord blood cells, some evidence of association between increasing TCE levels and decreased IL-4 >75th percentile OR: 0.6 (95% CI: 0.2, 2.1), <25th percentile OR 4.4 (95% CI: 1.1, 17.8) increased IFN-γ >75th percentile OR: 3.6 (95% CI: 0.9, 14.9) <25th percentile OR: 0.7 (95% CI: 0.2, 2.2) Similar trends not seen with tumor necrosis factor-α or IL-2	Lehmann et al. (2002) Germany. 1995–1996. n = 85 newborns
Cytokine secreting CD3+ and CD8+ T-cell populations blood sample, indoor air sampling of 28 volatile organic chemicals in child's bedroom	TCE exposure not associated with percentages of IL-4 CD3+ or IFN-γ CD8+ T-cells	Lehmann et al. (2001) Germany. 1995–1999. n = 200 36-mo old children
Cytokine concentration—serum urine sample (TCA concentration), blood sample, questionnaire (smoking history, age, residence), workplace TCE measures (personal samples, four exposed and four nonexposed workers)	Nonexposed workers similar to office controls for all cytokine measures. Compared to nonexposed workers, the TCE exposed workers had decreased IL-4 (mean 3.9 vs. 8.1 pg/mL) increased IL-2 (mean 798 vs. 706 pg/mL) increased IFN-γ (mean 37.1 vs. 22.9 pg/mL)	Iavicoli et al. (2005) Italy. n = 35 printers using TCE, 30 nonexposed workers (in same factory, did not use or were not near TCE), 40 office worker controls. All men. Mean age ~33 yrs

IFN = interferon; IL = interleukin

4.6.1.1.2. Generalized hypersensitivity skin diseases, with or without hepatitis

Occupational exposure to TCE has been associated with a severe, generalized skin disorder that is distinct from contact dermatitis in the clinical presentation of the skin disease (which often involves mucosal lesions), and in the accompanying systemic effects that can include lymphadenopathy, hepatitis, and other organ involvement. Kamijima et al. (2007) recently reviewed case reports describing 260 patients with TCE-related generalized skin disorders (Kamijima et al., 2007). Six of the patients were from the United States or Europe, with the remainder occurring in China, Singapore, Philippines, and other Asian countries. One study in Guangdong province, in southeastern China, included >100 of these cases in a single year (Huang et al., 2002). Kamijima et al. (2007) categorized the case descriptions as indicative of hypersensitivity syndrome (n = 124) or a variation of erythema multiforme, Stevens-Johnson syndrome, and toxic epiderma necrolysis (n = 115), with 21 other cases unclassified in either category. The fatality rate, approximately 10%, was similar in the two groups, but the prevalence of fever and lymphadenopathy was higher in the hypersensitivity syndrome patients. Hepatitis was seen in 92–94% of the multiforme, Stevens-Johnson syndrome, and toxic epiderma necrolysis patients, but the estimates within the hypersensitivity syndrome group were more variable (46–94%) (Kamijima et al., 2007).

Some of the case reports reviewed by Kamijima et al. (2007) provided information on the total number of exposed workers, working conditions, and measures of exposure levels. From the available data, generalized skin disease within a worksite occurred in 0.25–13% of workers in the same location, doing the same type of work (Kamijima et al., 2007). The measured concentration of TCE ranged from <50 to >4,000 mg/m^3, and exposure scenarios included inhalation only and inhalation with dermal exposures. Disease manifestation generally occurred within 2–5 weeks of initial exposure, with some intervals up to 3 months. Most of the reports were published since 1995, and the geographical distribution of cases reflects the newly industrializing areas within Asia.

Kamijima and colleagues recently conducted an analysis of urinary measures of TCE metabolites (TCA and TCOH) in 25 workers hospitalized for hypersensitivity skin disease in 2002 (Kamijima et al., 2008). Samples taken within 15 days of the last exposure to TCE exposure were available for 19 of the 25 patients, with a mean time of 8.4 days. Samples from the other patients were not used in the analysis because the half-life of U-TCA is 50–100 hours. In addition, 3–6 healthy workers doing the same type of work in the factories of the affected worker, and 2 control workers in other factories not exposed to TCE were recruited in 2002–2003 for a study of breathing zone concentration of volatile organochlorines and urinary measures of TCE metabolites. Worksite measures of TCE concentration were also obtained. Adjusting for time between exposure and sample collection, mean urinary concentration at the time of last exposure among the 19 patients was 206 mg/mL for TCA. Estimates for TCOH were not presented because of the shorter half-life for this compound. U-TCA levels in the

healthy exposed workers varied among the 4 factories, with means (± SDs) of 41.6 (± 18.0), 131 (±90.2), 180 (±92), and 395 (±684). The lower values were found in a factory in which the degreasing machine had been partitioned from the workers after the illnesses had occurred. TCE concentrations (personal TWAs) at the factories of the affected workers ranged from 164 to 2,330 mg/m^3 (30–431 ppm). At the two factories with no affected workers in the past 3 years, the mean personal TWA TCE concentrations were 44.9 mg/m^3 (14 ppm) and 1,803 mg/m^3 (334 ppm). There was no commonality of additives or impurities detected among the affected factories that could explain the occurrence of the hypersensitivity disorder.

To examine genetic influences on disease risk, Dai et al. (2004) conducted a case-control study of 111 patients with TCE-related severe generalized dermatitis and 152 TCE-exposed workers who did not develop this disease. Patients were recruited from May 1999 to November 2003 in Guangdong Province, and were employed in approximately 80 electronic and metal-plating manufacturing plants. Initial symptoms occurred within 3 months of exposure. The comparison group was drawn from the same plants as the cases, and had worked for >3 months without development of skin or other symptoms. Mean age in both groups was approximately 23 years. A blood sample was obtained from study participants for genotyping of TNF-α, TNF-β, and interleukin (IL)-4 genotypes. The genes were selected based on the role of TNF and of IL-4 in hypersensitivity and inflammatory responses. The specific analyses included two polymorphisms in the promoter region of TNF-α (G → A substitution at position –308); and a G → A substitution at position –238), a polymorphism at the first intron on TNF-β, and a polymorphism in the promoter region of IL-4 (C → T substitution at –590). There was no difference in the frequency of the TNF-α^{-238}, TNF-β, or IL-4 polymorphisms between cases and controls, but the wild-type TNF-α^{-308} genotype was somewhat more common among cases (94% in cases and 86% in controls).

Kamijima et al. (2007) note the similarities, particular with respect to specific skin manifestations, of the case presentations of TCE-related generalized skin diseases to conditions that have been linked to specific medications (e.g., carbamazepine, allopurinol, antibacterial sulfonamides), possibly in conjunction with reactivation of specific latent herpes viruses. A previous review by these investigators discussed insights with respect to drug metabolism that may be useful in developing hypotheses regarding susceptibility to TCE-related generalized skin disorders (Nakajima et al., 2003). Based on consideration of metabolic pathways and intermediaries, variability in CYP2E1, UDP-glucoronyltransferase, GST, and N-acetyl transferase (NAT) activities could be hypothesized to affect the toxicity of TCE. NAT2 is most highly expressed in liver, and the "slow" acetylation phenotype (which arises from a specific mutation) has been associated with adverse effects of medications, including drug-induced lupus (Lemke and McQueen, 1995) and hypersensitivity reactions (Spielberg, 1996). There are limited data pertaining to genetic or other sources of variability in these enzymes on risk of TCE-related generalized skin diseases, however. In a study in Guangdong province, CYP1A1, GSTM1,

GSTP1, GSTT1, and NAT2 genotypes in 43 cases of TCE-related generalized skin disease were compared to 43 healthy TCE-exposed workers (Huang et al., 2002). The authors reported that the NAT2 slow acetylation genotype was associated with disease, but the data pertaining to this finding were not presented.

4.6.1.1.3. Cytokine profiles and lymphocyte subsets

Cytokines are produced by many of the immune regulatory cells (e.g., macrophages, dendritic cells), and have many different effects on the immune system. The T-helper Type 1 (Th1) cytokines, are characterized as "pro-inflammatory" cytokines, and include TNF-α and interferon (IFN)-γ. Although this is a necessary and important part of the innate immune response to foreign antigens, an aberrant pro-inflammatory response may result in a chronic inflammatory condition and contribute to development of scarring or fibrotic tissue, as well as to autoimmune diseases. Th2 cytokines are important regulators of humoral (antibody-related) immunity. IL-4 stimulates production of IgE and thus influences IgE-mediated effects such as allergy, atopy, and asthma. Th2 cytokines can also act as "brakes" on the inflammatory response, so the balance between different types of cytokine production is also important with respect to risk of conditions resulting from chronic inflammation. Several studies have examined cytokine profiles in relation to occupational or environmental TCE exposure (see Table 4-69).

The Lehmann et al. (2001) study of 36-month-old children (described above) also included a blood sample taken at the 3-year study visit, which was used to determine the percentages of specific cytokine producing T-cells in relation to the indoor VOCs exposures measured at birth. There was no association between TCE exposure and either IL-4 CD3+ or IFN-γ CD8+ T-cells (Lehmann et al., 2001).

Another study by Lehmann et al. (2002) examined the relationship between indoor exposures to VOCs and T-cell subpopulations measured in cord blood of newborns. The study authors randomly selected 85 newborns (43 boys and 42 girls) from a larger cohort study of 997 healthy, full-term babies, recruited between 1997 and 1999 in Germany. Exclusion criteria included a history in the mother of an autoimmune disease or infectious disease during the pregnancy. Twenty-eight VOCs were measured via passive indoor sampling in the child's bedroom for a period of 4 weeks after birth (a period that is likely to reflect the exposures during the prenatal period close to the time of delivery). The levels were generally similar or slightly higher than the levels seen in the previous study using samples from the bedrooms of the 36-month-old children. The highest levels of exposure were seen for limonene (median 24.3 $\mu g/m^3$), α-pinene (median 19.3 $\mu g/m^3$), and toluene (median 18.3 $\mu g/m^3$), and the median exposure of TCE was 0.6 $\mu g/m^3$ (0.2 and 1.0 $\mu g/m^3$ for the 25th and 75th percentiles, respectively). Flow cytometry was used to measure the presence of CD3 T-cells obtained from the cord blood labeled with antibodies against IFN-γ, tumor necrosis factor-α, IL-2, and IL-4. There was some evidence of a decreased level of IL-2 with higher TCE exposure in the

univariate analysis, with median percentage of IL-2 cells of 46.1 and 33.0% in the groups that were below the 75th percentile and above the 75th percentile of TCE exposure, respectively. In analyses adjusting for family history of atopy, gender, and smoking history of the mother during pregnancy, there was little evidence of an association with either IL-2 or IFN-γ, but there was a trend of increasing TCE levels associated with decreased IL-4 and increased IFN-γ.

Iavicoli et al. (2005) examined cytokine levels in 35 TCE-exposed workers (Group A) from a printing area of a factory in Italy. Their work involved use of TCE in degreasing. Two comparison groups were included. Group B consisted of 30 other factory workers who were not involved in degreasing activities and did not work near this location, and Group C consisted of 40 office workers at the factory. All study participants were male and had worked at their present position for at least 3 years, and all were considered healthy. Personal breathing zone air samples from four workers in Group A and four workers in Group B were obtained in three consecutive shifts (24 total samples) to determine air concentration of TCE. A urine sample was obtained from each Group A and Group B worker (end of shift at end of work week) for determination of TCA concentrations (corrected for creatinine), and blood samples were collected for assessment of IL-2, IL-4, and IFN-γ concentrations in serum using enzyme-linked immunosorbent assays. Among exposed workers, the mean TCE concentration was approximately 35 mg/m^3 (30.75 ± SD 9.9, 37.75 ± 23.0, and 36.5 ± 8.2 mg/m^3 in the morning, evening, and night shifts, respectively). The U-TCA concentrations were much higher in exposed workers compared with nonexposed workers (mean ± SD, Group A 13.3 ± 5.9 mg/g creatinine; Group B 0.02 ± 0.02 mg/g creatinine). There was no difference in cytokine levels between the two control groups, but the exposed workers differed significantly (all *p*-values < 0.01 using Dunnett's test for multiple comparisons) from each of the two comparison groups. The observed differences were a decrease in IL-4 levels (mean 3.9, 8.1, and 8.1 pg/mL for Groups A, B, and C, respectively), and an increase in IL-2 levels (mean 798, 706, and 730 pg/mL for Groups A, B, and C, respectively) and in IFN-γ levels (mean 37.1, 22.9, and 22.8 pg/mL for Groups A, B, and C, respectively).

The available data from these studies (Iavicoli et al., 2005; Lehmann et al., 2002; Lehmann et al., 2001) provide some evidence of an association between increased TCE exposure and modulation of immune response involving an increase in pro-inflammatory cytokines (IL-2, IFN-γ) and a decrease in Th2 (allergy-related) cytokines (e.g., IL-4). These observations add support to the influence of TCE in immune-related conditions affected by chronic inflammation.

Lan et al. (2010) examined lymphocyte subsets among 80 TCE-exposed workers and 96 controls in Guangdong, China. Six factories using TCE for cleaning metals, optical lenses, or circuit boards were included in this study. These factories did not use other solvents (benzene, styrene, ethylene oxide, formaldehyde, or epichlorohydrin), based on an exposure screening using Dräger tubes and 3M Badges. Eighty workers from these factories and 96 unexposed controls (frequency matched by sex and 5-year age groups to controls) from clothes

manufacturers, a food production factory, and a hospital, were included in the study. The study was conducted in 2006. Study participants provided a blood sample, buccal cells, postshift and overnight urine samples, and completed a questionnaire with demographic, alcohol and smoking history, and occupational history data. A blood sample was used for a complete blood count and differential lymphocyte subset analysis. At the time of the blood draw, a clinical examination, including measurement of height and weight, and symptoms of recent respiratory infection (which could affect the differential blood cell counts) was conducted. TCE monitoring was conducted using full-shift personal air exposure measurements. The median level of exposure, based on the mean of two measurements taken for each participant in the month before the blood draw, among the 80 TCE-exposed workers was 12 ppm. The analysis used this level to categorize workers into high (≥12 ppm; mean 38 ppm) and low (<12 ppm; mean 5 ppm) exposures. Among the controls, the mean TCE exposure was <0.03 ppm. The total number of lymphocytes, T cells, CD4+ T cells, CD8+ T cells, B cells and NK cells was significantly lower among TCE-exposed workers compared with controls, with the largest decrease seen in the higher exposure group. For example, the age- and sex-adjusted lymphocyte count was 2,154, 2,012, and 1,671 cells/μL blood in the controls, <12 and ≥12 ppm groups, respectively (trend p = <0.0001). Plasma concentrations of soluble CD27 and CD30, two costimulators involved in the regulation of T cells, were also decreased in both exposure groups compared with controls. Similar patterns were seen when limited to the 77 workers with exposure levels <100 ppm, and when limited to the 60 workers with exposure levels <25 ppm. Granulocytes, monocytes and platelet counts did not differ by exposure. The authors noted that the immunosuppression and decreased lymphocyte activation seen in this study provide support the biological plausibility of a role of TCE exposure in NHL.

4.6.1.1.4. Autoimmune disease

4.6.1.1.4.1. Disease clusters and geographic-based studies

Reported clusters of diseases have stimulated interest in environmental influences on systemic autoimmune diseases. These descriptions include investigations into reported clusters of systemic lupus erythematosus (Dahlgren et al., 2007; Balluz et al., 2001) and Wegener granulomatosis (Albert et al., 2005). Wegener granulomatosis, an autoimmune disease involving small vessel vasculitis, usually with lung or kidney involvement, is a very rare condition, with an incidence rate of 3–14 per million per year (Mahr et al., 2006). TCE was one of several groundwater contaminants identified in a recent study investigating a cluster of seven cases of Wegener granulomatosis around Dublin, Pennsylvania. Because of the multiple contaminants, it is difficult to attribute the apparent disease cluster to any one exposure.

In addition to the study of asthma and infectious disease history among residents of Woburn, Massachusetts (Lagakos et al., 1986) (see Section 4.6.1.1.1), Byers et al. (1988) provided data pertaining to immune function from 23 family members of leukemia patients in

Woburn, Massachusetts. Serum samples were collected in May and June of 1984 and in November of 1985 (several years after 1979, when the contaminated wells had been closed). Total lymphocyte counts and lymphocyte subpopulations (CD3, CD4, and CD8) and the CD4/CD8 ratio were determined in these samples, and in samples from a combined control group of 30 laboratory workers and 40 residents of Boston selected through a randomized probability area sampling process. The study authors also assessed the presence of antinuclear antibodies (ANA) or other autoantibodies (antismooth muscle, antiovarian, antithyroglobulin, and antimicrosomal antibodies) in the family member samples and compared the results with laboratory reference values. The age distribution of the control group, and stratified analyses by age, are not provided. The lymphocyte subpopulations (CD3, CD4, and CD8) were higher and the CD4/CD8 ratio was lower in the Woburn family members compared to the controls in both of the samples taken in 1984. In the 1985 samples, however, the lymphocyte subpopulation levels had decreased and the CD4/CD8 ratio had increased; the values were no longer statistically different from the controls. None of the family member serum samples had antithyroglobulin or antimicrosomal antibodies, but 10 family-member serum samples (43%) had ANA (compared to <5% expected based on the reference value). Because the initial blood sample was taken in 1984, it is not possible to determine the patterns at a time nearer to the time of the exposure. The co-exposures that occurred also make it difficult to infer the exact role of TCE in any alterations of the immunologic parameters.

Kilburn and Warshaw (1992a) reported data from a study of contamination by metal-cleaning solvents (primarily TCE) and heavy metals (e.g., chromium) of the aquifer of the Santa Cruz River in Tucson, Arizona (1992a). Exposure concentrations >5 ppb (6–500 ppb) had been documented in some of the wells in this area. A study of neurological effects was undertaken between 1986 and 1989 (Kilburn and Warshaw, 1993b), and two of the groups within this larger study were also included in a study of symptoms relating to systemic lupus erythematosus. Residents of Tucson (n = 362) were compared to residents of southwest Arizona (n = 158) recruited through a Catholic parish. The Tucson residents were selected from the neighborhoods with documented water contamination (>5 ppb TCE for at least 1 year between 1957 and 1981). Details of the recruitment strategy are not clearly described, but the process included recruitment of patients with lupus or other rheumatic diseases (Kilburn and Warshaw, 1993b, 1992a). The prevalence of some self-reported symptoms (malar rash, arthritis/arthralgias, Raynaud syndrome, skin lesions, and seizure or convulsion) was significantly higher in Tucson, but there was little difference between the groups in the prevalence of oral ulcers, anemia, low white blood count or low platelet count, pleurisy, alopecia, or proteinuria. The total number of symptoms reported was higher in Tucson than in the other southwest Arizona residents (14.3 vs. 6.4% reported four or more symptoms, respectively). Low-titer (1:80) ANA were seen in 10.6 and 4.7% of the Tucson and other Arizona residents, respectively ($p = 0.013$). However, since part of the Tucson

study group was specifically recruited based on the presence of rheumatic diseases, it is difficult to interpret these results.

4.6.1.1.4.2. Case-control studies

Interest in the role of organic solvents, including TCE, in autoimmune diseases was spurred by the observation of a scleroderma-like disease characterized by skin thickening, Raynaud's phenomenon, and acroosteolysis and pulmonary involvement in workers exposed to vinyl chloride (Gama and Meira, 1978). A case report in 1987 described the occurrence of a severe and rapidly progressive case of systemic sclerosis in a 47-year-old woman who had cleaned X-ray tubes in a tank of TCE for approximately 2.5 hours (Lockey et al., 1987).

One of the major impediments to autoimmune disease research is the lack of disease registries, which makes it difficult to identify incident cases of specific diseases. There are no cohort studies of the incidence of autoimmune diseases in workers exposed to TCE. Most of the epidemiologic studies of solvents and autoimmune disease rely on general measures of occupational exposures to solvents, organic solvents, or chlorinated solvents exposures. A two- to threefold increased risk of systemic sclerosis (scleroderma) (Maitre et al., 2004; Garabrant et al., 2003; Aryal et al., 2001), rheumatoid arthritis (Sverdrup et al., 2005; Lundberg et al., 1994), undifferentiated connective tissue disease (Lacey et al., 1999), and antineutrophil-cytoplasmic antibody (ANCA)-related vasculitis (Beaudreuil et al., 2005; Lane et al., 2003) has generally been seen in these studies, but there was little evidence of an association between solvent exposure and systemic lupus erythematosus in two recent case-control studies (Finckh et al., 2006; Cooper et al., 2004).

Two case-control studies of scleroderma (Bovenzi et al., 2004; Maitre et al., 2004) and two of rheumatoid arthritis (Olsson et al., 2004; Olsson et al., 2000) provide data concerning solvent exposure that occurred among metal workers or in jobs that involved cleaning metal (i.e., types of jobs that were likely to use TCE as a solvent). There was a twofold increased risk among male workers in the two studies of rheumatoid arthritis from Sweden (Olsson et al., 2004; Olsson et al., 2000). The results from the smaller studies of scleroderma were more variable, with no exposed cases seen in one study with 93 cases and 206 controls (Maitre et al., 2004), and an OR of 5.2 (95% CI: 0.7, 37) seen in a study with 56 cases and 171 controls (Bovenzi et al., 2004).

Five other case-control studies provide data specifically about TCE exposure, based on industrial hygienist review of job history data (see Table 4-70). Three of these studies are of scleroderma (Garabrant et al., 2003; Diot et al., 2002; Nietert et al., 1998), one is of undifferentiated connective tissue disease (Lacey et al., 1999), and one is of small vessel vasculitidies involving ANCAs (Beaudreuil et al., 2005).

These studies included some kind of expert review of job histories, but only two studies included a quantification of exposure (e.g., a cumulative exposure metric, or a "high" exposure

group) (Diot et al., 2002; Nietert et al., 1998). Most of the studies present data stratified by sex, and as expected, the prevalence of exposure (either based on type of job or on industrial hygienist assessment) is considerably lower in women compared with men. In men, the studies generally reported ORs between 2.0 and 8.0, and in women, the ORs were between 1.0 and 2.0. The incidence rate of scleroderma in the general population is approximately 5–10 times higher in women compared with men, which may make it easier to detect large RRs in men.

The EPA conducted a meta-analysis of the three scleroderma studies with specific measures of TCE (Garabrant et al., 2003; Diot et al., 2002; Nietert et al., 1998), examining separate estimates for males and for females. The resulting combined estimate for "any" exposure, using a random effects model to include the possibility of nonrandom error between studies (DerSimonian and Laird, 1986), was OR: 2.5 (95% CI: 1.1, 5.4) for men and OR: 1.2 (95% CI: 0.58, 2.6) in women. (Because the "any" exposure variable was not included in the published report, Dr. Paul Nietert provided the EPA with a new analysis with these results, e-mail communication from Paul Nietert to Glinda Cooper, November 28, 2007.)

Specific genes may influence the risk of developing autoimmune diseases, and genes involving immune response (e.g., cytokines, major histocompatibility complex, B- and T-cell activation) have been the focus of research pertaining to the etiology of specific diseases. The metabolism of specific chemical exposures may also be involved (Cooper et al., 1999). Povey et al. (2001) examined polymorphisms of two CYP genes, CYP2E1 and CYP2C19, in relation to solvent exposure and risk of developing scleroderma. These specific genes were examined because of their hypothesized role in metabolism of many solvents, including TCE. Seven scleroderma patients who reported a history of solvent exposure were compared to 71 scleroderma patients with no history of solvent exposure and to 106 population-based controls. The CYP2E1*3 allele and the CYP2E1*4 allele were more common in the seven solvent-exposed patients (each seen in two of the seven patients; 29%) than in either of the comparison groups (approximately 5% for CYP2E1*3 and 14% for CYP2E1*4). The authors present these results as observations that require a larger study for corroboration and further elucidation of specific interactions.

Table 4-70. Case-control studies of autoimmune diseases with measures of TCE exposure

Disease, source of data	Results: exposure prevalence, OR, 95% CI	Reference, location, sample size, age
Scleroderma		
Structured interview (specific jobs and materials; jobs held ≥1 yrs). Exposure classified by self-report and by expert review (JEM).	Men Maximum intensity 30% cases, 10% controls; OR: 3.3 (95% CI: 1.0, 10.3) Cumulative intensity 32% cases, 21% controls; OR: 2.0 (95% CI: 0.7, 5.3) Maximum probability 16% cases, 3% controls; OR: 5.1 (95% CI: not calculated) Women Maximum intensity 6% cases, 7% controls; OR: 0.9 (95% CI: 0.3, 2.3) Cumulative intensity 10% cases, 9% controls; OR: 1.2 (95% CI: 0.5, 2.6) Maximum probability 4% cases, 5% controls; OR: 0.7 (95% CI: 0.2, 2.2)	Nietert et al. (1998) South Carolina. Prevalent cases, 178 cases (141 women, 37 men), 200 hospital-based controls. Mean age at onset 45.2 yrs
Structured interview (specific jobs and materials; jobs held ≥6 mo). Exposure classified by expert review.	Men and women Any exposure: cases 16%, controls 8%; OR: 2.4 (95% CI: 1.0, 5.4) High exposure:[a] cases 9%, controls 1%; OR: 7.6 (95% CI: 1.5, 37.4) Men Any exposure: cases 64%, controls 27%; OR: 4.7 (95% CI: 0.99, 22.0) Women Any exposure: cases 9%, controls 4%; OR: 2.1 (95% CI: 0.65, 6.8)	Diot et al. (2002) France. Prevalent cases, 80 cases (69 women, 11 men), 160 hospital controls. Mean age at diagnosis 48 yrs
Structured interview (specific jobs and materials; jobs held ≥3 mo). Exposure classified by self-report and by expert review.	Women Self report: cases 1.3%, controls 0.7%; OR: 2.0 (95% CI: 0.8, 4.8) Expert review: cases 0.7%, controls 0.4%; OR: 1.9 (95% CI: 0.6, 6.6)	Garabrant et al. (2003) Michigan and Ohio. Prevalent cases, 660 cases (all women), 2,227 population controls.[b] Ages 18 and older
Undifferentiated connective tissue disease		
Structured interview (specific jobs and materials; jobs held ≥3 mo). Exposure classified by self-report and by expert review.	Women Self report: cases 0.5%, controls 0.7%; OR: 0.88 (95% CI: 0.11, 6.95) Expert review: cases 0.5%, controls 0.4%; OR: 1.67 (95% CI: 0.19, 14.9)	Lacey et al. (1999), Michigan and Ohio. Prevalent cases, 205 cases (all women), 2,095 population controls. Ages 18 and older
ANCA-related diseases[c]		
Structured interview (specific jobs and materials; jobs held ≥6 mo). Exposure classified by expert review.	Men and women (data not presented separately by sex) cases 18.3%, controls 17.5%; OR: 1.1 (95% CI: 0.5, 2.4)	Beaudreuil et al. (2005) France. Incident cases, 60 cases (~50% women), 120 hospital controls. Mean age 61 yrs

[a]Cumulative exposure defined as product of probability × intensity × frequency × duration scores, summed across all jobs; scores of >1 classified as "high."
[b]Total n; n with TCE data: self -report 606 cases, 2,138 control; expert review 606 cases, 2,137 controls.
[c]Diseases included Wegener glomerulonephritis (n = 20), microscopic polyangiitis (n = 8), pauci-immune glomerulonephritis (n = 10), uveitis (n = 6), Churg-Strauss syndrome (n = 4), stroke (n = 4), and other diseases (no more than 2 each).

4.6.1.2. Cancers of the Immune System, Including Childhood Leukemia

4.6.1.2.1. Description of studies

Human studies have reported cancers of the immune system resulting from TCE exposure. Lymphoid tissue neoplasms arise in the immune system and result from events that occur within immature lymphoid cells in the bone marrow or peripheral blood (leukemias), or more mature cells in the peripheral organs (NHL). As such, the distinction between lymphoid leukemia and NHL is largely distributional with overlapping entities, such that a particular lymphoid neoplasm may manifest both lymphomatous and leukemic features during the course of the disease (Weisenburger, 1992). The broad category of lymphomas can be divided into specific types of cancers, including NHL, Hodgkin lymphoma, multiple myeloma, and various types of leukemia (e.g., acute and chronic forms of lymphoblastic and myeloid leukemia). The classification criteria for these cancers has changed over the past 30 years, reflecting improved understanding of the underlying stem cell origins of these specific subtypes. Lymphomas are grouped according to the World Health Organization (WHO) classification as B-cell neoplasms, T-cell/NK-cell neoplasms, and Hodgkin lymphoma, formerly known as Hodgkin disease (Harris et al., 2000). For example, hairy cell leukemia, CLL, NHL, and multiple myeloma may arise from mature B cells and are types of NHLs according to the WHO‘s lymphoma classification system (Morton et al., 2007, 2006). Most of the studies of TCE exposure evaluate NHL defined as lymphosarcoma, reticulum-cell sarcoma, and other lymphoid tissue neoplasms with recently published studies reporting on total B-cell or specific B-cell neoplasms.

Numerous studies are found in the published literature on NHL and either broad exposure categories or occupational title. The NHL studies generally report positive associations with organic solvents or job title as aircraft mechanic, metal cleaner or machine tool operator, and printers, although associations are not observed consistently across all studies, specific solvents are not identified, and different lymphoma classifications are adopted (Cocco et al., 2010; Orsi et al., 2010; Schenk et al., 2009; Wang et al., 2009; 't Mannetje et al., 2008; Karunanayake et al., 2008; Richardson et al., 2008; Alexander et al., 2007b; Boffetta and de Vocht, 2007; Seidler et al., 2007; Vineis et al., 2007; Dryver et al., 2004; Chiu and Weisenburger, 2003; Lynge et al., 1997; Tatham et al., 1997; Figgs et al., 1995; Blair et al., 1993). A major use of TCE is the degreasing, as vapor or cold state solvent, of metal and other products with potential exposure in jobs in the metal industry, printing industry, and aircraft maintenance or manufacturing industry (Bakke et al., 2007). The recent NHL case-control study of Purdue et al. (2009) examined degreasing tasks, specifically, and reported an increasing positive trend between NHL risk in males and three degreasing exposure surrogates: average frequency (hours/year) ($p = 0.02$), maximal frequency (hours/year), ($p = 0.06$), or cumulative number of hours ($p = 0.04$).

As described in Appendix B, the EPA conducted a thorough and systematic search of published epidemiological studies of cancer risk and TCE exposure using the PubMed,

TOXNET®, and EMBASE© bibliographic database. The EPA also requested unpublished data pertaining to TCE from studies that may have collected these data but did not include it in their published reports. ATSDR and state health department peer-reviewed studies were also reviewed. Information from each of these studies relating to specified design and analysis criteria was abstracted. These criteria included aspects of study design, representativeness of study subjects, participation rate/loss to follow-up, latency considerations, potential for biases related to exposure misclassification, disease misclassification, and surrogate information, consideration of possible confounding, and approach to statistical analysis. All studies were considered for hazard identification, but those studies more fully meeting the objective criteria provided the greater weight for identifying a cancer hazard.

The body of evidence on NHL and TCE is comprised of occupational cohort studies, population-based case-control studies, and geographic studies. Four case-control studies and four geographic studies also examined childhood leukemia and TCE. Most studies reported observed risk estimates and associated CIs for NHL and overall TCE exposure. The studies included a broad but sometimes slightly different group of lymphosarcoma, reticulum-cell sarcoma, and other lymphoid tissue neoplasms, with the exception of the Nordstrom et al. (1998) case-control study, which examined hairy cell leukemia, now considered a NHL, the Zhao et al. (2005) cohort study, which reported only results for *all* lymphohematopoietic cancers, including nonlymphoid types and excluding CLL, and the Greenland et al. (1994) nested case-control study which reported results for NHL and Hodgkin lymphoma combined. Persson and Fredrikson (1999) do not identify the classification system for defining NHL, and Hardell et al. (1994) define NHL using the Rappaport classification system. Miligi et al. (2006) used an NCI classification system and considered CLLs and NHL, classified as lymphosarcoma, reticulosarcoma, and other lymphoid tissue neoplasms, together, while Cocco et al. (2010), used the WHO classification system, which reclassifies lymphocytic leukemias and NHLs as lymphomas of B-cell or T-cell origin. EPA staff, additionally, was able to obtain results generally consistent with the traditional NHL definition from Dr. Cocco, although lymphomas not otherwise specified were excluded (Cocco, 2010). The cohort studies [except for Zhao et al. (2005)] and the nested case-control study of Greenland et al. (1994) have some consistency in coding NHL, with NHL defined as lymphosarcoma and reticulum-cell sarcoma (ICD code 200) and other lymphoid tissue neoplasms (ICD code 202) using the ICD Revisions 7, 8, or 9. Revisions 7 and 8 are essentially the same with respect to NHL; under Revision 9, the definition of NHL was broadened to include some neoplasms previously classified as Hodgkin lymphomas (Banks, 1992). Wang et al. (2009) refer to their cases as ―NHL‖ cases and according to the ICD-O classification system that they used, their cases are more specifically NHL subtypes such as diffuse, lymphosarcoma, or follicular lymphoma (9590–9642, 9690–9701) or mast cell tumors (9740–9750) which is consistent with the traditional definition of NHL (i.e., ICD-7, -8, -9 codes 200 + 202) (Morton et al., 2003). NHL cases in Purdue et al. (2011) were also classified

according to ICD-O (2nd Edition converted to ICO-O 3rd Edition codes), included diffuse, follicular T-cell and all other NHL subtypes, which is generally consistent with the traditional definition of NHL, although this grouping does not include the malignant lymphomas of unspecified type coded as M-9590-9599. Fewer studies in published papers presented this information for cell-specific lymphomas, leukemia, leukemia cell type, or multiple myeloma (Gold et al., 2011; Cocco et al., 2010; Costantini et al., 2008; Radican et al., 2008; Boice et al., 2006b; Hansen et al., 2001; Raaschou-Nielsen et al., 2001; Boice et al., 1999; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994).

The seven cohort studies with data on the incidence of lymphopoietic and hematopoietic cancer in relation to TCE exposure range in size from 803 (Hansen et al., 2001) to 86,868 (Chang et al., 2005), and were conducted in Denmark, Sweden, Finland, Taiwan, and the United States (see Table 4-71; for additional study descriptions, see Appendix B). Some subjects in the Hansen et al. (2001) study are also included in a study reported by Raaschou-Nielsen et al. (2003); however, any contribution from the former to the latter are minimal given the large differences in cohort sizes of these studies (Raaschou-Nielsen et al., 2003; Hansen et al., 2001). The exposure assessment techniques used in all studies except Chang et al. (2005) and Sung et al. (2007) included a detailed JEM (Zhao et al., 2005; Blair et al., 1998), biomonitoring data (Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994), or reference to industrial hygiene records on TCE exposure patterns and factors that affected exposure, indicating a high probability of TCE exposure potential (Raaschou-Nielsen et al., 2003) with high probability of TCE exposure to individual subjects. Subjects in Chang et al. (2005) and Sung et al. (2007), two studies with overlapping subjects employed at an electronics plant in Taiwan, have potential exposure to several solvents including TCE; all subjects are presumed as ―exposed‖ because of employment in the plant although individual subjects would be expected to have differing exposure potentials. The lack of attribution of exposure intensity to individual subjects yields a greater likelihood for exposure misclassification compared to the six other studies with exposure assessment approaches supported by information on job titles, tasks, and industrial hygiene monitoring data. Incidence ascertainment in two cohorts began 21 (Blair et al., 1998) and 38 years (Zhao et al., 2005) after the inception of the cohort. Specifically, Zhao et al. (2005) noted that their results may not accurately reflect the effects of carcinogenic exposure that resulted in nonfatal cancers before 1988. Because of the issues concerning case ascertainment raised by this incomplete coverage, observations must be interpreted in light of possible bias reflecting incomplete ascertainment of incident cases.

Table 4-71. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk

Population exposure group		Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
		RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	
Aerospace workers (Rocketdyne), California										Zhao et al. (2005)
	Any TCE exposure	Not reported		Not reported						n = 5,049 (2,689 with high cumulative TCE exposure), began work before 1980, worked at least 2 yrs, alive with no cancer diagnosis in 1988, follow-up from 1988 to 2000, JEM (intensity), internal referents (workers with no TCE exposure). Leukemia and multiple myeloma observations included in NHL category.
	Low cumulative TCE score			1.0 (referent)	28					
	Medium cumulative TCE score			0.88 (0.47, 1.65)	16					
	High cumulative TCE score			0.20 (0.03, 1.46)	1					
	(*p* for trend)			(0.097)						
Electronic workers, Taiwan										Chang et al. (2005); Sung et al. (2007)
	All employees	0.67 (0.42, 1.01)	22							n = 88,868 (n = 70,735 female), follow-up 1979–1997, does not identify TCE exposure to individual subjects (Chang et al., 2005).
	Males	0.73 (0.27, 1.60)	6	Not reported		Not reported		Not reported		
	Females	0.65 (0.37, 1.05)	16	Not reported		Not reported		Not reported		
	Females					0.78 (0.49, 1.17)	23	Not reported		n = 63,982 females, follow-up 1979–2001, does not identify TCE exposure to individual subjects (Sung et al., 2007).

Table 4-71. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population exposure group		Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
		RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	
Blue-collar workers, Denmark										Raaschou-Nielsen et al. (2003)
	Any exposure	1.1 (1.0, 1.6)	229	1.2 (1.0, 1.5)	96	1.2 (0.9, 1.4)	82	1.03 (0.70, 1.47)	31	n = 40,049 (14,360 with presumed higher level exposure to TCE), worked for at least 3 mo, follow-up from 1968 to 1997, documented TCE use[c]. EPA based the lymphopoietic cancer category on summation of ICD codes 200–204.
	Subcohort w/higher exposure[d]	Not reported		1.5 (1.2, 2.0)	65	Not reported		Not reported		
	Employment duration									
	1–4.9 yrs			1.5 (1.1, 2.1)	35					
	≥5 yrs			1.6 (1.1, 2.2)	30					
Biologically-monitored workers, Denmark										Hansen et al. (2001)
	Any TCE exposure	2.0 (1.1, 3.3)	15	3.1 (1.3, 6.1)	8	2.0 (0.7, 4.4)	6	0.71 (0.02, 3.98)	1	n = 803, U-TCA or air TCE samples, follow-up 1968–1996 [subset of Raaschlou-Nielsen et al. (2003) cohort]. EPA based the lymphopoietic cancer category on summation of ICD codes 200–204.
	Cumulative exposure (Ikeda), males	Not reported				Not reported		Not reported		
	<17 ppm-yr			3.9 (0.8, 11)	3					
	≥17 ppm-yr			3.1 (0.6, 9.1)	3					
	Mean concentration (Ikeda), males	Not reported				Not reported		Not reported		
	<4 ppm			3.9 (1.1, 10)	4					
	4+ ppm			3.2 (1.1, 10)	4					
	Employment duration, males	Not reported				Not reported		Not reported		
	<6.25 yr			2.5 (0.3, 9.2)	2					
	≥6.25 yr			4.2 (1.1, 11)	4					

Table 4-71. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population exposure group		Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
		RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	
Aircraft maintenance workers, Hill Air Force Base, Utah										Blair et al. (1998)
	TCE Subcohort	Not reported		Not reported		Not reported		Not reported		n = 10,461 men and 3,605 women (total n = 14,066, n = 7,204 with TCE exposure), employed at least 1 yr from 1952 to 1956, follow-up 1973–1990, JEM (intensity), internal referent (workers with no chemical exposures).
	Males, cumulative exposure									
	0	1.0 (referent)		1.0 (referent)		1.0 (referent)		1.0 (referent)	9	
	<5 ppm-yr	0.8 (0.4, 1.7)	12	0.9 (0.3, 2.6)	8	0.4 (0.1, 2.0)	2	0.8 (0.1, 12.7)	1	
	5–25 ppm-yr	0.7 (0.3, 1.8)	7	0.7 (0.2, 2.6)	4		0	3.8 (0.4, 37.4)	3	
	>25 ppm-yr	1.4 (0.6, 2.9)	17	1.0 (0.4, 2.9)	7	0.9 (0.2, 3.7)	4	5.1 (0.6, 43.7)	5	
	Females, cumulative exposure									
	0	1.0 (referent)		1.0 (referent)		1.0 (referent)		1.0 (referent)		
	<5 ppm-yr	1.2 (0.3, 4.4)	3	0.6 (0.1, 5.0)	1		0	Not reported	2	
	5–25 ppm-yr	1.9 (0.4, 8.8)	2		0	2.4 (0.3, 21.8)	1	Not reported	1	
	>25 ppm-yr	0.9 (9.2, 3.3)	3	0.9 (0.2, 4.5)	2		0	Not reported	1	
Biologically-monitored workers, Finland										Anttila et al. (1995)
	Any TCE exposure	1.51 (0.92, 2.33)	20	1.81 (0.78, 3.56)	8	1.08 (0.35, 2.53)	5	1.62 (0.44, 4.16)	4	n = 3,089 men and women, U-TCA samples, follow-up 1967–1992.
	Mean air-TCE (Ikeda extrapolation)									
	<6 ppm	1.36 (0.65, 2.49)	10	2.01 (0.65, 4.69)	5	0.39 (0.01, 2.19)	1	1.48 (0.18, 5.35)	2	
	6+ ppm	2.08 (0.95, 3.95)	9	1.40 (0.17, 5.04)	2	2.65 (0.72, 6.78)	4	2.41 (0.29, 6.78)	2	

Table 4-71. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population exposure group		Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
		RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	RR (95% CI)[a]	n[a]	
Biologically-monitored workers, Sweden										Axelson et al. (1994)
	Males	1.17 (0.47, 2.40)	7	1.56 (0.51, 3.64)	5	Not reported		0.57 (0.01, 3.17)	1	n = 1,421 men and 249 women (total 1,670), U-TCA samples, follow-up 1958–1987. EPA based the lymphopoietic cancer category includes ICD-7 200–203.
	0–17 ppm (Ikeda extrapolation)	Not reported		1.44 (0.30, 4.20)	3	Not reported				
	18–35 ppm (Ikeda extrapolation)			(0, 8.58)	0					
	≥36 ppm (Ikeda extrapolation)			6.25 (0.16, 34.8)	1					
	Females	Not reported		Not reported		Not reported				

[a]n = number of observed cases.
[b]SIRs using an external population referent group unless otherwise noted.
[c]Exposure assessment based on industrial hygiene data on TCE exposure patterns and factors that affect such exposure (Raaschou-Nielsen et al., 2002), with high probability of TCE exposure potential to individual subjects. Companies included iron and metal (48%), electronics (11%), painting (11%), printing (8%), chemical (5%), dry cleaning (5%), and other industries.
[d]Defined as at least 1 year duration and first employed before 1980.

Eighteen cohort or PMR studies describing mortality risks from lymphopoietic and hematopoietic cancer are summarized in Table 4-72 (for additional study descriptions, see Appendix B). Two studies examined cancer incidence, Radican et al. (2008), who updated mortality in Blair et al. (1998) cohort, and Zhao et al. (2005), and are identified above. In 10 of the 18 studies presenting mortality risks (Clapp and Hoffman, 2008; Sung et al., 2007; ATSDR, 2004a; Chang et al., 2003; Henschler et al., 1995; Sinks et al., 1992; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Wilcosky et al., 1984), a relatively limited exposure assessment methodology was used, study participants may not represent the underlying population, or there was a low exposure prevalence of TCE exposure. For reasons identified in the systematic review, these studies are given less weight in the overall evaluation of the literature than the eight other cohort studies that better met the ideals of evaluation criteria (Radican et al., 2008; Boice et al., 2006b; Zhao et al., 2005; Boice et al., 1999; Ritz, 1999a; Blair et al., 1998 and extended follow-up by; Morgan et al., 1998; Greenland et al., 1994).

Case-control studies of NHL from United States (Connecticut), Germany, Italy, Sweden, and Canada were identified, and are summarized in Table 4-73 (for additional study descriptions, see Appendix B). These studies identified cases from hospital records (Cocco et al., 2010; Costantini et al., 2008; Seidler et al., 2007; Mester et al., 2006; Miligi et al., 2006; Persson and Fredrikson, 1999; Hardell et al., 1994; Siemiatycki, 1991); the SEER Cancer Registry—Connecticut residents (Wang et al., 2009), Iowa, Los Angeles County, and Seattle and Detroit metropolitan area residents (Purdue et al., 2011), or Seattle and Detroit metropolitan area residents (Gold et al., 2011); or the Swedish Cancer Registry (Nordström et al., 1998), and hospital or population controls. These studies assign potential occupational TCE exposure to cases and controls using self-reported information obtained from a mailed questionnaire (Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994) or from direct interview with study subjects, with industrial hygienist ratings of exposure potential and a JEM (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Costantini et al., 2008; Seidler et al., 2007; Miligi et al., 2006; Siemiatycki, 1991). Additionally, large multiple center lymphoma case-control studies examine specific types of NHL (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Miligi et al., 2006), leukemia (Costantini et al., 2008), or multiple myeloma (Purdue et al., 2011; Cocco et al., 2010; Costantini et al., 2008).

Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk

Population, exposure group		Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
		RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	
Computer manufacturing workers (IBM), New York										Clapp and Hoffman (2008)
	Males	2.24 (1.01, 4.19)	9	Not reported		Not reported		Not reported	3	n = 115 cancer deaths from 1969 to 2001, proportional cancer mortality ratio, does not identify TCE exposure to individual subjects. EPA based the lymphopoietic cancer category on ―all lymphatic cancers.”
	Females	Not reported	0	Not reported		Not reported		Not reported	0	
Aerospace workers (Rocketdyne), California										Boice et al. (2006b)
	Any TCE (utility/eng flush)	0.74 (0.34, 1.40)	9	0.21 (0.01, 1.18)	1	1.08 (0.35, 2.53)	5	0.50 (0.01, 2.77)	1	n = 41,351 (1,111 Santa Susana workers with any TCE exposure), employed on or after 1948–1999, worked ≥6 mo, follow-up to 1999, JEM without quantitative estimate of TCE intensity.

Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group		Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
		RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	
Aerospace workers (Rocketdyne), California (continued)										Zhao et al. (2005)
	Any TCE exposure	Not reported		Not reported	60	Not reported		Not reported		n = 6,044 (n = 2,689 with high cumulative level exposure to TCE), began work and worked at least 2 yrs in 1950 or later–1993, follow-up to 2001, JEM (intensity), internal referents (workers with no TCE exposure). Leukemia and multiple myeloma observations included in NHL category.
	Low cumulative TCE score			1.0 (referent)	27					
	Medium cumulative TCE score			1.49 (0.86, 2.57)	27					
	High TCE score			1.30 (0.52, 3.23)	6					
	(*p* for trend)			(0.370)						
View-Master employees, Oregon										ATSDR (2004a)
	Males	0.58 (0.11, 1.69)	3	0.69 (0.08, 2.49)	2	0.50 (0.01, 2.79)	1			n = 616 deaths from 1989 to 2001, PMR, does not identify TCE exposure to individual subjects. EPA based the NHL cancer category on ~~–o~~ther lymphopoietic tissue" which included NHL and multiple myeloma.
	Females	0.64 (0.28, 1.26)	8	0.52 (0.14, 1.33)	4	0.67 (0.14, 1.96)	3			

Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	
Electronic workers, Taiwan									Chang et al. (2003)
All employees									n = 88,868 (n = 70,735 female), began work 1978–1997, follow-up 1985–1997, does not identify TCE exposure to individual subjects.
Males	Not reported		1.27 (0.41, 2.97)	5	0.44 (0.05, 1.59)	2	Not reported		
Females	Not reported		1.14 (0.55, 2.10)	10	0.54 (0.23, 1.07)	8	Not reported		
Aerospace workers (Lockheed), California									Boice et al. (1999)
Routine TCE									n = 77,965 (n = 2,267 with routine TCE exposure and n = 3.016 with intermittent-routine TCE exposure), began work ≥1960, worked at least 1 yr, follow-up from 1960 to 1996, JEM without quantitative estimate of TCE intensity.
Any TCE exposure	1.5 (0.81, 1.60)	36	1.19 (0.65, 1.99)	14	1.05 (0.54, 1.84)	12	0.91 (0.34, 1.99)	6	
Routine-intermittent									
Any TCE exposure	Not reported		Not reported		Not reported				
Exposure duration	Not reported				Not reported				
0 yr			1.0 (referent)	32			1.0 (referent)	24	
<1 yr			0.74 (0.32, 1.72)	7			0.45 (0.13, 1.54)	3	
1–4 yrs			1.33 (0.64, 2.78)	10			1.48 (0.64, 3.41)	8	
≥5 yrs			1.62 (0.82, 3.22)	14			0.51 (0.15, 1.76)	3	
p for trend			0.20				>0.20		

Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	
Uranium-processing workers (Fernald), Ohio									Ritz (1999a)
Any TCE exposure	Not reported		Not reported		Not reported		Not reported		n = 3,814 (n = 2,971 with TCE), began work 1951–1972, worked ≥3 mo, follow-up to 1989, internal referents (workers with no TCE exposure).
No TCE exposure	1.0 (referent)		Not reported		Not reported		Not reported		
Light TCE exposure, >2 yrs	1.45 (0.68, 3.06)[c]	18	Not reported		Not reported		Not reported		
Moderate TCE exposure, >2 yrs	1.17 (0.15, 9.00)[c]	1	Not reported		Not reported		Not reported		
Aerospace workers (Hughes), California									Morgan et al. (1998)
TCE subcohort	0.99 (0.64, 1.47)	25	0.96 (0.20, 2.81)[d]	3	1.05 (0.50, 1.93)	10	1.08 (0.35, 2.53)[e]	5	n = 20,508 (4,733 with TCE exposure), worked ≥6 mo 1950–1985, follow-up to 1993, external and internal (all non-TCE exposed workers) workers referent, JEM (intensity).
TCE subcohort			1.01 (0.46, 1.92)[e]	9					
Low intensity (<50 ppm)	1.07 (0.51, 1.96)	10	1.79 (0.22, 6.46)[d]	2	0.85 (0.17, 2.47)	3			
High intensity (>50 ppm)	0.95 (0.53, 1.57)	15	0.50 (0.01, 2.79)[d]	1	1.17 (0.47, 2.41)	7			
TCE subcohort (Cox Analysis)									
Never exposed	1.0 (referent)	82	1.0 (referent)	8	1.0 (referent)	32			
Ever exposed	1.05 (0.67, 1.65)[f]	25	1.36 (0.35, 5.22)[d,f]	3	0.99 (0.48, 2.03)[f]	10			
Peak									
No/Low	1.0 (referent)	90	1.0 (referent)	9	1.0 (referent)	35			
Medium/High	1.08 (0.64, 1.82)	17	1.31 (0.28, 6.08)[d]	2	1.10 (0.49, 2.49)	7			

Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	
Aerospace workers (Hughes), California (continued)									
Cumulative									
Referent	1.0 (referent)	82	1.0 (referent)	8	1.0 (referent)	32			
Low	1.09 (0.56, 2.14)	10	2.25 (0.46, 11.1)[d]	2	0.69 (0.21, 2.32)	3			
High	1.03 (0.59, 1.79)	15	0.81 (0.10, 6.49)[d]	1	1.14 (0.5, 2.60)	7			
Aircraft maintenance workers, Hill Air Force Base, Utah									Blair et al. (1998); Radican et al. (2008)
TCE subcohort	1.1 (0.7, 1.8)[g]	66	2.0 (0.9, 4.6)[g]	28	0.6 (0.3, 1.2)[g]	16	1.3 (0.5, 3.4)	14	n = 14,066 (n = 7,204 ever exposed to TCE), employed at least 1 yr from 1952 to 1956, follow-up to 1990 (Blair et al., 1998) or to 2000 (Radican et al., 2008), JEM, internal referent (workers with no chemical exposures).
Males, cumulative exposure									
0	1.0 (referent)		1.0 (referent)		1.0 (referent)		1.0 (referent)		
<5 ppm-yr	1.1 (0.6, 2.1)	21	1.8 (0.6, 5.4)	10	1.0 (0.3, 3.2)	7	1.0 (0.2, 4.2)	4	
5–25 ppm-yr	1.0 (0.4, 2.1)	11	1.9 (0.6, 6.3)	6		0	0.8 (0.1, 4.4)	2	
>25 ppm-yr	1.3 (0.7, 2.5)	21	1.1 (0.3, 3.8)	5	1.2 (0.4, 3.6)	7	1.2 (0.3, 4.7)	4	
Females, cumulative exposure									
0	1.0 (referent)				1.0 (referent)		1.0 (referent)		
<5 ppm-yr	1.5 (0.6, 4.0)	6	3.8 (0.8, 18.9)	3	0.4 (0.1, 3.2)	1	3.2 (0.5, 19.8)	2	
5–25 ppm-yr	0.7 (0.1, 4.9)	1		0		0	4.3 (0.4, 23.4)	1	
>25 ppm-yr	1.1 (0.4, 3.0)	6	3.6 (0.8, 16.2)	4	0.3 (0.1, 2.4)	1	1.3 (0.1, 13.2)	1	
TCE subcohort	1.06 (0.75, 1.51)[h]	106	1.36 (0.77, 2.39)[h]	46	0.64 (0.35, 1.18)[h]	27	1.35 (0.62, 2.93)	25	

Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group		Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
		RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	
Aircraft maintenance workers, Hill Air Force Base, Utah (continued)										
	Males, cumulative exposure	1.12 (0.72, 1.73)	88	1.56 (0.79, 4.21)	37	0.77 (0.37, 1.62)	24	1.08 (0.43, 2.71)	19	
	0	1.0 (referent)		1.0 (referent)		1.0 (referent)		1.0 (referent)		
	<5 ppm-yr	1.04 (0.63, 1.74)	34	1.83 (0.79, 4.21)	18	0.86 (0.36, 2.02)	11	0.69 (0.21, 2.27)	5	
	5–25 ppm-yr	1.06 (0.49, 1.88)	21	1.17 (0.42, 3.24)	7	0.51 (0.16, 1.63)	4	1.58 (0.53, 4.71)	7	
	>25 ppm-yr	1.25 (0.75, 2.09)	33	1.50 (0.61, 3.69)	12	0.87 (0.35, 2.14)	9	1.19 (0.40, 3.54)	7	
	Females, cumulative exposure	1.00 (0.55, 1.83)	18	1.18 (0.49, 2.85)	9	0.36 (0.10, 1.32)	3	2.37 (0.67, 8.44)	6	
	0	1.0 (referent)		1.0 (referent)		1.0 (referent)		1.0 (referent)		
	<5 ppm-yr	1.10 (0.48, 2.54)	7	1.48 (0.47, 4.66)	4	0.35 (0.05, 2.72)	1	2.20 (0.40, 12.02)	2	
	5–25 ppm-yr	0.38 (0.05, 2.79)	1		0		0	2.79 (0.31, 25.05)	1	
	>25 ppm-yr	1.11 (0.53, 2.31)	10	1.30 (0.45, 3.77)	5	0.48 (0.10, 2.19)	2	2.38 (0.53, 10.67)	3	
Cardboard manufacturing workers, Arnsburg, Germany										Henschler et al. (1995)
	TCE-exposed subjects	1.10 (0.03, 6.12)	1							n = 169 TCE exposed and n = 190 unexposed men, employed ≥1 yr from 1956 to 1975, follow-up to 1992, local population referent, qualitative exposure assessment.
	Unexposed subjects from same factory	1.11 (0.03, 6.19)	1							

Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group	Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	
GE plant, Pittsfield, Massachusetts			0.76 (0.24, 2.42)[i,j]	15	1.1 (0.46, 2.66)[i]	22			Greenland et al. (1994)
									Nested case-control study, n = 512 cancer (cases) and 1,202 noncancer (controls) male deaths reported to pension fund between 1969 and 1984 among workers employed <1984 and with job history record, JEM-ever held job with TCE exposure. Hodgkin lymphoma in NHL grouping.
Cardboard manufacturing workers, Atlanta, Georgia									Sinks et al. (1992)
	0.3 (0.0, 1.6)	1	Not reported		Not reported		Not reported		n = 2,050, employed on or before 1957–1988, follow-up to 1988, Material Data Safety Sheets used to identify chemicals used in work areas.

Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group		Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
		RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	
U.S. Coast Guard employees										Blair et al. (1989)
	Marine inspectors	1.57 (0.91, 2.51)	17	1.75 (0.48, 4.49)	4	1.55 (0.62, 3.19)	7	Not reported		n = 3,781 males (1,767 marine inspectors), employed 1942–1970, follow-up to 1980. TCE and nine other chemicals identified as potential exposures; no exposure assessment to individual subjects.
	Noninspectors	0.60 (0.24, 1.26)	7	0.41 (0.01, 2.30)	1	0.66 (0.14, 1.94)	3	Not reported		
Aircraft manufacturing employees, Italy										Costa et al. (1989)
	All male subjects	0.80 (0.41, 1.40)	12	Not reported		Not reported		Not reported		n = 7,676 males, employed on or before 1954–1981, followed to 1981, job titles of white- and blue-collar workers, technical staff, and administrative clerks, does not identify TCE exposure to individual subjects.
Aircraft manufacturing, San Diego, California										Garabrant et al. (1988)
	All employees	0.82 (0.56, 1.15)	32	0.82 (0.44, 1.41)[d]	13	0.82 (0.47, 1.32)	10	Not reported		n = 14,067, employed at least 4 yrs with company and ≥1 d at San Diego plant from 1958 to 1982, followed to 1982, does not identify TCE exposure to individual subjects.
				0.65 (0.21, 1.52)[k]	5					

Table 4-72. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group		Lymphopoietic cancer		NHL		Leukemia		Multiple myeloma		Reference(s) and study description[b]
		RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	RR (95% CI)	n[a]	
Solvent-exposed rubber workers										Wilcosky et al. (1984)
		2.4[i]	3	0.81	3					Nested case-control study, n = 9 lymphosarcoma and 10 leukemia (cases) and 20% random sample of all other deaths (controls) between 1964 and 1973 in cohort of n = 6,678, exposure assessment by company record for use in work area.

[a]n = number of observed cases.
[b]Unless otherwise noted, all studies reported standardized mortality ratios using an external population referent group.
[c]Logistic regression analysis with 15 lag for TCE exposure (Ritz, 1999a).
[d]In Morgan et al. (1998) and Garabrant et al. (1988), this category was based on lymphosarcoma and reticulosarcoma.
[e]As presented in Mandel et al. (2006) for NHL, this category defined as ICD-7, ICDA-8, and ICD-9 codes of 200 and 202. As presented in Alexander et al. (2006) for multiple myeloma.
[f]Risk ratio from Cox Proportional Hazard Analysis, stratified by age and sex, from Environmental Health Strategies (1997) Final Report to Hughes Corporation (Communication from Paul A. Cammer, President, Trichloroethylene Issues Group to Cheryl Siegel Scott, U.S. EPA, December 22, 1997).
[g]Estimated RRs from Blair et al. (1998) from Poisson regression models adjusted for date of hire, calendar year of death and sex.
[h]Estimated RRs from Radican et al. (2008) from Cox proportional hazard models adjusted for age and sex.
[i]OR from nested case-control analysis.
[j]Lymphomas, lymphosarcomas, reticulosarcomas, and Hodgkin lymphoma (ICDA-8 200-202) in Greenland et al. (1994).
[k]Other lymphatic and hematopoietic tissue neoplasms (Garabrant et al., 1988).

Table 4-73. Case-control studies of TCE exposure and lymphopoietic cancer, leukemia or multiple myeloma

Population	Cancer type and exposure group	OR (95% CI)	n exposed cases	Reference(s)
Men and women aged 20–74 in Iowa, Los Angeles County (California), Seattle and Detroit metropolitan areas	**NHL**			Gold et al. (2011); Purdue et al. (2011)
	Any TCE exposure			
	Possible	1.1 (0.9, 1.3)	545	
	Probable	1.4 (0.8, 2.4)	45	
	Average weekly exposure[a]			
	0 ppm-hr/wk	1.0	341	
	1–60 ppm-hr/wk	1.6 (0.7, 3.8)	15	
	61–150 ppm-hr/wk	0.5 (0.2, 1.4)	7	
	>150 ppm-hr/wk	2.5 (1.1, 6.1)	23	
	(*p* for linear trend)	0.02		
	Cumulative exposure[a]			
	0	1.0	341	
	1–46,800 ppm-hr	1.4 (0.6, 3.3)	14	
	46,801–112,320 ppm-hr	0.6 (0.2, 1.7)	7	
	>112,320 ppm-hr	2.3 (1.0, 5.0)	24	
	(*p* for linear trend)	0.08		
	NHL types			
	Probable TCE exposure			
	Diffuse	0.9 (0.5, 2.0)	155	
	Follicular	2.1 (1.0, 4.2)	13	
	CLL	2.7 (1.2, 5.8)	11	

Table 4-73. Case-control studies of TCE exposure and lymphopoietic cancer, leukemia or multiple myeloma (continued)

Population	Cancer type and exposure group	OR (95% CI)	n exposed cases	Reference(s)
Men and women aged 20–74 (continued)	**Multiple myeloma**			Gold et al. (2011); Purdue et al. (2011) (continued)
	Any TCE exposure	1.4 (0.9, 2.1)	66	
	High confidence exposure[b]	1.7 (1.0, 2.7)	43	
	Cumulative exposure[b]			
	0	1.0	139	
	1–471 ppm-hr	1.1 (0.4, 2.9)	6	
	472–3,000 ppm-hr	1.6 (0.7, 3.5)	11	
	3,001–7,644 ppm-hr	1.5 (0.6, 3.9)	7	
	7,645–570,000 ppm-hr	2.3 (1.1, 5.0)	17	
	(*p* for linear trend)	0.03		
Men and women aged ≥17 yrs in Czech Republic, Finland, France, Germany, Ireland, Italy, and Spain (Epilymph study)	**All Centers:**			Cocco et al. (2010)
	B-cell NHL[b]			
	Any TCE exposure	0.8 (0.6, 1.1)	71	
	Cumulative Exposure			
	Low	0.9 (0.6, 1.6)	26	
	Medium	0.5 (0.3, 0.9),	16	
	High	1.0 (0.6, 1.6)	29	
	(*p* for linear trend)	0.16		

Table 4-73. Case-control studies of TCE exposure and lymphopoietic cancer, leukemia or multiple myeloma (continued)

Population	Cancer type and exposure group	OR (95% CI)	n exposed cases	Reference(s)
Men and women aged ≥17 yrs in Czech Republic, Finland, France, Germany, Ireland, Italy, and Spain (Epilymph study) (continued)	**NHL types**[c]			Cocco et al. (2010) (continued)
	Diffuse large B-cell	0.7 (0.4, 1.1)	17	
	Follicular	1.2 (0.6, 2.3)	11	
	CLL	0.9 (0.5, 1.5)	18	
	Multiple myeloma	0.6 (0.3, 1.2)	9	
	T-cell lymphoma	0.9 (0.4, 2.2)	6	
	German centers:			Seidler et al. (2007); Mester et al. (2006)
	NHL			
	Any TCE exposure	Not reported		
	Cumulative TCE			
	0 ppm-yr	1.0	610	
	>0– ≤4 ppm-yr	0.7 (0.4, 1.1)	40	
	4.4– <35 ppm-yr	0.7 (0.5, 1.2)	32	
	High exposure, >35 ppm-yr	2.1 (1.0, 4.8)	21	
	(*p* for linear trend)	0.14		
	>35 ppm-yr, 10-yr lag	2.2 (1.0, 4.9)		
Women aged 21–84 in Connecticut, United States	**NHL**			Wang et al. (2009)
	Any TCE exposure	1.2 (0.9, 1.8)	77	
	Low intensity TCE exposure	1.1 (0.8, 1.6)	64	
	Medium-high intensity TCE exposure	2.2 (0.9, 5.4)	13	
	(*p* for linear trend)	0.06		
	Low probability TCE exposure	1.1 (0.7, 1.8)	43	
	Medium-high probability TCE exposure	1.4 (0.9, 2.4)	34	
	(*p* for linear trend)	0.37		
	Low intensity TCE exposure/low probability	0.9 (0.6, 1.5)	30	Wang et al. (2009) (continued)
	Low intensity/medium-high probability	1.4 (0.9, 2.4)	34	
	Medium-high intensity/low probability	2.2 (0.9, 5.4)	13	
	Medium-high intensity/medium-high probability		0	

Table 4-73. Case-control studies of TCE exposure and lymphopoietic cancer, leukemia or multiple myeloma (continued)

Population	Cancer type and exposure group	OR (95% CI)	n exposed cases	Reference(s)
Population in eight Italian regions	**NHL**			Miligi et al. (2006); Costantini et al. (2008)
	Any TCE exposure	Not reported		
	TCE exposure intensity			
	Very low/low	0.8 (0.5, 1.3)	35	
	Medium/high	1.2 (0.7, 2.0)	35	
	(*p* for linear trend)	0.8		
	Duration exposure, medium/high TCE intensity			
	≤15 yrs	1.1 (0.6, 2.1)	22	
	>15 yrs	1.0 (0.5, 2.6)	12	
	(*p* for linear trend)	0.72		
	Other NHL			
	TCE exposure intensity, medium/high			
	Small lymphocytic NHL	0.9 (0.4, 2.1)	7	
	Follicular NHL	Not presented	3	
	Diffuse NHL	1.9 (0.9, 3.7)	13	
	Other NHL	1.2 (0.6, 2.4)	11	
	Multiple myeloma	0.9 (0.3, 2.4)	27	

Table 4-73. Case-control studies of TCE exposure and lymphopoietic cancer, leukemia or multiple myeloma (continued)

Population	Cancer type and exposure group	OR (95% CI)	n exposed cases	Reference(s)
Population in eight Italian regions (continued)	**Leukemia**			Miligi et al. (2006); Costantini et al. (2008) (continued)
	Any TCE exposure	Not reported		
	TCE exposure intensity			
	Very low/low	1.0 (0.5, 1.8)	17	
	Medium/high	0.7 (0.4, 1.5)	11	
	CLL			
	Any TCE exposure	Not reported		
	TCE exposure intensity			
	Very low/low	1.2 (0.5, 2.7)	8	
	Medium/high	0.9 (0.3, 2.6)	4	
Population of Örebro and Linköping, Sweden	**B-cell NHL**			Persson and Fredrikson (1999)
	Any TCE exposure	1.2 (0.5, 2.4)	16	
Population of Sweden	**Hairy cell lymphoma**			Nordstrom et al. (1998)
	Any TCE exposure	1.5 (0.7, 3.3	9	
Population of Umea, Sweden	**NHL**			Hardell et al. (1994)
	Any exposure to TCE	7.2 (1.3, 42)	4	
Population of Montreal, Canada	**NHL**			Siemiatycki et al. (1991)
	Any TCE exposure	1.1 (0.6, 2.3)[d]	6	
	Substantial TCE exposure	0.8 (0.2, 2.5)[d]	2	

[a]For Purdue et al. (2011), OR for subjects interviewed using computer-assisted personal interview with job modules and includes subjects assessed as unexposed or with probably exposure, defined as holding one or more jobs with an assigned probability of TCE exposure of ≥50%.
[b]For Gold et al. (2011) subjects with jobs assessed with low confidence considered as unexposed.
[c]For Cocco et al. (2010), OR for subjects with high confidence assessment of TCE exposure.
[d]90% CI.

Four geographic-based studies on NHL in adults are summarized in Table 4-74 (for additional study descriptions, see Appendix B) and subjects in three studies are identified based upon their residence in a community where TCE was detected in water serving the community (ATSDR, 2006a; Cohn et al., 1994b; Vartiainen et al., 1993). Both Cohn et al. (1994b) and ATSDR (2006a) also present estimates for childhood leukemia and these observations are discussed below with other studies reporting on childhood leukemia. A subject is assumed to have a probability of exposure due to residence likely receiving water containing TCE. Most studies do not include statistical models of water distribution networks, which may influence TCE concentrations delivered to a home, nor a subject's ingestion rate to estimate TCE exposure to individual study subjects. ATSDR (2006a) adopts exposure modeling of soil vapor contamination to define study area boundaries and to identify census tracts with a higher probability of exposure to volatile organic solvents without identifying exposure concentrations to TCE and other solvents. In these studies, one level of exposure to all subjects in a geographic area is assigned, although there is some inherent measurement error and misclassification bias because not all subjects are exposed uniformly.

NHL risk is statistically significantly elevated in three studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review [3.1, 95% CI: 1.3, 6.1 (Hansen et al., 2001); 1.5, 95% CI: 1.2, 2.0, subcohort with higher exposure (Raaschou-Nielsen et al., 2003), 2.3, 95% CI: 1.0, 5.0, >112,320-ppm hours cumulative TCE exposure, 2.5, 95% CI: 1.1, 6.1, >150-ppm hours average weekly TCE exposure (Purdue et al., 2011)]. Two of these incidence studies report statistically significantly associations for NHL for subjects with longer employment duration as a surrogate of TCE exposure [≥6.25 year, 4.2, 95% CI: 1.1, 11 (Hansen et al., 2001); ≥5 year, 1.6, 95% CI: 1.1, 2.2 (Raaschou-Nielsen et al., 2003)] and Purdue et al. (2011) report a positive trend with NHL and cumulative TCE exposure ($p = 0.08$) or average weekly TCE exposure ($p = 0.02$). Hansen et al. (2001) also examined two other exposure surrogates, cumulative exposure and exposure intensity, with estimated risk larger in low exposure groups than for high exposure groups. A fourth study from Sweden reports a large and imprecise risk with TCE [7.2, 95% CI: 1.3, 42 (Hardell et al., 1994)] based on four exposed cases. Cohort mortality studies and other case-control studies, except Cocco et al. (2010), observed a 10–50% increased risk between NHL and any TCE exposure [1.2, 95% CI: 0.65, 1.99 (Boice et al., 1999); 1.36, 95% CI: 0.35, 5.22 (Morgan et al., 1998); 1.5, 95% CI: 0.7, 3.3 (Nordström et al., 1998); 1.2, 95% CI: 0.5, 2.4 (Persson and Fredrikson, 1999); 1.36, 95% CI: 0.77, 2.39 (Radican et al., 2008); 1.1, 95% CI: 0.6, 2.3 (Siemiatycki, 1991); 1.2, 95% CI: 0.9, 1.8 (Wang et al., 2009)].

Table 4-74. Geographic-based studies of TCE and NHL or leukemia in adults

Population	Exposure group	NHL		Leukemia		Reference[a]
		RR (95% CI)	n exposed cases	RR (95% CI)	n exposed cases	
Two study areas in Endicott, New York		0.54 (0.22, 1.12)	7	0.79 (0.34, 1.55)	8	ATSDR (2006a)
Residents of 13 census tracts in Redlands, California		1.09 (0.84, 1.38)	111	1.02 (0.74, 1.35)	77	Morgan and Cassady (2002)
Population in New Jersey	Males, maximum estimated TCE concentration (ppb) in municipal drinking water					Cohn et al. (1994b)
	<0.1	1.00	493	1.00	438	
	0.1–0.5	1.28 (1.10, 1.48)	272	0.85 (0.71, 1.02)	162	
	≥5.0	1.20 (0.94, 1.52)	78	1.10 (0.84, 1.90)	63	
	Females, maximum estimated TCE concentration (ppb) in municipal drinking water					
	<0.1	1.00	504	1.00; 315		
	0.1–0.5	1.02 (0.87, 1.2)	26	1.13 (0.93, 1.37)	156	
	>5.0	1.36 (1.08, 1.70)	87	1.43 (1.43, 1.90)	56	
Population in Finland	Residents of Hausjarvi	0.6 (0.3, 1.1)	14	1.2 (0.8, 1.7)	33	Vartiainen et al. (1993)
	Residents of Huttula	1.4 (1.0, 2.0)	13	0.7 (0.4, 1.1)	19	

[a]No geographic-based study reported an RR estimate for multiple myeloma except Vartiainen et al. (1993) who observed SIRs of 0.7 (95% CI: 0.3, 1.3) and 0.6 (95% CI; 0.2, 1.3) for residents of Hausjarvi and Huttula, respectively..

ORs are higher for diffuse or follicular NHL, primarily B-cell lymphomas, than for all NHLs in both studies that examine forms of lymphoma, although based on few exposed cases and inconsistently reported (Purdue et al., 2011; Cocco et al., 2010; Miligi et al., 2006) (see Table 4-74). Observations in the two other studies of B-cell lymphomas (Wang et al., 2009; Persson and Fredrikson, 1999) appear consistent with Miligi et al. (2006) and Purdue et al. (2011). Together, these observations suggest that the associations between TCE and specific NHL types are stronger than the associations seen with other forms of NHL, and that disease misclassification may be introduced in studies examining TCE and NHL as a broader category. Mortality observations in other occupational cohorts (Sung et al., 2007; Boice et al., 2006b; ATSDR, 2004a; Chang et al., 2003; Ritz, 1999a; Henschler et al., 1995; Greenland et al., 1994; Costa et al., 1989; Garabrant et al., 1988; Wilcosky et al., 1984) included a risk estimate of 1.0 in 95% CIs; these studies neither add to nor detract from the overall weight of evidence given their lower likelihood for TCE exposure due to inferior exposure assessment approaches, lower prevalence of exposure, lower statistical power, and fewer exposed deaths.

Seven studies presented estimated risks for leukemia and overall TCE exposure: Antilla et al. (1995); Blair et al. (1998) and its update by Radican et al. (2008); Morgan et al. (1998); Boice et al. (1999); Boice et al. (2006b); Hansen et al. (2001); and Raaschou-Nielsen et al. (2003). Only three studies also presented estimated risks for a high exposure category (Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995). Three case-control studies presented estimated risk for leukemia categories and overall TCE exposure or low or high TCE exposure category (Purdue et al., 2011; Cocco et al., 2010; Costantini et al., 2008). Risk estimates in these cohort studies ranged from 0.64 (95% CI: 0.35, 1.18) (Radican et al., 2008) to 2.0 (95% CI: 0.7, 4.44) (Hansen et al., 2001). The largest study, with 82 observed incident leukemia cases, reported an RR estimate of 1.2 (95% CI: 0.9, 1.4) (Raaschou-Nielsen et al., 2003). Case-control studies which examined all leukemias (Costantini et al., 2008) or CLL (Purdue et al., 2011; Cocco et al., 2010; Costantini et al., 2008), and TCE exposure are quite limited in statistical power. Risk estimates in the four case-control studies ranged from 0.7 (95% CI: 0.4, 1.5) for all leukemias and medium to high exposure intensity [Costantini et al. (2008) to 2.7 (95% CI: 1.2, 5.8) for CLL] and probable TCE exposure (Purdue et al., 2011).

Eight cohort studies presented estimated risks for multiple myeloma and overall TCE exposure Antilla et al. (1995); Axelson et al. (1994); Blair et al. (1998) and its update by Radican et al. (2008); Morgan et al. (1998); Boice et al. (1999); Boice et al. (2006b); Hansen et al. (2001); and Raaschou-Nielsen et al. (2003). Only three studies also presented estimated risks for a high exposure category (Radican et al., 2008; Boice et al., 1999; Anttila et al., 1995). Three case-control studies presented estimated risk for multiple myeloma and overall TCE exposure or low or high TCE exposure category (Gold et al., 2011; Cocco et al., 2010; Costantini et al., 2008). Risk estimates in these cohort studies ranged from 0.57 (95% CI: 0.01, 3.17) (Axelson et al., 1994) to 1.62 (95% CI: 0.44, 4.16) (Anttila et al., 1995). The largest cohort study, with

31 observed incident multiple myeloma cases, reported an RR estimate of 1.03 (95% CI: 0.70, 1.47) (Raaschou-Nielsen et al., 2003). The largest case-control study of 43 exposed multiple myeloma cases with high confidence TCE exposure reported an OR of 1.7 (95% CI: 1.0, 2.7) and a positive trend with increasing cumulative TCE exposure ($p = 0.03$) (Gold et al., 2011).

The number of studies of childhood lymphoma including acute lymphatic leukemia and TCE is much smaller than the number of studies of TCE and adult lymphomas, and consists of four case-control studies (Costas et al., 2002; Shu et al., 1999; McKinney et al., 1991; Lowengart et al., 1987) and four geographic-based studies (ATSDR, 2008b, 2006a; ADHS, 1995; Cohn et al., 1994b; Aickin et al., 1992; ADHS, 1990) (see Table 4-75). An additional publication, focusing on ras mutations, based on one of the case-control studies is also available (Shu et al., 2004). All four case-control studies evaluate maternal exposure, and three studies also examine paternal occupational exposure (Shu et al., 2004; Shu et al., 1999; McKinney et al., 1991; Lowengart et al., 1987). There are relatively few cases with maternal exposure (range 0–16) in these case-control studies, and only Shu et al. (2004; 1999) used a large number (n = 136) of cases with paternal exposure. The small numbers of exposed case parents limit examination of possible susceptibility time windows. Overall, evidence for association between parental TCE exposure and childhood leukemia is not robust or conclusive.

The results from the studies of Costas et al. (2002) and Shu et al. (2004; 1999) suggest a fetal susceptibility to maternal exposure during pregnancy, with RRs observed for this time period equal or higher than the RRs observed for periods before conception or after birth (see Table 4-75). The studies by Lowengart et al. (1987) and McKinney et al. (1991) do not provide informative data pertaining to this issue due to the small number (n = <3) of exposed case mothers. A recent update of a cohort study of electronics workers at a plant in Taiwan (2005; Chang et al., 2003) reported a fourfold increased risk (3.83; 95% CI: 1.17, 12.55) (Sung et al., 2008) for childhood leukemia risk among the offspring of female workers employed during the 3 months before to 3 months after conception. Exposures at this factory included TCE, perchloroethylene, and other organic solvents (Sung et al., 2008). The lack of TCE assignment to individual subjects in this study decrease its weight in the overall analysis.

Table 4-75. Selected results from epidemiologic studies of TCE exposure and childhood leukemia

		RR (95% CI)	n observed events	Reference(s)
Cohort studies (solvents)				
Childhood leukemia among offspring of electronic workers				Sung et al. (2008)
	Nonexposed	1.0[a]	9	
	Exposed pregnancy to organic solvents	3.83 (1.17, 12.55)	6	
Case-control studies				
Children's Cancer Group Study (children ≤15 yrs old)				
	Acute lymphocytic leukemia			
	Maternal occupational exposure to TCE			Shu et al. (1999)
	Anytime	1.8 (0.8, 4.1)	15	
	Preconception	1.8 (0.8, 5.2)	9	
	During pregnancy	1.8 (0.5, 6.4)	6	
	Postnatal	1.4 (0.5, 4.1)	9	
	Paternal occupational exposure to TCE			
	Anytime	1.1 (0.8, 1.5)	136	
	Preconception	1.1 (0.8, 1.5)	100	
	During pregnancy	0.9 (0.6, 1.4)	56	
	Postnatal	1.0 (0.7, 1.3)	77	
	K-ras + acute lymphocytic leukemia			Shu et al., (2004)
	Maternal occupational exposure to TCE			
	Anytime	1.8 (0.6, 4.8)	5	
	Preconception	2.0 (0.7, 6.3)	4	
	During pregnancy	3.1 (1.0, 9.7)	4	
	Postnatal		0	
	Paternal occupational exposure to TCE			
	Anytime	0.6 (0.3, 1.4)	9	
	Preconception	0.6 (0.3, 1.5)	8	
	During pregnancy	0.3 (0.1, 1.2)	2	
	Postnatal	0.4 (0.1, 1.4)	3	

Table 4-75. Selected results from epidemiologic studies of TCE exposure and childhood leukemia (continued)

		RR (95% CI)	n observed events	Reference(s)
Residents of ages ≤19 in Woburn, Massachusetts				Costas et al. (2002)
	Maternal exposure 2 yrs before conception to diagnosis			
	Never	1.00	3	
	Least	5.00 (0.75, 33.5)	9	
	Most	3.56 (0.51, 24.8)	7	
	(*p* for linear trend)	≥0.05		
	Maternal exposure 2 yrs before conception			
	Never	1.00	11	
	Least	2.48 (0.42, 15.2)	4	
	Most	2.82 (0.30, 26.4)	4	
	(*p* for linear trend)	≥0.05		
	Birth to diagnosis			
	Never	1.00	7	
	Least	1.82 (0.31, 10.8)	7	
	Most	0.90 (0.18, 4.56)	5	
	(*p* for linear trend)	≥0.05		
	Maternal exposure during pregnancy			
	Never	1.00	9	
	Least	3.53 (0.22, 58.1)	3	
	Most	14.3 (0.92, 224)	7	
	(*p* for linear trend)	<0.05		
Population ≤14 yrs of age in 3 areas north England, United Kingdom				McKinney et al. (1991)
	Acute lymphocytic leukemia and NHL			
	Maternal occupation exposure to TCE			
	Preconception	1.16 (0.13, 7.91)	2	
	Paternal occupational exposure to TCE			
	Preconception	2.27 (0.84, 6.16)	9	
	Periconception and gestation	4.49 (1,15, 21)	7	
	Postnatal	2.66 (0.82, 9.19)	7	

Table 4-75. Selected results from epidemiologic studies of TCE exposure and childhood leukemia (continued)

		RR (95% CI)	n observed events	Reference(s)
Los Angeles Cancer Surveillance Program				Lowengart et al. (1987)
	Acute lymphocytic and nonlymphocytic leukemia, ≤10 yrs old			
	Maternal occupational exposure to TCE		0	
	Paternal occupational exposure to TCE			
	One yr before pregnancy	2.0 (p = 0.16)	6/3[b]	
	During pregnancy	2.0 (p = 0.16)	6/3[b]	
	After delivery	2.7 (0.64, 15.6)	8/3[b]	
Geographic-based studies				
Two study areas in Endicott, New York				ATSDR (2006a)
	Leukemia, ≤19 yrs old	Not reported	<6	
Population in New Jersey				
	Acute lymphocytic leukemia			
	Maximum estimated TCE concentration (ppb) in municipal drinking water			Cohn et al. (1994b)
	Males			
	<0.1	1.00	45	
	0.1–0.5	0.91 (0.53, 1.57)	16	
	≥5.0	0.54 (0.17, 17.7)	3	
	Females			
	<0.1	1.00	25	
	0.1–0.5	1.85 (1.03, 3.70)	22	
	≥5.0	2.36 (1.03, 5.45)	7	
Resident of Tucson Airport Area, Arizona				ADHS (1995, 1990)
	Leukemia, ≤19 yrs old			
	1970–1986	1.48 (0.74, 2.65)	11	
	1987–1991	0.80 (0.31, 2.05)	3	
Resident of West Central Phoenix, Arizona				Aickin et al. (1992)
	Leukemia, ≤19 yrs old	1.95 (1.43, 2.63)	38	

[a]Internal referents, live born children among female workers not exposed to organic solvents.
[b]Discordant pairs.

The evidence for an association between childhood leukemia and paternal exposure to solvents is quite strong (Colt and Blair, 1998); however, for studies of TCE exposure, the small numbers of exposed case fathers in two studies (McKinney et al., 1991; Lowengart et al., 1987) and, for all three studies, likelihood of misclassification resulting from a high percentage of paternal occupation information obtained from proxy interviews, limits observation interpretations. Both Lowengart et al. (1987) and McKinney et al. (1991) provide some evidence for a two- to fourfold increase of childhood leukemia risk and paternal occupational exposure although the population study of Shu et al. (2004; 1999), with 13% of case father's occupation reported by proxy respondents, does not appear to support the earlier and smaller studies.

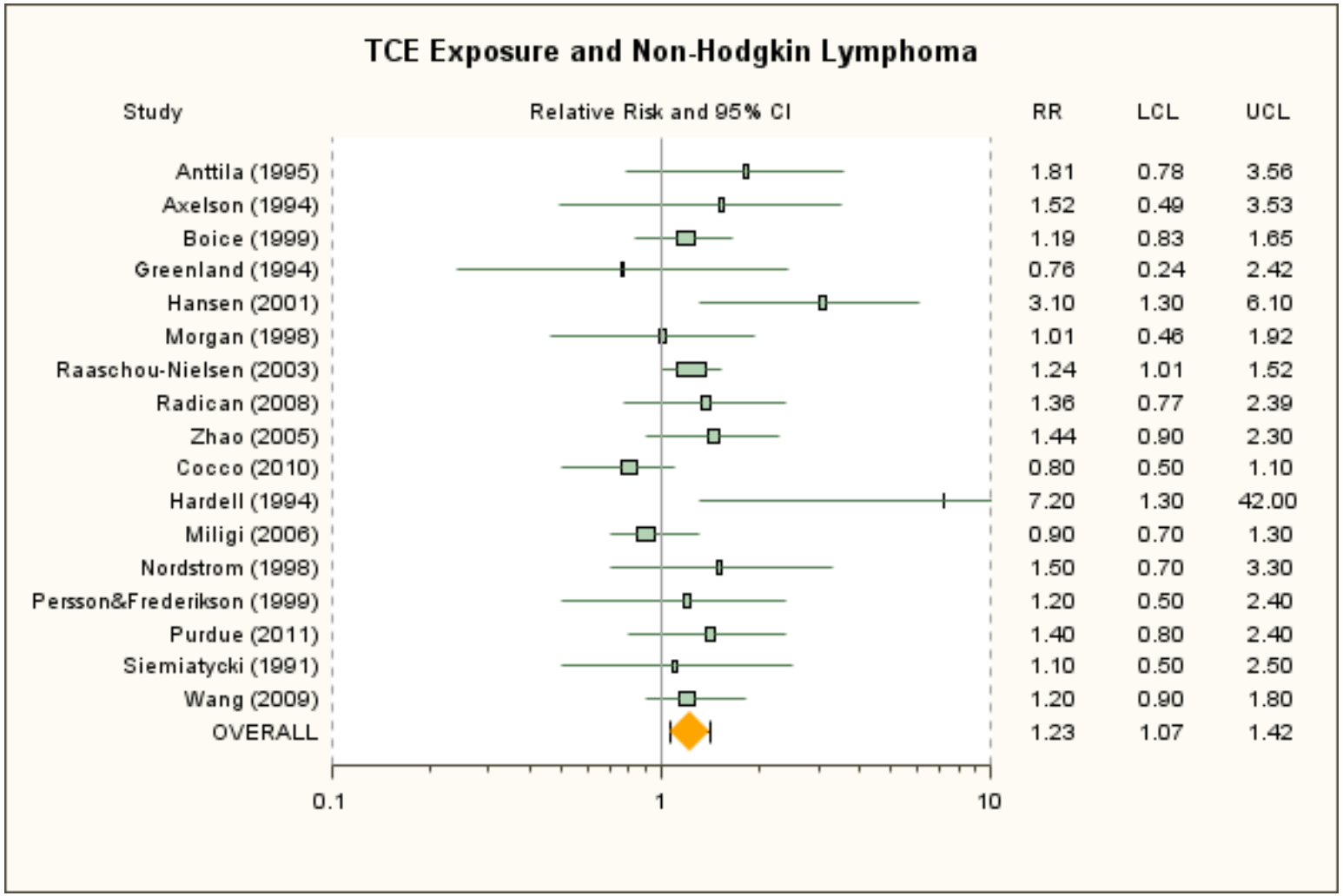
The geographic-based studies for adult lymphopoietic (see Table 4-74) or childhood leukemias (see Table 4-75) do not greatly contribute to the overall weight of evidence. While some studies observed statistically significantly elevated risks for NHL or childhood cancer, these studies generally fulfilled only the minimal of evaluation criteria with questions raised about subject selection (Morgan and Cassady, 2002), their use of less sophisticated exposure assessment approaches and associated assumption of an average exposure to all subjects (all studies), and few cases with high level parental exposure (all studies).

4.6.1.2.2. Meta-analysis of NHL risk

Meta-analysis is adopted as a tool for examining the body of epidemiologic evidence on NHL and TCE exposure and to identify possible sources of heterogeneity. The meta-analysis of NHL examines 17 cohort and case-control studies identified through a systematic review and evaluation of the epidemiologic literature on TCE exposure (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Radican et al., 2008; Miligi et al., 2006; Zhao et al., 2005; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Persson and Fredrikson, 1999; Morgan et al., 1998; Nordström et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Hardell et al., 1994; Siemiatycki, 1991) and two studies as alternatives (Boice et al., 2006b; Blair et al., 1998). These 19 studies of NHL and TCE had high likelihood of exposure, were judged to have met, to a sufficient degree, the criteria of epidemiologic design and analysis, and reported estimated risks for overall TCE exposure; 13 of these studies, also, presented estimated NHL risk with high level TCE exposure (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Radican et al., 2008; Miligi et al., 2006; Zhao et al., 2005; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Siemiatycki, 1991). Full details of the systematic review, criteria to identify studies for including in the meta-analysis, and meta-analysis methodology and findings are discussed in Appendices B and C.

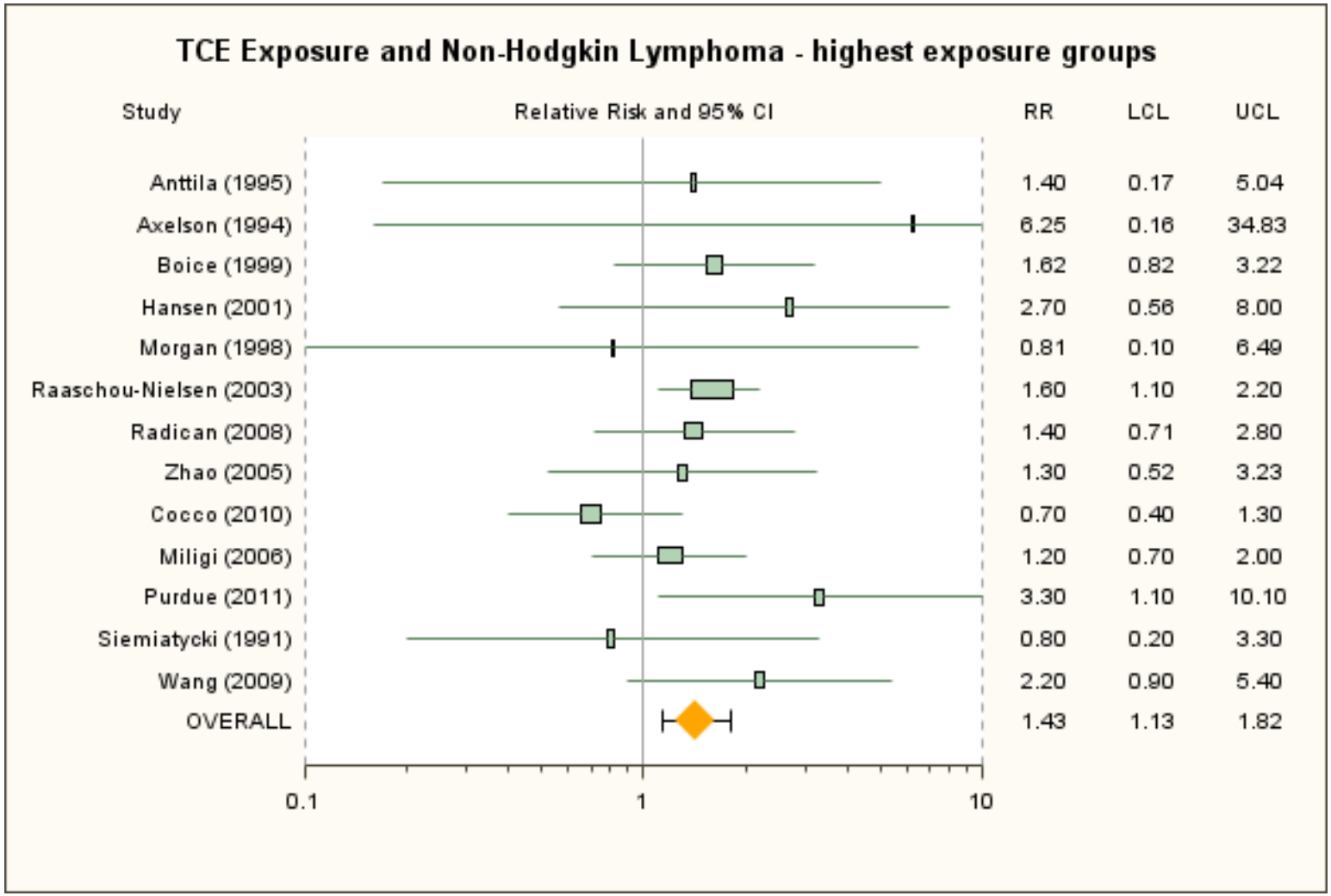
The meta-analyses of the overall effect of TCE exposure on NHL suggest a small, robust, and statistically significant increase in NHL risk. The summary estimate from the primary random effect meta-analysis (RRm) was 1.23 (95% CI: 1.07, 1.42) (see Figure 4-16). This result and its statistical significance were not influenced by individual studies. Removal of individual studies resulted in RRm estimates between 1.18 (with the removal of Hansen et al., 2001) and 1.27 (with the removal of Miligi et al. (2006) or Cocco et al. (2010)), and lower 95% CIs excluded 1.0 (all p-values were $p < 0.02$). The result is similarly not sensitive to individual risk ratio estimate selections. Use of six alternative selections, individually, resulted in RRm estimate that ranged from 1.20 (95% CI: 1.03, 1.39) (with estimated overall RR for incidence in Zhao et al., 2005) to 1.28 (95% CI: 1.09, 1.49) (with Raaschou-Nielsen et al. (2003) subgroup). Nor was the RRm estimate highly sensitive to restriction of the meta-analysis to only those studies for which RR estimates for the traditional definition of NHL were available. An alternate

analysis that omitted Miligi (which included CLLs), Nordstrom (which was a study of hairy cell leukemias), Persson and Frederikson (for which the classification system not specified), and Greenland (which included Hodgkin lymphomas) and which included Boice (2006b) instead of Zhao (which included all lymphohematopoietic cancers) yielded an RRm estimate of 1.27 (95% CI: 1.05, 1.55). Meta-analysis of the highest exposure groups, either duration, intensity, or their product, cumulative exposure, results in an RRm of 1.43 (95% CI: 1.13, 1.82), which is greater than the RRm from the overall exposure analysis, and provides additional support for an association between NHL and TCE (see Figure 4-17). No single study was overly influential; removal of individual studies resulted in RRm estimates that were all statistically significant (all with $p \leq 0.025$) and that ranged from 1.38 [with the removal of Purdue et al. (2011)] to 1.57 [with the removal of Cocco et al. (2010)]. In addition, the RRm estimate was not highly sensitive to alternate RR estimate selections. Use of the nine alternate selections, individually, resulted in RRm estimates that were all statistically significant (all with $p < 0.025$) and all in the narrow range from 1.40 (95% CI: 09, 1.80) [with Blair et al. (1998) incidence RR instead of Radican et al. (2008) mortality hazard ratio] to 1.49 (95% CI: 1.14, 1.93) [with Hansen et al. (2001) duration]. The highest exposure category groups have a reduced likelihood for exposure misclassification because they are believed to represent a greater differential TCE exposure compared to people identified with overall TCE exposure. Observation of greater risk associated with higher exposure category compared to overall (typically any vs. none) exposure comparison additionally suggests an exposure-response gradient between NHL and TCE, although estimation of a level of exposure associated with the RRm is not possible.



The summary estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the RRm estimate.

Figure 4-16. Meta-analysis of NHL and overall TCE exposure.



The summary estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the RRm estimate.

Figure 4-17. Meta-analysis of NHL and TCE exposure—highest exposure groups.

Low-to-moderate heterogeneity in RRm is observed across the results of the 17 studies in the meta-analysis of the overall effect of TCE and the 13 studies with highest exposure groups, but it was not statistically significant ($p = 0.16$ and $p = 0.30$, respectively). The I^2-values were 26% for overall exposure and 14% for highest exposure groups, suggesting low-to-moderate and low heterogeneity, respectively. To investigate the heterogeneity, subgroup analyses were done examining the cohort and case-control studies separately. Difference between cohort and case-control studies could explain much of the observed heterogeneity. In the subgroup analysis of overall exposure and of highest exposure groups, increased risk of NHL was strengthened in analysis limited to cohort studies and reduced in the case-control study analysis. Examination of heterogeneity in cohort and case-control studies of overall exposure separately was not statistically significant in either case (I^2-values for the cohort studies were 12%, suggesting low heterogeneity and 27% for the case-control studies, suggesting low-to-moderate heterogeneity), although some may be present given that statistical tests of heterogeneity are generally insensitive in cases of minor heterogeneity. Subgroup analyses examining the cohort and case-control studies highest exposure groups, separately, showed no residual heterogeneity in the cohort subgroup ($I^2 = 0\%$) and moderate heterogeneity in the case-control subgroup (I^2-value was 53%) that was not statistically significant ($p = 0.08$). Although no further attempt was made to quantitatively investigate potential sources of heterogeneity, the removal of the Cocco et al. (2010) study, an influential study, eliminates all of the heterogeneity, suggesting that the RR estimate for the highest exposure group from that study is a relative outlier.

In general, sources of heterogeneity are uncertain and may reflect several features known to influence epidemiologic studies. Study design itself is unlikely to be an underlying cause of heterogeneity and, to the extent that it may explain some of the differences across studies, is more probably a surrogate for some other difference(s) across studies that may be associated with study design. Furthermore, other potential sources of heterogeneity may be masked by the broad study design subgroupings. The true source(s) of heterogeneity across these studies is an uncertainty.

One reason may be differences in exposure assessment and in overall TCE exposure concentration between cohort and case-control studies. Several cohort and case-control studies included TCE assignment from information on job and task exposures, e.g., a JEM (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Radican et al., 2008; Boice et al., 2006b; Miligi et al., 2006; Zhao et al., 2005; Boice et al., 1999; Morgan et al., 1998; Siemiatycki, 1991), or from an exposure biomarker in either breath or urine (Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994). Three case-control studies (Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994) relied on self-reported TCE exposure. No information is available to judge the degree of possible misclassification bias associated with a particular exposure assessment approach; it is quite possible that in some cohort studies, in which past exposure is inferred from various data sources, exposure misclassification may be as great as in population- or hospital-

based case-control studies. In addition, a low overall TCE exposure prevalence is anticipated in population case-control studies, which would typically assess a large number of workplaces and operations, where exposures are less well defined, and where case and control subjects identified as exposed to TCE probably have minimal contact (NRC, 2006). Observed higher risk ratios with higher exposure categories in NHL case-control studies support exposure differences as a source of heterogeneity.

Diagnostic inaccuracies are likely another source of heterogeneity in the meta-analysis through study differences in NHL groupings and in lymphoma classification schemes, although restricting the meta-analysis to only those studies for which RR estimates based on the traditional NHL definition were available did not eliminate all heterogeneity. All studies include a broad but slightly different group of lymphosarcoma, reticulum-cell sarcoma, and other lymphoid tissue neoplasms (Codes 200 and 202), except Nordstrom et al. (1998), Zhao et al. (2005), and Greenland et al. (1994). Cohort studies have some consistency in coding NHL, with NHL defined as lymphosarcoma and reticulum-cell sarcoma (200) and other lymphoid tissue neoplasms (202) using the ICD, Revision 7, 200 and 202—four studies (Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994), ICD-Adapted, Revision 8 (Blair et al., 1998), and ICD-7, -8, -9, and -10, per the version in use at the time of death (as presented in Morgan et al. (1998) [as presented in Mandel et al. (2006), Boice et al. (1999), Radican et al. (2008)], as does the case-control study of Siemiatycki (1991) whose coding scheme for NHL is consistent with ICD 9, 200 and 202. Case-control studies, on the other hand, have adopted other classification systems for defining NHL including the NCI Working Formulation (Miligi et al., 2006), Rappaport (Hardell et al., 1994), or else do not identify the classification system for defining NHL (Persson and Fredrikson, 1999). Cocco et al. (2010) used the WHO/Revised European-American Lymphoma (REAL) classification system, which reclassifies lymphocytic leukemias and NHLs as lymphomas of B-cell or T-cell origin and considers CLLs and multiple myelomas as (non-Hodgkin) lymphomas; however, U.S. EPA was able to obtain results generally consistent with the traditional NHL definition from Dr. Cocco, although lymphomas not otherwise specified were excluded. Wang et al. (2009) defined NHL using ICD-O-2 codes (M-9590-9595, 9670-9688, 9690-9698, 9700-9723), which is consistent with the traditional definition of NHL (i.e., ICD-7, -8, -9 codes 200 + 202). Purdue et al. (2011) used ICD-O-3 codes 967-972, which is generally consistent with the traditional definition of NHL, although this grouping does not include the malignant lymphomas of unspecified type coded as M-9590-9599.

There is some evidence of potential publication bias in this data set; however, it is uncertain that this is actually publication bias rather than an association between SE and effect size resulting for some other reason, e.g., a difference in study populations or protocols in the smaller studies. Furthermore, if there is publication bias in this data set, it does not appear to account completely for the finding of an increased NHL risk.

NRC (2006) deliberations on TCE commented on two prominent evaluations of the then-current epidemiologic literature using meta-analysis techniques. These studies were by Wartenberg et al. (2000), and by Kelsh et al. (2005), submitted by Exponent-Health Sciences to NRC during their deliberations and subsequently published in a paper on NHL (Mandel et al., 2006) and a paper on multiple myeloma and leukemia (Alexander et al., 2006). The NRC found weaknesses in the techniques used in each of these studies, and suggested that EPA conduct a new meta-analysis of the epidemiologic data on TCE using objective and transparent criteria so as to improve on the past analyses. EPA staff conducted their analysis according to NRC (2006) suggestions for transparency, systematic review criteria, and examination of both cohort and case-control studies. The EPA analysis of NHL analysis considered a larger number of studies than in the previous analyses (Mandel et al., 2006; Wartenberg et al., 2000), includes recently published studies (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Radican et al., 2008; Boice et al., 2006b; Miligi et al., 2006; Zhao et al., 2005), and combines both cohort and case-control studies.

4.6.2. Animal Studies

The immunosuppressive and immunomodulating potential of TCE has not been fully evaluated in animal models across various exposure routes, over various relevant durations of exposure, across representative lifestages, and/or across a wide variety of endpoints. Nevertheless, the studies that have been conducted indicate a potential for TCE-induced immunotoxicity, both following exposures in adult animals and during immune system development (i.e., in utero and preweaning exposures).

4.6.2.1. Immunosuppression

A number of animal studies have indicated that moderate to high concentrations of TCE over long periods have the potential to result in immunosuppression in animal models, dependant on species and gender. These studies are described in detail below and summarized in Table 4-76.

4.6.2.1.1. Inhalation exposures

Mature cross-bred dogs (5/group) were exposed to 0-, 200-, 500-, 700-, 1,000-, 1,500-, or 2,000 ppm TCE for 1 hour or to 700 ppm TCE for 4 hours, by tracheal intubation under i.v. sodium pentobarbital anesthesia. An additional group of dogs was exposed by venous injection of 50 mg/kg TCE administered at a rate of 1 mL/minute (Hobara et al., 1984). Blood was sampled pre- and postexposure for erythrocyte and leukocyte counts. Marked, transient decreases in leukocyte counts were observed at all exposure levels 30 minutes after initiation of exposure. At the end of the exposure period, all types of leukocytes were decreased (by 85%); neutrophils were decreased 33%, and lymphocytes were increased 40%. There were no treatment-related changes in erythrocyte counts, hematocrit values, or thrombocyte counts.

Table 4-76. Summary of TCE immunosuppression studies

Exposure route/vehicle, duration, dose	NOAEL; LOAEL[a]	Results	Reference, species/strain sex/number
Inhalation exposure studies[b]			
Single 1-hr exposure to all dose groups; plus single 4-hr exposure at 700 ppm[c] 0, 200, 500, 700, 1,000, 1,500, or 2,000 ppm	LOAEL: 200 ppm	Marked transient ↓ leukocyte counts at all exposure levels 30 min after initiating exposure. At end of exposure, 85% ↓ leukocyte counts (33% ↓ neutrophils, 40% ↓ lymphocytes).	Hobara et al. (1984) Dog, cross-bred, both sexes, 5/group
Single 3-hr exposure. Also, 3 hrs/d on 5 d at lowest dose 0, 2.6, 5.2, 10.6, 25.6, or 48 ppm	NOAEL: 2.6 ppm LOAEL: 5.2 ppm	Challenged with *Streptococcus zooepidemicus* to assess susceptibility to infection and *Klebsiella pneumoniae* to assess bacterial clearance. For single exposure: dose-related statistically significant ↑ mortality at ≥5.2 ppm over 14 d. Statistically significant ↓ in bactericidal activity at 10.6 ppm.	Aranyi et al. (1986) Mouse, CD-1 females, 4–5 wks old, approximately 30 mice/group, 5–10 replications; for pulmonary bactericidal activity assay, 17–24 mice/group
Single 3-hr exposure. 0, 5, 10, 25, 50, 100, 200 ppm	NOAEL: 25 ppm LOAEL: 50 ppm	Challenged with *Streptococcus zooepidemicus* to assess susceptibility to infection and bacterial clearance. For single exposure: dose-related statistically significant ↑ mortality at ≥50 ppm over 20 d. Dose dependent responses also observed in the clearance of bacteria from the lung at ≥50 ppm, the number of mice with delayed bacterial clearance at various postinfection time points at ≥50 ppm, and the phagocytic function of alveolar macrophages at 200 ppm.	Selgrade and Gilmour (2010) Mouse, CD-1 females, 5–6 wks old, at least 38 mice/group
Single 3-hr exposure, 50–200 ppm[d]		Challenged with *Streptococcus zooepidemicus*. Dose-related ↑ mortality, bacterial antiphagocytic capsule formation, and bacterial survival. Dose-related impairment of alveolar macrophages; increased neutrophils in bronchoalveolar fluid at 3 d postinfection.	Park et al. (1993) (abstract) Mouse, CD-1, (sex and number/group not specified)
4-wk, 6 hrs/d, 5 d/wk 0, 100, 300, or 1,000 ppm	**NOAEL: 300 ppm LOAEL: 1,000 ppm**	**At 1,000 ppm, 64% ↓ plaque-forming cell assay response.**	**Woolhiser et al. (2006) Rat, Sprague-Dawley, female, 16/group**

Table 4-76. Summary of TCE immunosuppression studies (continued)

Exposure route/vehicle, duration, dose	NOAEL; LOAEL[a]	Results	Reference, species/strain sex/number
Oral exposure studies			
Gavage in 10% Emulphor, 14 d, daily, 0, 24, or 240 mg/kg-d	LOAEL: 24 mg/kg-d	Statistically significant ↓ cell-mediated immune response to SRBC at both dose levels.	Sanders et al. (1982b) Mouse, CD-1, male, 9–12/group
Drinking water with 1% Emulphor, 4–6 mo 0, 0.1, 1.0, 2.5, or 5.0 mg/mL	**LOAEL: 0.1 mg/kg-d**	**In females, humoral immunity ↓ at 2.5 and 5 mg/mL TCE, whereas cell-mediated immunity ↓ and bone marrow stem cell colonization ↓ at all four concentrations. The males were relatively unaffected after both 4 and 6 mo.**	**Sanders et al. (1982b) Mouse, CD-1, male and female, 7–25/group**
Gavage, 14 d, 0, 14.4, or 144 mg/kg-d CH	NOAEL: 144 mg/kg-d	No treatment-related effects.	Kauffmann et al. (1982) Mouse, CD-1, male, 12/group
Drinking water, 90 d, 0, 0.07, or 0.7 mg/mL CH. (M: 0, 16, or 160 mg/kg-d; F: 0, 18, or 173 mg/kg-d)	NOAEL: 0.07 mg/mL LOAEL: 0.7 mg/mL	Statistically significant ↓ cell-mediated immune response (plasma hemagglutination titers and spleen antibody-producing cells of mice sensitized to SRBC) in females at 0.7 mg/mL.	Kauffmann et al. (1982) Mouse, CD-1, male and female, 15–20/group
Drinking water, From mating to PND 21 or 56, (Emulphor concentration not provided) 0 (Emulphor), 1, or 10 ppm	LOAEL: 1 ppm	At 10 ppm, ↓ body weight and length at PND 21. IgM antibody response to SRBC challenge suppressed in both ♂ and ♀ pups at 10 ppm, and ♂ pups at 1 ppm, ↓ in splenic CD4+CD8-T-cells. At 56 PND, striking ↑ in NK cell activity seen at both doses.	Adams et al. (2003) (abstract) Mouse, $B6C3F_1$, both sexes, numbers of pups not stated
Drinking water, from GDs 0 to 3 or 8 wks of age, 0, 1,400, or 14,000 ppb	LOAEL: 1,400 ppb	Suppressed PFC responses in both sexes and ages at 14,000 ppb, in males at both ages at 1,400 ppb, and in females at 8 wks at 1,400 ppb. Numbers of spleen B220+ cells ↓ at 3 wks at 14,000 ppb. Pronounced ↑ thymus T-cell populations at 8 wks.	Peden-Adams et al. (2006) Mouse, $B6C3F_1$, dams and both sexes offspring, 5 litters/group; 5–7 pups/group at 3 wks; 4–5 pups/sex/group at 8 wks
Drinking water, from GD 0 to 7–8 wks of age; 0, 0.5, or 2.5 mg/mL	LOAEL: 0.5 mg/mL	At 0.5 mg/mL: statistically significant ↓ postweaning weight; statistically significant↑ IFNγ produced by splenic CD4+ cells at 5–6 wks; statistically significant ↓ splenic CD8+ and B220+ lymphocytes; statistically significant↑ IgG2a and histone; statistically significant altered CD4–/CD8– and CD4+/CD8+ thymocyte profile At 2.5 mg/mL: statistically significant ↓ postweaning weight; statistically significant ↑ IFNγ produced by splenic CD4+ and CD8+ cells at 4–5 and 5–6 wks; statistically significant ↓ splenic CD4+, CD8+, and B220+ lymphocytes; statistically significant altered CD4+/CD8+ thymocyte profile.	Blossom and Doss (2007) Mouse, MRL +/+, dams and both sexes offspring, 3 litters/group; 8–12 pups/group

Table 4-76. Summary of TCE immunosuppression studies (continued)

Exposure route/vehicle, duration, dose	NOAEL; LOAEL[a]	Results	Reference, species/strain sex/number
Drinking water, from GD 0 to PND 42; 0 or 0.1 mg/mL; maternal dose = 25.7 mg/kg-d; offspring PNDs 24–42 dose = 31.0 mg/kg-d	LOAEL: 0.1 mg/mL	At 0.1 mg/mL: at PND 20, statistically significant ↑ thymocyte cellularity and distribution, associated with statistically significant ↑ in thymocyte subset distribution; statistically significant ↑ reactive oxygen species generation in total thymocytes; statistically significant ↑ in splenic CD4+ T-cell production of IFN-γ and IL-2 in females and TNF-α in males at PND 42.	Blossom et al. (2008) Mouse, MRL +/+, dams and both sexes offspring, 8 litters/group; 3–8 pups/group
Drinking water, from GD 0 to 12 mo of age; 0 (1% Emulphor), 1,400, or 14,000 ppb	LOAEL: 1,400 ppb	At 1,400 ppb: splenic CD4–/CD8– cells statistically significant ↑ in females; thymic CD4+/CD8+ cells statistically significant ↓ in males; 18% ↑ in male kidney weight. At 14,000 ppb: thymic T-cell subpopulations (CD8+, CD4/CD8–, CD4+) statistically significant ↓ in males.	Peden-Adams et al. (2008) Mouse, MRL +/+, dams and both sexes offspring, unknown number litters/group, 6–10 offspring/sex/group
i.p. injection exposure studies			
3 d, single daily injection, 0, 0.05, 0.5, or 5 mmol/kg-d	NOAEL: 0.05 mmol/kg-d LOAEL: 0.5 mmol/kg-d	↓ NK cell activity at 0.5 and 5 mmol/kg-d. ↓ splenocyte counts at 5 mmol/kg-d.	Wright et al. (1991) Rat, Sprague-Dawley
3 d, single daily injection, 0 or 10 mmol/kg-d	LOAEL: 10 mmol/kg-d	↓ NK cell activity and ↓ spleen weights at 10 mmol/kg-d.	Wright et al. (1991) Mouse, $B6C3F_1$

[a]NOAEL and LOAEL are based upon reported study findings.
[b]**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5).
[c]Inhalation, tracheal intubation under anesthesia.
[d]Exact dose levels not specified.

↓, ↑ = decreased, increased; PFC = plaque-forming cell; SRBC = sheep red blood cells

In a study that examined the effects of a series of inhaled organic chemical air contaminants on murine lung host defenses, Aranyi et al. (Aranyi et al., 1986) exposed female CD-1 mice to single 3-hour exposures of TCE at time-weighted concentrations of 0, 2.6, 5.2, 10.6, 25.6, or 48 ppm. Additionally, at the dose at which no adverse treatment-related effect occurred with a single exposure (i.e., 2.6 ppm), a multiple exposure test (5 days, 3 hours/day) was conducted. Susceptibility to *Streptococcus zooepidemicus* aerosol infection and pulmonary bactericidal activity to inhaled *Klebsiella pneumoniae* were evaluated. There was a significant ($p < 0.0001$) treatment by concentration interaction for mortality, with the magnitude of the effect increasing with concentration. A significant ($p < 0.0001$) treatment by concentration interaction was also found for bactericidal activity. Single 3-hour exposures at 10.6, 25.6, and 48 ppm resulted in significant increases in mortality, although increases observed after single exposures at 5.2 or 2.6 ppm or five exposures at 2.6 ppm were not significant. Pulmonary bactericidal activity was significantly decreased after a single exposure at 10.6 ppm, but single exposures to 2.6 or 5.2 ppm resulted in significant increases.

Suppression of pulmonary host defenses and enhanced susceptibility to respiratory bacterial infection was studied in female CD-1 mice by Selgrade and Gilmour (2010). The mice (5–6 weeks of age; at least 38 per exposure group) were exposed via inhalation for 3 hours to concentrations of 0, 5, 10, 25, 50, 100, or 200 ppm TCE. The mice were then challenged by aerosol doses of *S. zooepidemicus*. Bacterial clearance (based upon organisms present in lung lavage fluid) and a phagocytic index (percentage of phagocytic cells in lung lavage fluid and the number of bacteria ingested per phagocytic cell) were assessed. Mortality due to infection was significantly increased with TCE exposure concentration at exposures of 50 ppm and higher (NOAEL = 25 ppm). Dose-dependent responses were also observed for the clearance of bacteria from the lung at ≥25 ppm, the number of mice with delayed bacterial clearance at various postinfection time points at ≥25 ppm, and the phagocytic function of alveolar macrophages at 200 ppm. The higher NOAEL for mortality observed in this study compared to Aranyi et al. (1986) (i.e., 25 vs. 5 ppm) was attributed to the use of unencapsulated bacteria in this study; the study authors suggested that this may be more representative of the human condition.

In a host-resistance assay, CD-1 mice (sex and number/group not specified) exposed to TCE by inhalation for 3 hours at 50–200 ppm were found to be more susceptible to increased infection following challenge with *S. zooepidemicus* administered via aerosol (Park et al., 1993). Dose-related increases in mortality, bacterial antiphagocytic capsule formation, and bacterial survival were observed. Alveolar macrophage phagocytosis was impaired in a dose-responsive manner, and an increase in neutrophils in bronchoalveolar lavage fluid was observed in exposed mice 3 days post infection.

A guideline (OPPTS 870.3800) 4-week inhalation immunotoxicity study was conducted in female Sprague-Dawley rats (Woolhiser et al., 2006). The animals (16/group) were exposed to TCE at nominal levels of 0, 100, 300, or 1,000 ppm for 6 hours/day, 5 days/week. Effects on

the immune system were assessed using an antigen response assay, relevant organs weights, histopathology of immune organs, and hematology parameters. Four days prior to study termination, the rats were immunized with sheep red blood cells (SRBC), and within 24 hours following the last exposure to TCE, a plaque-forming cell (PFC) assay was conducted to determine effects on splenic anti-SRBC IgM response. Minor, transient effects on body weight and food consumption were noted in treated rats for the first 2 weeks of exposure. Mean relative liver and kidney weights were significantly ($p = 0.05$) increased at 1,000 ppm as compared to control, while lung, spleen, and thymus weights were similar to control. No treatment-related effects were observed for hematology, white blood cell differential counts, or histopathological evaluations (including spleen, thymus, and lung-associated lymph nodes). At 1,000 ppm, rats demonstrated a 64% decrease in PFC assay response. LDH, total protein levels, and cellular differentiation counts evaluated from bronchoalveolar lavage (BAL) samples were similar between control and treated groups. A phagocytic assay using BAL cells showed no alteration in phagocytosis, although these data were not considered fully reliable since: (1) the number of retrieved macrophage cells was lower than expected and pooling of samples was conducted and (2) samples appear to have been collected at 24 hours after the last exposure (rather than within approximately 2 hours of the last exposure), thereby allowing for possible macrophage recovery. The NOAEL for this study was considered by the study authors to be 300 ppm, and the LOAEL was 1,000 ppm; however, the effect level may have actually been lower. It is noted that the outcome of this study does not agree with the studies by Aranyi et al. (1986) and Park et al. (1993), both of which identified impairment of macrophage phagocytic activity in BAL following inhalation TCE exposures.

4.6.2.1.2. Oral exposures

In a study by Sanders et al. (1982b), TCE was administered to male and female CD-1 mice for 4 or 6 months in drinking water at concentrations of 0, 0.1, 1, 2.5, or 5 mg/mL(Sanders et al., 1982b). In females, humoral immunity was suppressed at 2.5 and 5 mg/mL, while cell-mediated immunity and bone marrow stem cell activity were inhibited at all dose levels. Male mice were relatively unaffected either at 4 or 6 months, even though a preliminary study in male CD-1 mice (exposed to TCE for 14 days by gavage at 0, 24, or 240 mg/kg-day) had demonstrated a decrease in cell-mediated immune response to SRBC in male mice at both treatment levels.

A significant decrease in humoral immunity (as measured by plasma hemagglutination titers and the number of spleen antibody producing cells of mice sensitized to sheep erythrocytes) was observed by Kaufmann et al. (1982) in female CD-1 mice (15–20/group) following a 90-day drinking water exposure to 0, 0.07, or 0.7 mg/mL (equivalent to 0, 18, or 173 mg/kg) CH, a metabolite of TCE. Similar responses were not observed in male CD-1 mice

exposed for 90 days in drinking water (at doses of 0, 16, or 160 mg/kg-day), or when administered CH by gavage to 12/group for 14 days at 14.4 or 144 mg/kg-day.

The potential for developmental immunotoxicity was assessed in $B6C3F_1$ mice administered TCE in drinking water at dose levels of 0, 1,400 or 14,000 ppb from GD 0 to either 3 or 8 weeks of age (Peden-Adams et al., 2006; Adams et al., 2003 [preliminary data]). At 3 and 8 weeks of age, offspring lymphocyte proliferation, NK cell activity, SRBC-specific IgM production (PFC response), splenic B220+ cells, and thymus and spleen T-cell immunophenotypes were assessed. Delayed-typed hypersensitivity and autoantibodies to double-stranded DNA (dsDNA) were evaluated in offspring at 8 weeks of age. Observed positive responses consisted of suppressed PFC responses in males at both ages and both TCE treatment levels, and in females at both ages at 14,000 ppb and at 8 weeks of age at 1,400 ppb. Spleen numbers of B220+ cells were decreased in 3-week-old pups at 14,000 ppb. Pronounced increases in all thymus T-cell subpopulations (CD4+, CD8+, CD4+/CD8+, and CD4–/CD8–) were observed at 8 weeks of age. Delayed hypersensitivity response was increased in 8-week-old females at both treatment levels and in males at 14,000 ppb only. No treatment-related increase in serum anti-dsDNA antibody levels was found in the offspring at 8 weeks of age.

In a study designed to examine potential susceptibility of the young (Blossom and Doss, 2007), TCE was administered to groups of pregnant MRL +/+ mice in drinking water at occupationally-relevant levels of 0, 0.5, or 2.5 mg/mL. A total of 3 litters per treatment group were maintained following delivery (i.e., a total of 11 pups at 0 mg/mL TCE, 8 pups at 0.5 mg/mL TCE, and 12 pups at 2.5 mg/mL TCE), and TCE was continuously administered to the offspring until young adulthood (i.e., 7–8 weeks of age). Although there were no effects on reproduction, offspring postweaning body weights were significantly decreased in both treated groups. Additionally, TCE exposure was found to modulate the immune system following developmental and early life exposures. Decreased spleen cellularity and reduced numbers of CD4+, CD8+, and B220+ lymphocyte subpopulations were observed in the postweaning offspring. Thymocyte development was altered by TCE exposures, as evidenced by significant alterations in the proportions of double-negative subpopulations and inhibition of in vitro apoptosis in immature thymocytes. TCE was also shown to induce a dose-dependent increase in CD4+ and CD8+ T-lymphocyte IFNγ in peripheral blood by 4–5 weeks of age, although these effects were no longer observed at 7–8 weeks of age. Serum antihistone autoantibodies and total IgG_{2a} were significantly increased in treated offspring; however, no histopathological signs of autoimmunity were observed in the liver and kidneys at sacrifice.

This increase in T-cell hyperactivity was further explored in a study by Blossom et al. (2008). In this study, MRL +/+ mice were treated with 0 or 0.1 mg/mL TCE in the drinking water. Based on drinking water consumption data, average maternal doses of TCE were 25.7 mg/kg-day, and average offspring (PNDs 24–42) doses of TCE were 31.0 mg/kg-day. Treatment was initiated at the time of mating, and continued in the females (8/group) throughout

gestation and lactation. Pups were weaned at PND 24, and the offspring were continued on drinking water treatment in a group-housed environment until study termination (PND 42). Subsets of offspring were sacrificed at PNDs 10 and 20, at which time developmental and functional endpoints in the thymus were evaluated (i.e., total cellularity, CD4+/CD8+ ratios, CD24 differentiation markers, and double-negative subpopulation counts). Indicators of oxidative stress were measured in the thymus at PNDs 10 and 20, and in the brain at PND 42. Mitogen-induced intracellular cytokine production by splenic CD4+ and CD8+ T-cells was evaluated in juvenile mice and brain tissue was examined at PND 42 for evidence of inflammation. Behavioral testing was also conducted; these methods and results are described in Section 4.3. TCE treatment did not affect reproductive capacity, parturition, or ability of dams to maintain litters. The mean body weight of offspring was not different between the control and treated groups. Evaluation of the thymus identified a significant treatment-related increase in cellularity, accompanied by alterations in thymocyte subset distribution, at PND 20 (sexes combined). TCE treatment also appeared to promote T-cell differentiation and maturation at PND 42, and ex vivo evaluation of cultured thymocytes indicated increased reactive oxygen species generation. Evaluation of peripheral blood indicated that splenic CD4+ T-cells from TCE-exposed PND 42 mice produced significantly greater levels of IFN-γ and IL-2 in males and TNF-α in both sexes. There was no effect on cytokine production on PND 10 or 20. The dose of TCE that resulted in adverse offspring outcomes in this study (i.e., 0.1 mg/mL, equivalent to 25.7–31.0 mg/kg-day) is comparable to that which has been previously demonstrated to result in immune system alterations and autoimmunity in adult MRL +/+ mice (i.e., 0.1 mg/mL, equivalent to 21 mg/kg-day (Griffin et al., 2000b).

Another study that examined the effects of developmental exposure to TCE on the MRL+/+ mouse was conducted by Peden-Adams et al. (2008). In this study, MRL/MpJ (i.e., MRL +/+) mice (unspecified number of dams/group) were exposed to TCE (solubilized with 1% Emulphor) in drinking water at levels of 0, 1,400, or 14,000 ppb from GD 0 and continuing until the offspring were 12 months of age. TCE concentrations in the drinking water were reported to be analytically confirmed. Endpoints evaluated in offspring at 12 months of age included final body weight; spleen, thymus, and kidney weights; spleen and thymus lymphocyte immunophenotyping (CD4 or CD8); splenic B-cell counts; mitogen-induced splenic lymphocyte proliferation; serum levels of autoantibodies to dsDNA and glomerular antigen, periodically measured from 4 to 12 months of age; and urinary protein measures. Reported sample sizes for the offspring measurements varied from 6 to 10 per sex per group; the number of source litters represented within each sample was not specified. The only organ weight alteration was an 18% increase in kidney weight in the 1,400 ppb males. Splenic CD4–/CD8– cells were altered in female mice (but not males) at 1,400 ppm only. Splenic T-cell populations, numbers of B220+ cells, and lymphocyte proliferation were not affected by treatment. Populations of thymic T-cell subpopulations (CD8+, CD4–/CD8–, and CD4+) were significantly decreased in male but not

female mice following exposure to 14,000-ppb TCE, and CD4+/CD8+ cells were significantly reduced in males by treatment with both TCE concentrations. Autoantibody levels (anti-dsDNA and anti-glomerular antigen) were not increased in the offspring over the course of the study, indicating that TCE did not contribute to the development of autoimmune disease markers following developmental exposures that continued into adult life.

Overall, the studies by Peden-Adams et al. (2008; 2006), Blossom and Doss (2007), and Blossom et al. (2008), which examined various immunotoxicity endpoints following exposures that spanned the critical periods of immune system development in the rodent, were generally not designed to assess issues such as posttreatment recovery, latent outcomes, or differences in severity of response that might be attributed to the early life exposures.

4.6.2.1.3. i.p. administration

Wright et al. reported that following 3 days of single i.p. injections of TCE in Sprague-Dawley rats at 0, 0.05, 0.5, or 5 mmol/kg-day and B6C3F_1 mice at 0 or 10 mmol/kg-day, NK cell activity was depressed in the rats at the mid- and high-dose levels, and in the mice at the high-dose level (Wright et al., 1991). Also at the highest dose levels tested, decreased splenocyte counts and relative spleen weight were observed in the rats and mice, respectively. In vitro assays demonstrated treatment-related decreases in splenocyte viability, inhibition of lipopolysaccharide-stimulated lymphocyte mitogenesis, and inhibited NK cell activity suggesting the possibility that compromised immune function may play a role in carcinogenic responses of experimental animals treated with TCE.

4.6.2.2. Hypersensitivity

Evidence of a treatment-related increase in delayed hypersensitivity response has been observed in guinea pigs following dermal exposures with TCE and in mice following exposures that occurred both during development and postnatally (see Table 4-77).

In a modified guinea pig maximization test, Tang et al. (2002) evaluated the contact allergenicity potential of TCE and three metabolites (TCA, TCOH, and CH) in four animals (FMMU strain, sex not specified) per group (Tang et al., 2002). Edema and erythema indicative of skin sensitization (and confirmed by histopathology) were observed. Sensitization rates were reported to be 71.4% for TCE and 58.3% for TCA, as compared to a reference positive control response rate (i.e., 100% for 2,4-dinitrochlorobenzene). In this study, the mean response scores for TCE, TCA, and 2,4-dinitrochlorobenzene were 2.3, 1.1, and 6.0, respectively. TCE was judged to be a strong allergen and TCA was a moderate allergen, according to the criteria of Magnusson and Kligman (1969). TCOH and CH were not found to elicit a dermal hypersensitivity response.

Table 4-77. Summary of TCE hypersensitivity studies[a]

Exposure route/vehicle, duration, dose	NOAEL; LOAEL[b]	Results	Reference, species/strain sex/number
Induction by single intradermal injection, then challenge by dermal application at 21 d 0 or 0.1 mL induction; 0 or 0.2 mL challenge TCE, TCA, TCOH, and CH		Edema and erythema (confirmed by histopathology) indicative of skin sensitization for TCE (strong sensitizer) and TCA (moderate sensitizer)	Tang et al. (2002) Guinea pig, FMMU strain, sex not specified, 4/group
Intradermal injection, 0, 167, 500, 1,500, or 4,500 mg/kg Dermal patch, 0 or 900 mg/kg Hypersensitivity: total dose from induction through challenge <340 mg/kg	Intradermal NOAEL: 500 mg/kg Intradermal LOAEL: 1,500 mg/kg Dermal patch NOAEL: 900 mg/kg	Intradermal injection: At 1,500 mg/kg: statistically significant ↑ AST; at 4,500 mg/kg, statistically significant ↑ ALT and AST, statistically significant ↓ total protein and globulin; fatty degeneration of liver Dermal patch: no effects of treatment Hypersensitivity: sensitization rate of 66% (strong sensitizer), with edema and erythema; statistically significant ↑ ALT, AST, and LDH; statistically significant ↑ relative liver weight; statistically significant ↓ albumin, IgA, and GGT; hepatic lesions (ballooning changes)	Tang et al. (2008) Guinea pig, FMMU strain, female, 5–6/group for intradermal/dermal patch study, 10/group for hypersensitivity study, female
Drinking water, from GD 0 to 8 wks of age 0, 1,400, or 14,000 ppb	**LOAEL: 1,400 ppb**	**Statistically significant ↑ swelling of foot pad in females at 1,400 and in both sexes at 14,000 ppb**	**Peden-Adams et al. (2006) Mouse, B6C3F$_1$, both sexes, 5 litters/group; 4–5 pups/sex/group at 8 wks[c]**

[a]**Bolded study** carried forward for consideration in dose-response assessment (see Chapter 5).

[b]NOAEL and LOAEL are based upon reported study findings.
[c]Subset of immunosuppression study.

Immune-mediated hepatitis associated with dermal hypersensitivity reactions in the guinea pig following TCE exposures was characterized by Tang et al. (2008). In this study, FMMU strain female guinea pigs (5–6/group) were treated with intradermal injection of 0, 167, 500, 1,500, or 4,500 mg/kg TCE or with a dermal patch containing 0 or 900 mg/kg TCE and sacrificed at 48 hours posttreatment. At the intradermal dose of 1,500 mg/kg, a significant increase ($p < 0.05$) in serum AST level was observed. At 4,500 mg/kg, significantly ($p < 0.01$) increased ALT and AST levels were reported, and total protein and globulin decreased significantly ($p < 0.05$). Histopathological examination of the liver revealed fatty degeneration, hepatic sinusoid dilation, and inflammatory cell infiltration. No changes were observed at the intradermal doses of ≤500 mg/kg, or the dermal patch dose of 900 mg/kg. A Guinea Pig Maximization Test was also conducted according to the procedures of Magnusson and Kligman on 10 FMMU females/group, in which the total TCE dosage from induction through challenge phases was below 340 mg/kg. TCE treatment resulted in dermal erythema and edema, and the sensitization rate was 66% (i.e., classified as a strong sensitizer). Significant increases ($p < 0.05$) in ALT, AST, LDH, and relative liver weight, and significant decreases ($p < 0.05$) in albumin, IgA, and GGT were observed. Additionally, hepatic lesions (diffuse ballooning changes without lymphocyte infiltration and necrotic hepatocytes) were noted. It was concluded that TCE exposure to guinea pigs resulted in delayed type hypersensitivity reactions with hepatic injury that was similar to occupational medicamentosa-like dermatitis disorders observed in human occupational studies.

Also, as indicated in Section 4.6.2.1.2, in a developmental immunotoxicity-type study in $B6C3F_1$ mice, administration of TCE in drinking water at dose levels of 0, 1,400, or 14,000 ppb from GD 0 through to 8 weeks of age resulted in an increased delayed hypersensitivity response in 8-week-old female offspring at both treatment levels and in males at the high dose of 14,000 ppb (Peden-Adams et al., 2006).

In an in vitro study that evaluated a number of chlorinated organic solvents, nonpurified rat peritoneal mast cells (NPMC) and rat basophilic leukemia (RBL-2H3) cells were sensitized with anti-DNP (dinitrophenol) monoclonal IgE antibody and then stimulated with DNP-conjugated bovine serum albumin plus TCE (Seo et al., 2008). TCE enhanced antigen-induced histamine release from NPMC and RBL-2H3 cells in a dose-related manner, and increased IL-4 and TNF-α production from the RBL-2H3 cells. In an in vivo study, i.p.-injected TCE was found to markedly enhance passive cutaneous anaphylaxis reaction in antigen-challenged rats. These results suggest that TCE increases histamine release and inflammatory mediator production from antigen-stimulated mast cells via the modulation of immune responses; TCE exposure may lead to the enhancement of allergic disease through this response.

4.6.2.3. Autoimmunity

A number of studies have been conducted to examine the effects of TCE exposure in mouse strains (i.e., MRL +/+, MRL –lpr, or NZB × NZW) which are all known to be genetically susceptible to autoimmune disease. The studies have demonstrated the potential for TCE to induce autoimmune disease (as demonstrated in Table 4-78, which summarizes those studies which assessed serology, ex vivo assays of cultured splenocytes, and/or clinical or histopathology). These and other studies conducted in susceptible mouse strains have proven to be useful tools in exploring various aspects of the mode of action for this response.

Khan et al. (1995) used the MRL +/+ mouse model to evaluate the potential for TCE and one of its metabolites, DCAC to elicit an autoimmune response. Female mice (4–5/group) were dosed by i.p. injection with 10 mmol/kg TCE or 0.2 mmol/kg DCAC every 4th day for 6 weeks and then sacrificed. Spleen weights and IgG were increased. ANA and anti-ssDNA (single-stranded DNA) antibodies were detected in the serum of TCE- and DCAC-treated mice; anticardiolipin antibodies were detected in the serum of DCAC-treated mice. A greater magnitude of response observed with DCAC treatment suggested that the metabolite may be important to the mechanism of TCE-induced autoimmunity.

Other studies in female MRL +/+ mice (8/group) examined exposure via drinking water. In one of these studies, mice were treated with 2.5 or 5.0 mg/mL (455 or 734 mg/kg-day) TCE in drinking water for up to 22 weeks (Griffin et al., 2000a; Gilbert et al., 1999). Serial sacrifices were conducted at weeks 4, 8, and 22. Significant increases in ANA and total serum immunoglobulin were found at 4 weeks of TCE treatment (indicating an autoimmune response), but not at 22 weeks. Increased expression of the activation marker C44 on splenic CD4+ cells was observed at 4 weeks, with the highest expression seen in the highest exposure group. In addition, at 4 weeks, splenic T-cells from treated mice secreted more IFN-γ and less IL-4 than control T-cells (significant at 0.5 and 2.5 mg/mL), consistent with a Th1 immune or inflammatory response. By 22 weeks of TCE treatment, a specific immune serum antibody response directed against dichloroacetylated proteins was activated in hepatic tissues, indicating the presence of protein adducts.

In a subsequent study that assessed occupationally relevant concentrations, TCE was administered to female MRL +/+ mice (8/group) in drinking water at treatment levels of 0.1, 0.5, or 2.5 mg/mL (21, 100, or 400 mg/kg-day) for 4 and 32 weeks (Griffin et al., 2000b). At 4 weeks, significant increases in serum antinuclear antibody levels were observed at 0.1 and 0.5 mg/kg-day. A dose-related increase in the percentage of activated CD4+ T-cells in lymph nodes of treated mice was observed at 32 weeks, and a dose-related increase in secretion in IFN-γ by the CD4+ T-cells was also observed at 4 and 32 weeks. There was a slight but statistically significant increase in serum ALT levels at 32 weeks at 0.5 mg/mL. Histopathological evaluation at 32 weeks revealed extensive hepatic lymphocytic cell infiltration at 0.5 and 2.5 mg/mL; all treated groups contained significantly more hepatocyte reactive changes (i.e.,

presence of multinucleated hepatocytes, variations in hepatocyte morphology, and hepatocytes in mitosis) than controls.

A similar response was observed by Cai et al. following chronic (48 weeks) exposure of TCE to female MRL +/+ mice (5/group) in drinking water at 0 or 0.5 mg/mL (approximately 60 μg/g/day) (Cai et al., 2008). After 11 weeks of treatment, a statistically significant decrease in body-weight gain was observed. After 24 weeks of exposure, serum ANA was consistently elevated in treated mice as compared to controls, although statistical significance was not achieved. Apparent treatment-related effects on serum cytokines included decreased IL-6 after 36 and 48 weeks, decreased TNF-α after 48 weeks, and increased granulocyte colony stimulating factor (G-CSF) after 36 weeks of treatment. After 36 weeks of treatment, ex vivo cultured splenocytes secreted higher levels of IFN-γ than control splenocytes. Although there were no observed effects on serum aminotransferase liver enzymes at termination, statistically significant incidences of hepatocytic necrosis and leukocyte infiltration (including CD3+ T lymphocytes) into liver lobules were observed in treated mice after 48 weeks of exposure. Hepatocyte proliferation was also increased. TCE treatment for 48 weeks also induced necrosis and extensive infiltration of leukocytes in the pancreas, infiltration of leukocytes into the perivascular and peribronchial regions of the lungs, and thickening of the alveolar septa in the lungs. At 36 and 48 weeks of exposure, massive perivascular infiltration of leukocytes (including CD3+ T lymphocytes) was observed in the kidneys, and immunoglobulin deposits were found in the glomeruli.

Table 4-78. Summary of autoimmune-related studies of TCE and metabolites in mice and rats (by sex, strain, and route of exposure)[a]

Number/group, vehicle, dose, duration	NOAEL; LOAEL[b]	Results			Reference
		Serology	Ex vivo assays of cultured splenocytes	Clinical and histopathology	
Autoimmune-prone: female MRL +/+ mice, drinking water					
8 per group, 0, 2.5, or 5 mg/mL TCE (average 0, 455, or 734 mg/kg-d), 4, 8, or 22 wks	LOAEL: 2.5 mg/mL	Increased ANA at 4 and 8 wks, no difference between groups at 22 wks	Increased activated CD4+ T-cells and IFN-γ secretion across doses at 4 wks, these effects were reversed at 22 wks; decreased IL-4 secretion (4 and 22 wks)	No evidence of liver or renal damage, based on serum ALT, SDH, and BUN.	Griffin et al. (2000a)
8 per group, 0, 0.1, 0.5, or 2.5 mg/mL TCE (0, 21, 100, or 400 mg/kg-d), 4 or 32 wks	**LOAEL: 0.1 mg/mL**	**Increased ANA in all treated groups at 4 wks, but not at 32 wks**	**Increased activated CD4+ T-cells (32 wks), IFN-γ secretion (4 and 32 wks), no effect on IL-4 secretion**	**Extensive hepatic mononuclear cellular infiltrate in 0.5 and 2.5 mg/mL groups, and hepatocyte reactive changes in all treated groups at 32 wks.**	**Griffin et al. (2000b)**
6–8 per group, 0, 0.1, or 0.9 mg/mL trichloroacetaldehyde hydrate (0, 24, or 220 mg/kg-d) or TCA (0, 27, or 205 mg/kg-d), 4 wks	LOAEL: 0.1 mg/mL	Increased ANA and antihistone antibodies at 0.9 mg/mL trichloroacetaldehyde hydrate[c]	Increased activated CD4+ T-cells at 0.1 and 0.9 g/mL doses of both metabolites. At 0.9 mg/mL, increased IFN-γ secretion, no effect on IL-4 secretion	No evidence of liver of kidney damage, based on serum ALT, liver and kidney histology.	Blossom et al. (2004)
8 per group, 0, 0.1, 0.3, or 0.9 mg/mL trichloroacetaldehyde hydrate (0, 13, 46, or 143 mg/kg-d), 40 wks	LOAEL: 0.9 mg/mL	Slightly suppressed anti-ssDNA, anti-dsDNA, and antihistone antibody expression; differences not statistically significant	Increased activated CD4+ T-cells and increased INF-γ secretion, no effect on IL-4 secretion	Diffuse alopecia, skin inflammation and ulceration, mononuclear cell infiltration, mast cell hyperplasia, dermal fibrosis. Statistically significant increase at 0.9 mg/mL dose group, but also increased at lower doses. No liver or kidney histopathology effects seen.	Blossom et al. (2007)

Table 4-78. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)

Number/group, vehicle, dose, duration	NOAEL; LOAEL[b]	Results			Reference
		Serology	Ex vivo assays of cultured splenocytes	Clinical and histopathology	
5 per group, 0 or 0.5 mg/mL TCE (mean 60 µg/g-d), 48 wks	**LOAEL: 0.5 mg/mL**	**Increased ANA after 24 wks but not statistically significant**	**Increased INF-γ secretion after 36 wks but not statistically significant**	**Hepatic necrosis; hepatocyte proliferation; leukocyte infiltrate in the liver, lungs, and kidneys; no difference in serum aminotransferase liver enzymes.**	**Cai et al. (2008)**
Autoimmune-prone: male and female offspring MRL +/+ mice, drinking water					
3 litters/group, 8–12 offspring/group; 0, 0.5, or 2.5 mg/mL, GD 0 to 7–8 wks of age	LOAEL: 0.5 mg/mL	Increased antihistone antibodies and total IgG_{2a} in treated groups	Dose-dependent increase in IFN-γ secretion at 4–5 wks of age but not 7–8 wks of age	No histopathological effects in liver or kidneys.	Blossom and Doss (2007)
8 litters/group, 8–12 offspring/group; 0 or 0.1 mg/mL; maternal dose = 25.7 mg/kg-d; offspring PNDs 24–42 dose = 31.0 mg/kg-d; GD 0 to PND 42	LOAEL: 0.1 mg/mL	Not evaluated	Increased IFN-γ and IL-2 in females, increased TNF-α in both sexes	Not evaluated	Blossom et al. (2008)
Unknown number of litters/ group, 6–10 offspring/ sex/group; 0 (1% Emulphor), 1,400, or 14,000 ppb; GD 0 to 12 mo of age	NOAEL: 1,400 ppb	No increase in autoantibody levels	Not evaluated	Not evaluated	Peden-Adams et al. (2008)

Table 4-78. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)

Number/group, vehicle, dose, duration	NOAEL; LOAEL[b]	Results			Reference
		Serology	Ex vivo assays of cultured splenocytes	Clinical and histopathology	
Autoimmune-prone: female MRL +/+ mice, i.p. injection					
4–5 per group, 0 (corn oil), 10 mmol/kg TCE, or 0.2 mmol/kg DCAC, every 4th d for 6 wks	LOAEL: 10 mmol/kg TCE, 0.2 mmol/kg DCAC	In both groups, increased ANA and anti-ssDNA antibodies. In DCAC group, anticardiolipin antibodies. No difference in antihistone, -Sm, or -DNA antibodies	Not evaluated	Not evaluated	Khan et al. (1995)
6 per group, 0 (corn oil), 0.2 mmol/kg DCAC, or 0.2 mmol/kg dichloroacetic anhydride, 2 times per wk for 6 wks	LOAEL: 0.2 mmol/kg TCE, 0.2 mmol/kg dichloroacetic anhydride	In both treated groups, increased ANA	In both treated groups, increased IL-1α, IL-1β, IL-3, IL-6, IFN-γ, G-CSF and KC secretion; decreased IL-5. In DCAC group, increased IL-17 and INF-α[d]	In both treated groups, increased lymphocytes in spleen, thickening of alveolar septa with lymphocytic interstitial infiltration.	Cai et al. (2006)
Autoimmune-prone: female NZB × NZW mice, drinking water					
6 per group, 0, 1,400, or 14,000 ppb TCE[e,f], 27 wks exposure	LOAEL: 1,400 ppb	Increased anti-dsDNA antibodies at 19 wks and at 32–32 wks in the 1,400 ppb group	Not evaluated	At 14,000 ppb, proteinuria increased beginning at 20 wks; renal pathology scores increased, no evidence of liver disease.	Gilkeson et al. (2004)
10 per group, 0, 1,400, or 14,000 ppb TCE[f], 27 wks exposure	**LOAEL: 1,400 ppb**	**Increased anti-dsDNA antibodies at 19 wks and at 32–32 wks in the 1,400 ppb group**	**No effect on splenocyte NK activity**	**No effect on renal pathology score; liver disease not examined.**	**Keil et al. (2009)**

Table 4-78. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)

Number/group, vehicle, dose, duration	NOAEL; LOAEL[b]	Results			Reference
		Serology	Ex vivo assays of cultured splenocytes	Clinical and histopathology	
Autoimmune-prone: male MRL—*lpr/lpr* mice, inhalation					
5 per group, 0, 500, 1,000, or 2,000 ppm TCE, 4 hrs/d, 6 d/wk, 8 wks	**LOAEL: 500 ppm**			**At ≥500 ppm, dose-related liver inflammation, splenomegaly and hyperplasia of lymphatic follicles; at 1,000 ppm, immunoblastic cell formation in lymphatic follicles, no changes in thymus.**	**Kaneko et al. (2000)**
Autoimmune-inducible: female brown Norway Rat, gavage					
6–8 per group, 0, 100, 200, 400 mg/kg, 5 d/wk, 6 wks followed by 1 mg/kg $HgCl_2$ challenge	NOAEL 500 mg/kg	Not reported[g]	Not evaluated	Not evaluated	White et al. (2000)
Nonautoimmune-prone: female B6C3F$_1$ mice, drinking water					
6 per group, 0, 1,400, or 14,000 ppb TCE,[e,f] 30 wks exposure	LOAEL: 1,400 ppb	Anti-dsDNA increased in 1,400 ppb group beginning at age 32 wks and in the 14,000 ppb group beginning at age 26 wks	No effect on splenocyte NK activity	No renal disease observed.	Gilkeson et al. (2004)

Table 4-78. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)

Number/group, vehicle, dose, duration	NOAEL; LOAEL[b]	Results			Reference
		Serology	Ex vivo assays of cultured splenocytes	Clinical and histopathology	
10 per group, 0, 1,400, or 14,000 ppb TCE,[f] 30 wks exposure	**LOAEL: 1,400 ppb**	**Anti-dsDNA increased beginning at 26 wks in the 14,000 ppb group and at 32 wks of age in the 1,400 ppb group; increases in anti-ssDNA antibodies seen in both groups at 32 wks. Anti-glomerular antigen were not affected**	**No effect on splenocyte NK activity**	**Increased renal pathology scores in 1,400 ppb group; Significant decrease in thymus weight in both groups**	**Keil et al. (2009)**

[a]**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5); selected endpoints, based on those reported across the majority of studies. Lupus-prone mouse strains develop lupus-like condition spontaneously, with virtually complete penetrance. The autoimmune-inducible (Brown Norway) rat has been used as a model of mercuric chloride induced glomerulonephritis and experimental autoimmune myasthenia gravis.
[b]NOAEL and LOAEL are based upon reported study findings.
[c]No difference reported in anti-dsDNA, -ssDNA, -ribonucleosome, -SSA, -SSB, -Sm, -Jo-1, or -Scl-70 antibodies.
[d]No difference reported in secretion of other cytokines measured: IL-2, IL-4, IL-10, IL-12, TNF-α, granulocyte monocyte colony stimulating factor, macrophage inflammatory protein-1α, and RANTES (CCL-5).
[e]Dose levels cited in the report (Gilkeson et al., 2004) were incorrect; corrections provided by personal communication from Margie Peden-Adams (Medical University of South Carolina) to Glinda Cooper (U.S. EPA) on 13 August 2008; dose levels in this table are correctly reported.
[f]Dose in mg/kg-day not given.
[g]Anti-dsDNA tests were described in the methods section; no effect of TCE on serum IgE levels was seen, and it is not clear if the additional serological tests were conducted in the TCE portion of this study or if they were conducted but not reported because no effect was seen.

G-CSF = granulocyte colony stimulating factor; KC = keratinocyte-derived chemokine; SDH = sorbitol dehydrogenase

To examine the role of metabolic activation in the autoimmune response, Griffin et al. (2000c) treated MRL +/+ mice with 2.5 mg/mL (300 mg/kg-day) TCE in drinking water for 4 weeks (Griffin et al., 2000c). Immune responses were examined in the presence or absence of subcutaneous doses of 200 mg/kg-day diallyl sulfide, a specific inhibitor of CYP2E1, which is known to be a primary CYP that is active in TCE metabolism. With diallyl sulfide cotreatment that resulted in a decreased level of CYP2E1 apoprotein in liver microsomes, the enhanced mitogen-induced proliferative capacity of T-cells was inhibited and the reduction in IL-4 levels secreted by CD4+ T-cells was reversed for TCE-treated MRL +/+ mice. This study suggests that metabolism of TCE by CYP2E1 is responsible, at least in part, for the treatment-related CD4+ T-cell alterations.

The TCE metabolite, trichloroacetaldehyde (TCAA) or trichloroacetaldehyde hydrate (TCAH), was also evaluated in MRL +/+ mice (Blossom et al., 2007; Blossom and Gilbert, 2006; Gilbert et al., 2004) in order to determine if outcomes similar to the immunoregulatory effects of TCE would be observed, and to attempt to further characterize the role of metabolism in the mode of action for TCE. At concentrations ranging from 0.04 to 1 mM, TCAA stimulated proliferation of murine Th1 cells treated with anti-CD3 antibody or antigen in vitro. At similar concentrations, TCAA induced phenotypic alterations consistent with upregulation of CD28 and downregulation of CD62L in cloned memory Th1 cells and DC4+ T-cells from untreated MRL +/+ mice. Phosphorylation of activating transcription factor 2 (ATF-2) and c-Jun (two components of the activator protein-a transcription factor) was also observed with TCAA-induced Th1 cell activation. Higher concentrations of TCAA formed a Schiff base on T-cells, which suppressed the ability of TCAA to phosphorylate ATF-2. These findings suggested that TCAA may promote T-cell activation by stimulating the mitogen-activated protein kinase pathway in association with Schiff base formation on T-cell surface proteins (Gilbert et al., 2004).

In order to determine whether metabolites of TCE could mediate the immunoregulatory effects previously observed with TCE treatment (i.e., the generation of lupus and autoimmune hepatitis, associated with activation of IFN-γ-producing CD4+ T-cells), Blossom et al. (2004) administered TCE metabolites, TCAH and TCA, to MRL +/+ mice (6–8/group) in drinking water for 4 weeks. Drinking water concentrations were 0, 0.1, or 0.9 mg/mL; average daily doses were calculated as 0, 24, or 220 mg/kg-day for TCAH and 0, 27, or 205 mg/kg-day for TCA. These treatment levels were considered to be physiologically relevant and to reflect occupational exposure. A phenotypic analysis of splenic and lymph node cells, cytokine profile analysis, evaluation of apoptosis in CD4+ T-cells, and examination of serum markers of autoimmunity (anti-ssDNA, antihistone, or ANA) were conducted. Exposure to TCAH or TCA at both treatment levels was found to promote CD4+ T-cell activation, as shown by significant ($p < 0.05$) increases in the percentage of $CD62L^{lo}$ CD4+ T-cells in the spleens and lymph nodes of the MRL +/+ mice. Increased levels of IFN-γ were secreted by CD4+ T-cells from mice

treated by TCAH and TCA. No significant changes in body weight were observed; spleen weights were similar between control and treated mice with the exception of a significant decrease in spleen weight from mice treated with 0.9 mg/mL TCA. Liver and kidney histology were not affected, and serum ALT levels were similar for control and treated mice. A generalized trend towards an increase in serum autoantibodies (anti-ssDNA) was observed in TCAH-treated mice, and slight but significant increases in antihistone and antinuclear antibody production were observed in mice treated with 0.9 mg/mL-day TCAH.

The autoimmune response of female MRL +/+ mice to DCAC, a metabolite of TCE, and to dichloroacetic anhydride (DCAA) a similar acylating agent, was evaluated by Cai et al. (2006). Six mice/group were injected intraperitoneally, twice weekly for 6 weeks, with 0.2 mmol/kg DCAC or DCAA in corn oil. Body weight gain was significantly decreased after 5 or 6 weeks treatment with DCAC and DCAA. DCAC treatment resulted in significant increases in total serum IgG (77% increase over control) and IgG1 (172% increase over control), as well as the induction of DCAC-specific IgG and IgG1. Serum IgM levels were significantly decreased by 25 and 18% in DCAC and DCAA-treated mice, respectively. IgE levels were increased 100% over controls in DCAC-treated mice. Of eight Th1/Th2 cytokines measured, only IL-5 was decreased in DCAC- and DCAA-treated mice. Serum ANA were detected in both DCAC- and DCAA-treated mice. Treatment-related increases in cytokine and chemokine secretion in cultured splenocytes were observed for DCAC and DCAA (IL-1, G-CSF, keratinocyte-derived chemokine, IL-3, and IL-6). DCAC-treated splenocytes also secreted more IL-17 and IFN-α than controls. Histopathological changes were observed in the spleens of DCAC and DCAA-treated mice (lymphocyte population increases in the red pulp). With both DCAC and DCAA treatment, the alveolar septa were thickened in the lungs, moderate levels of lymphocytic interstitial infiltrates were present in tissues, and alveolar capillaries were clogged with erythrocytes. These findings were attributed both to the predisposition of the MRL +/+ mice towards autoimmune disease and to the treatment-related induction of autoimmune responses.

Fas-dependant activation-induced cell death leading to autoimmune disease has been shown to be related to impaired Fas or FasL ligand expression in humans and mice, and defects in the Fas-signaling pathways have been described in autoimmune disease models. The study by Blossom and Gilbert (2006) examined the effects of TCAH on Fas-dependent autoimmune cell death). In this study, TCAH: (1) inhibited apoptosis of antigen-activated cells; (2) did not protect CD4+ T-cells from Fas-independent apoptosis; (3) did not inhibit autoimmune cell death induced by direct engagement of the Fas receptor; (4) inhibited the expression of FasL but not Fas on the surface of activated CD4+ T-cell; (5) increased release of FasL from CD4+ cells in a metalloprotein-dependent manner; and (6) increased metalloprotein MMP-7 expression.

Gilbert et al. (2006) studied the effect of treatment on apoptosis in CD4+ T-lymphocytes isolated from MRL +/+ female mice that had been exposed to TCE (0, 0.1, 0.5, or 2.5 mg/mL) in

the drinking water for 4 or 32 weeks or to TCAH (0.1, 0.3, or 0.9 mg/mL) in drinking water for 4 or 40 weeks. After only 4 weeks, decreased activation-induced apoptosis was associated with decreased FasL expression in the CD4+ T-cells, suggesting that TCE- and TCAH-induced autoimmune disease was promoted through suppression of the process that would otherwise delete activated self-reactive T-lymphocytes. By 32 weeks of treatment, TCE had induced autoimmune hepatitis, which was associated with the promotion of oxidative stress, the formation of liver protein adducts, and the stimulated production of antibodies to those adducts. TCAH-treated mice did not exhibit autoimmune hepatitis by 40 weeks, but developed a dose-dependent alopecia and skin inflammation (Blossom et al., 2007). TCAH appeared to modulate the CD4+ T-cell subset by promoting the expression of an activated/effector phenotype with an increased capacity to secrete the proinflammatory cytokine IFN-γ. A 4-week exposure to TCAH attenuated activation-induced cell death and the expression of the death receptor Fas in CD4+ cells; these effects were not seen after a 40-week exposure period. Differences in response were tentatively attributed to higher levels of metalloproteinases (specifically MMP-7) at 4 weeks of treatment, suggesting a possible mechanism for the promotion of skin pathology by TCAH.

The role of protein adduct formation in autoimmune response has been pursued by various researchers. Halmes et al. (1997) administered a single i.p. dose of TCE in corn oil to male Sprague-Dawley rats (2/group) at 0 or 1,000 mg/kg. Using antiserum that recognizes TCE covalently bound to protein, a single 50 kDa microsomal adduct was detected by Western blot in livers of treated rats. Using affinity chromatography, a 50 kDa dichloroacetyl protein was also isolated from rat plasma. The protein was reactive immunochemically with anti-CYP2E1 antibodies. The data suggest that the protein adduct may be CYP2E1 that has been released from TCE-damaged hepatocytes.

Cai et al. (2007) examined the role of protein haptenization in the induction of immune responses. In this study, MRL +/+ mice were immunized with albumin adducts of various TCE reactive intermediates of oxidative metabolism. Serum immunoglobulins and cytokine levels were measured to evaluate immune responses against the haptenized albumin. Antigen-specific IgG responses (subtypes: IgG1, IgG2a, and IgG2b) were found. Serum levels of G-CSF were increased in immunized mice, suggesting macrophage activation. Following immunization with formyl-albumin, lymphocyte infiltration in the hepatic lobule and portal area was increased. This study suggests that proteins that are haptenized by metabolites of TCE may act as antigens to induce humoral immune responses and T-cell-mediated hepatitis.

A possible role for oxidative stress in inflammatory autoimmune disease was proposed by Khan et al. (2001). A study was performed in which female MRL +/+ mice were treated with 10 mmol/kg TCE or 0.2 mmol/kg DCAC via i.p. injection every 4th day for 2, 4, 6, or 8 weeks. Antimalondialdehyde serum antibodies, a marker of lipid peroxidation and oxidative stress, were measured and were found to increase by 4 weeks of treatment, marginally for TCE and

significantly for DCAC. It was reported that antimalondialdehyde antibodies has also been found to be present in the serum of systemic lupus erythematosus-prone MRL-lpr/lpr mice.

In another study that addressed the association of oxidative and nitrosative stress, and the role of lipid peroxidation and protein nitration, in TCE-mediated autoimmune response, Wang et al. (2007b) treated female MRL +/+ mice with 0.5 mg/mL TCE in drinking water for 48 weeks . The formation of antibodies in the serum to lipid peroxidation-derived aldehyde protein adducts was evaluated. With TCE treatment, the serum levels of antimalondialdehyde and anti-4-hydroxynonenal protein adduct antibodies, inducible nitric oxide synthase, and nitrotyrosine were increased. These were associated with increases in antinuclear-, anti-ssDNA-, and anti-dsDNA antibodies. The involvement of lipid peroxidation-derived aldehyde protein adducts in TCE autoimmunity was further explored, using female MRL +/+ mice that were administered by i.p. injections of TCE at 10 mmol/kg, either every 4th day for 6 or 12 weeks (Wang et al., 2007a) or once per week for 4 weeks (Wang et al., 2008). Significant increases in malondialdehyde and 4-hydroxynonenal protein adducts, as well as significant induction of specific antibodies directed against these antigens were observed in both studies. Wang et al. (2008) also demonstrated a significant proliferation of CD4+ T-cells in TCE-treated mice, and splenic lymphocytes from TCE-treated mice released more IL-2 and IFN-γ when stimulated with MDA- or 4-hydroxynonenal-adducted mouse serum albumin. Overall, the result of these studies suggest a role for lipid peroxidation aldehydes in the induction and/or exacerbation of autoimmune response in the MRL +/+ animal model, and the involvement of Th1 cell activation.

In studies conducted in other rodent strains, less consistent outcomes have been observed. Inhalation exposure of an autoimmune-prone strain of male mice (MRL-lpr/lpr) to 0, 500, 1,000, or 2,000 ppm TCE for 4 hours/day, 6 days/week, for 8 weeks resulted in depressed serum IgG levels and increased numbers of lymphoblastoid cells (Kaneko et al., 2000). Also at 2,000 ppm, changes in T-cell helper to suppressor cell ratios were observed. At histopathological evaluation, dose-dependent inflammation and associated changes were noted in the liver at ≥500 ppm, hyperplasia of the lymphatic follicles of the spleen and splenomegaly were observed at ≥500 ppm, and the spleen exhibited the development of an immunoblastic-cell-like structure at 1,000 ppm.

A 26-week drinking water study of TCE in NZB × NZW (NZBWF1) autoimmune-prone mice demonstrated an increase in anti-dsDNA antibodies at 19 weeks and at 32 and 34 weeks in the 1,400 ppb group, and increased kidney disease at 14,000 ppb (i.e., increased proteinuria at 20 weeks; increased renal pathology scores were noted at termination, based upon glomerular proliferation, inflammation, and necrosis) (Gilkeson et al., 2004).[11] Also in that study, a small increase in anti-dsDNA antibody production, without kidney disease, was observed in B6C3F$_1$

[11]The study was reported in symposium proceedings. Dose levels cited in the proceedings were incorrect; however, corrections were provided by personal communication from Margie Peden-Adams (Medical University of South Carolina) to Glinda Cooper (U.S. EPA) on 13 August 2008, and dose levels are correctly reported here.

mice, with statistically significant ($p < 0.05$) or borderline ($p = 0.07$) effects seen in the 1,400 ppb group at observations between 32 and 39 weeks of age, and in the 14,000 ppb group at observations between 26 and 39 weeks of age.

Keil et al. (2009) also assessed the effects of TCE exposure on NZWBF1 mice, comparing the responses to those of TCE-exposed $B6C3F_1$ mice, which are not autoimmune prone (Keil et al., 2009). In this study, groups of NZWBF1 and $B6C3F_1$ female mice (10/dose level) were administered 0, 1,400, or 14,000 ppb TCE in the drinking water. Treatment was initiated at 9 weeks of age and continued until 36 weeks of age for the NZBWF1 mice and until 39 weeks of age for the $B6C3F_1$ mice. Body weight; spleen, thymus, liver, and kidney weight; spleen and thymus cellularity; and renal pathology were assessed. Splenic lymphocyte proliferation, autoantibody production (anti-dsDNA, anti-ssDNA, and antiglomerular), total serum IgG, NK cell activity, and mitogen-induced lymphocyte proliferation were conducted. Administration of TCE did not result in alterations in NK cell activity or T- or B-cell proliferation in either strain of mice. In the NZBWF1 mice, there was little evidence of an increase or of an acceleration in ssDNA antibody production with TCE exposure, but as was seen in the earlier study by these investigators (Gilkeson et al., 2004), dsDNA antibodies were increased at 19 weeks and at 32–34 weeks in the 1,400 ppb group. However, antiglomerular antibody levels were increased in NZBWF1 mice early in the study, returning to control levels by 23 weeks of age. In the $B6C3F_1$ mice, the number of activated T-cells (CD4++/CD44+) was increased (significantly at 14,000 ppb; $p \leq 0.05$) and thymus weights were significantly decreased ($p \leq 0.05$) in a dose-responsive manner. Renal pathology (as indicated by renal score based on assessment of glomerular inflammation, proliferation, crescent formation, and necrosis) was significantly increased ($p \leq 0.05$) at 1,400 ppb. Also in the $B6C3F_1$ mice, autoantibodies to dsDNA were increased relative to controls beginning at 26 weeks in the 14,000 ppb group and at 32 weeks of age in the 1,400 ppb group; increases in anti-ssDNA antibodies were seen in both groups at 32 weeks. Antiglomerular antibodies were not affected in $B6C3F_1$ mice. In summary, the authors concluded that this study showed that 27–30 weeks of TCE drinking water administration to NZBWF1 (autoimmune-prone) mice did not contribute to the progression of autoimmune disease, while similar administration to $B6C3F_1$ (nonautoimmune-prone) mice increased the expression of a number of markers that are associated with autoimmune disease. This study is important in that it demonstrates that autoimmune responses to TCE exposure in animal models are not solely dependent upon a genetic predisposition to autoimmune disease.

White et al. (2000) conducted a study in female Brown Norway rats, which have been shown to be susceptible to development of chemically-induced IgE mediated glomerulonephritis that is similar to the nephritic damage seen in systemic lupus erythematosus. TCE administered by gavage 5 days/week at 100, 200, or 400 mg/kg did not increase in IgE levels after 6 weeks exposure, or after an additional challenge with 1 mg/kg $HgCl_2$.

Several studies have examined the potential for autoimmune response following oral exposures during pre- and postnatal immune system development, as described in Section 4.6.2.1.2. Peden-Adams et al. (2008; 2006) conducted two such studies. In the first study, B6C3F_1 mice were treated with either 1,400 or 14,000 ppb TCE in drinking water from GD 0 to postnatal week 8 (Peden-Adams et al., 2006). No treatment-related increases in serum anti-dsDNA antibody levels were observed in the 8-week-old offspring, although it is noted that the mouse strain used in the experiment is not an autoimmune-prone animal model. A more recent study (Peden-Adams et al., 2008) exposed pregnant MRL +/+ mice to TCE in drinking water at levels of 0, 1,400, or 14,000 ppb from GD 0 and continued the exposures until the offspring were 12 months of age. Consistent with the findings of the 2006 publication, autoantibody levels (anti-dsDNA and antiglomerular) were not increased in the offspring over the course of the study. Contrasting with these negative studies, the lupus-prone MRL +/+ mouse model was utilized in two additional drinking water studies with developmental exposures in which there was some indication of a positive association between developmental exposures to TCE and the initiation of autoimmune disease. Blossom and Doss (2007) administered TCE to pregnant MRL +/+ mice in drinking water at levels of 0, 0.5, or 2.5 mg/mL and continued administration to the offspring until approximately 7–8 weeks of age. TCE exposure induced a dose-dependent increase in T-lymphocyte IFN-γ in peripheral blood at 4–5 weeks of age, but this effect was not observed in splenic T-lymphocytes at 7–8 weeks of age. Serum antihistone autoantibodies and total IgG_{2a} were significantly increased in the TCE-treated offspring; however, histopathological evaluation of the liver and kidneys did not reveal any treatment-related signs of autoimmunity. In a study by Blossom et al. (2008), pregnant MRL +/+ mice were administered TCE in the drinking water at levels of 0 or 0.1 mg/mL from GD 0 through lactation, and continuing postweaning in the offspring until GD 42. Significant treatment-related increases in pro-inflammatory cytokines (IFN-γ and Il-2 in males and TNF-α in both sexes) produced by splenic CD4+ T-cells were observed in PND 42 offspring.

In summary, TCE treatment induces and exacerbates autoimmune disease in genetically susceptible strains of mice, and has also been shown to induce signs of autoimmune disease in a nongenetically predisposed strain. Although the mechanism for this response is not fully understood, a number of studies have been conducted to examine this issue. The primary conclusion to date is that metabolism of the TCE to its chloral or DCA metabolites is at least partially responsible for activating T-cells or altering T-cell regulation and survival associated with polyclonal disease in susceptible mice strains.

4.6.2.4. Cancers of the Immune System

Cancers of the immune system that have been observed in animal studies and are associated with TCE exposure are summarized in Tables 4-79 and 4-80. The specific cancer

types observed are malignant lymphomas, lymphosarcomas, and reticulum cell sarcomas in mice and leukemias in rats.

Table 4-79. Malignant lymphomas incidence in mice exposed to TCE in gavage and inhalation exposure studies

Cancer type, species, and sex	Prevalence in exposure groups: n affected/n total (% affected)						Reference
Gavage exposure							
Malignant lymphomas	Vehicle control		1,000 mg/kg-d				NTP (1990)
$B6C3F_1$ mice, male	11/50 (22%)		13/50 (26%)				
$B6C3F_1$ mice, female	7/48 (15%)		13/49 (27%)				
Lymphosarcomas and reticulum cell sarcomas	Vehicle control		Low dose		High dose		NCI (1976)[b]
$B6C3F_1$ mice, male	1/20 (5%)		4/50 (8%)		2/48 (4%)		
$B6C3F_1$ mice, female	1/20 (5%)		5/50 (10%)		5/47 (11%)		
Malignant lymphomas	Control	TCE-pure	TCE-indust	TCE-EPC	TCE-BO	TCE-EPC-BO	Henschler et al. (1984) [c]
Swiss (ICR/HA) mice, male	19/50 (38%)	16/50 (32%)	17/49 (35%)	11/49 (22%)	11/49 (22%)	12/49 (24%)	
Swiss (ICR/HA) mice, female	28/50 (56%)	21/50 (42%)	19/50 (38%)	20/50 (40%)	23/48 (48%)	18/50 (36%)	
Inhalation exposure							
Malignant lymphomas	Control		96		480		Henschler et al. (1980)[d]
Han:NMRI mice, male	7/30 (23%)		7/29 (24%)		6/30 (20%)		
Han:NMRI mice, female[e]	9/29 (31%)		17/30 (57%)		18/28 (64%)		

[a]After 103 weeks of gavage exposure, beginning at 8 weeks of age.
[b]After 90 weeks of gavage exposure, beginning at 5 weeks of age. Low dose is 1,200 mg/kg-d for male mice, 900 mg/kg-d for female mice (5 days/week). High dose is 2,400 mg/kg-d for male mice, 1,800 mg/kg-d for female mice (5 days/week).
[c]After 72 weeks of gavage exposure (corn oil), beginning at 5 weeks of age. Male mice received 2,400 mg/kg-d, female mice received 1,800 mg/kg-d. Stabilizers were added in the percentage w/w: TCE-EPC, 0.8%, TCE-BO, 0.8%, TCE-EPC-BO, 0.25 and 0.25%.
[d]After 78 weeks of inhalation exposure. Administered daily concentration: low dose is 96 (mg/m^3) and high dose is 480 (mg/m^3), equivalent to 100 and 500 ppm (100 ppm = 540 mg/m^3), adjusted for 6 hours/day, 5 days/week exposure.
[e]Statistically significant by Cochran-Armitage trend test ($p < 0.05$).

Sources: NTP (1990) Tables 8 and 9; NCI (1976) Table ~~–~~XXXa"; Henschler et al. (1980) Table 3a.

Table 4-80. Leukemia incidence in rats exposed to TCE in gavage and inhalation exposure studies

Species and sex	Prevalence in exposure groups: n affected/n total (% affected)				Reference
Gavage exposure	Control	50 mg/kg	250 mg/kg		Maltoni et al. (1988; 1986)[a]
Sprague-Dawley rats, male	0/30 (0%)	2/30 (6.7%)	3/30 (10.0%)		
Sprague-Dawley rats, female	1/30 (3.3%)	0/30 (0%)	0/30 (0%)		
	Control	500 mg/kg	1,000 mg/kg		NTP (1988)[b]
August rats, female	0/50 (0%)	1/50 (2%)	5/50 (10%)		
Inhalation exposure	Control	100 ppm	300 ppm	600 ppm	Maltoni et al. (1988; 1986)[c]
Sprague-Dawley rats, male	9/135 (6.7)	13/130 (10.0)	14/130 (10.8)	15/130 (11.5)	
Sprague-Dawley rats, female	7/145 (4.8)	9/130 (6.9)	2/130 (1.5)	11/130 (8.5)	

[a]After 52 weeks of gavage exposure, beginning at 13 weeks of age, olive oil vehicle. Percentage affected and starting n given in reported; EPA calculated n affected.
[b]After 104 weeks of gavage exposure, beginning at 6.5–8 weeks of age, corn oil vehicle.
[c]After 104 weeks of inhalation exposure, BT304 and BT304bis. Percentage affected and starting n given in reported; EPA calculated n affected.

In the NCI (1976) study, the results for Osborne-Mendel rats were considered inconclusive due to significant early mortality, but exposure to B6C3F$_1$ mice were also analyzed. Limited increases in lymphomas over controls were observed in both sexes of mice exposed (see Table 4-79). The NCI study (1976) used technical-grade TCE, which contained two known carcinogenic compounds as stabilizers (epichlorohydrin and 1,2-epoxybutane). A later study (Henschler et al., 1984) in which mice were given TCE that was pure, industrial, and stabilized with one or both of these stabilizers did not find significant increases in lymphomas over historical controls. A gavage study by NTP (1988), which used TCE stabilized with diisopropylamine, did not see an increase in lymphomas in all four strains of rats (ACI, August, Marshall, and Osborne-Mendel). The final NTP study (1990) in male and female F344 rats and B6C3F$_1$ mice, using epichlorohydrin-free TCE, again reported early mortality in male rats. This study did not observe a significant increase in lymphomas over that of controls. Henschler et al. (1980) tested NMRI mice, WIST rats, and Syrian hamsters of both sexes, and observed a variety of tumors in both sexes, consistent with the spontaneous tumor incidence in these strains (Deerberg et al., 1974; Deerberg and Müller-Peddinghaus, 1970). Henschler et al. (1980) did not show an increase in lymphomas in rats or hamsters of either sex. Background levels of lymphomas in this mouse strain are high, making it difficult to determine if the increased lymphomas in female mice is a treatment effect. In a follow-up study, Henschler et al. (1984) examined the role of stabilizers of TCE in the lymphomas demonstrated in female mice in the

1980 paper. Each exposure group had ~50 SPF-bred ICR/HA-Swiss mice and exposure was for 18 months. Background incidence of tumors was high in all groups. Focusing just on malignant lymphomas (see Table 4-79), the high background incidence in unexposed animals again makes it difficult to determine if there is TCE and/or stabilizer-related incidence of lymphomas. There are no data at any other timepoint than 18 months. A high mortality rate in all animals as well as the increased incidence of _background' lymphomas in that report was also a problem and may have been related to the shorter time frame.

Maltoni et al. (1988; 1986) reported a nonsignificant increase in leukemias in male rats exposed via inhalation. Maltoni et al. (1988; 1986) demonstrates a borderline higher frequency of leukemias in male Sprague-Dawley rats following exposure by ingestion for 52 weeks, believed by the authors to be related to an increase in lymphoblastic lymphosarcomas (see Table 4-80). The gavage study by NTP (1988), which used TCE stabilized with diisopropylamine, observed leukemia in female August rats with a positive trend, but was not significantly greater than the vehicle controls.

In summary, overall there is limited available data in animals on the role of TCE in lymphomas and leukemias. There are few studies that analyze for lymphomas and/or leukemias. Lymphomas were described in four studies (NTP, 1990; Henschler et al., 1984; Henschler et al., 1980; NCI, 1976), but study limitations (high background rate) in most studies make it difficult to determine if these are TCE-induced. Three studies found positive trends in leukemia in specific strains and/or gender (Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986). Due to study limitations, these trends cannot be determined to be TCE-induced.

4.6.3. Summary

4.6.3.1. Noncancer Effects

The human and animal studies of TCE and immune-related effects provide strong evidence for a role of TCE in autoimmune disease and in a specific type of generalized hypersensitivity syndrome. The data pertaining to immunosuppressive effects is weaker. It should also be noted that immune-related and inflammatory effects, particularly cell-mediated immunity involving cytokine production and activation of macrophages and NK cells, may influence a variety of other conditions of considerable public health importance, including cancer (tumor surveillance) and atherosclerosis. Thus, the relevance of immune-related effects of TCE are not limited to diseases affecting organs and tissues within the immune system. The relation between systemic autoimmune diseases, such as scleroderma, and occupational exposure to TCE has been reported in several recent studies. A meta-analysis of scleroderma studies (Garabrant et al., 2003; Diot et al., 2002; Nietert et al., 1998) conducted by the EPA resulted in a statistically significant combined OR for any exposure in men (OR: 2.5, 95% CI: 1.1, 5.4), with a lower RR seen in women in women (OR: 1.2, 95% CI: 0.58, 2.6). The incidence of systemic sclerosis among men is very low (approximately 1 per 100,000 per year), and is approximately 10 times

lower than the rate seen in women (Cooper and Stroehla, 2003). Thus, the human data, at this time, do not allow for the determination of whether the difference in effect estimates between men and women reflects the relatively low background risk of scleroderma in men, gender-related differences in exposure prevalence or in the reliability of exposure assessment (Messing et al., 2003), a gender-related difference in susceptibility to the effects of TCE, or chance. Changes in levels of inflammatory cytokines were reported in an occupational study of degreasers exposed to TCE (Iavicoli et al., 2005) and a study of infants exposed to TCE via indoor air (Lehmann et al., 2002; Lehmann et al., 2001). Experimental studies support the biological plausibility of these effects. Numerous studies have demonstrated accelerated autoimmune responses in autoimmune-prone mice (Cai et al., 2008; Blossom et al., 2007; Blossom et al., 2004; Griffin et al., 2000a; Griffin et al., 2000b). With shorter exposure periods, effects include changes in cytokine levels similar to those reported in human studies. More severe effects, including autoimmune hepatitis, inflammatory skin lesions, and alopecia, were manifest at longer exposure periods, and interestingly, these effects differ somewhat from the "normal" expression in these mice. Immunotoxic effects, including increases in anti-dsDNA antibodies in adult animals and decreased PFC response with prenatal and neonatal exposure, have been also reported in B6C3F_1 mice, which do not have a known particular susceptibility to autoimmune disease (Peden-Adams et al., 2006; Gilkeson et al., 2004). Recent mechanistic studies have focused on the roles of various measures of oxidative stress in the induction of these effects by TCE (Wang et al., 2008; Wang et al., 2007b).

There have been a large number of case reports of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis, associated with occupational exposure to TCE, with prevalences as high as 13% of workers in the same location (Kamijima et al., 2008; Kamijima et al., 2007). Evidence of a treatment-related increase in delayed hypersensitivity response accompanied by hepatic damage has been observed in guinea pigs following intradermal injection (Tang et al., 2008; Tang et al., 2002), and hypersensitivity response was also seen in mice exposed via drinking water pre- and postnatally (GD 0 through to 8 weeks of age) (Peden-Adams et al., 2006).

Human data pertaining to TCE-related immunosuppression resulting in an increased risk of infectious diseases is limited to the report of an association between reported history of bacteria of viral infections in Woburn, Massachusetts (Lagakos et al., 1986). Evidence of localized immunosuppression, as measured by pulmonary response to bacterial challenge (i.e., risk of Streptococcal pneumonia-related mortality and clearance of *Klebsiella* bacteria) was seen in an acute exposure study in CD-1 mice (Aranyi et al., 1986). A 4-week inhalation exposure in Sprague-Dawley rats reported a decrease in PFC response at exposures of 1,000 ppm (Woolhiser et al., 2006).

4.6.3.2. Cancer

Associations observed in epidemiologic studies of lymphoma and TCE exposure suggest a causal relation between TCE exposure and NHL. Issues of study heterogeneity, potential publication bias, and weaker exposure-response results contribute uncertainty to the evaluation of the available data.

In a review of the NHL studies, studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs, biomarker monitoring, or industrial hygiene data on TCE exposure patterns and factors that affect such exposure) and which met, to a sufficient degree, the standards of epidemiologic design and analysis were identified. These studies generally reported excess RR estimates for NHL between 0.8 and 3.1 for overall TCE exposure. Statistically significant elevated RR estimates with NHL and overall TCE exposure were observed in two cohort (Raaschou-Nielsen et al., 2003; Hansen et al., 2001) and one case-control (Hardell et al., 1994) study. Both cohort studies reported statistically significant associations with NHL for subjects with longer employment duration as a surrogate of TCE exposure as does a second case-control study with high-quality exposure-assessment methodology reported statistically significant associations with highest cumulative TCE exposure or highest average-weekly TCE exposure (Purdue et al., 2011). Hardell et al. (1994) reported a strong but imprecise association, in part reflecting possible bias from subject-reported exposure history and few exposed cases. Other identified studies reported a 10–50% elevated RR estimate with overall TCE exposures that were not statistically significant, except for two population case-control studies of NHL, one of which did not report RR estimates with overall TCE exposure but did for medium-high intensity or cumulative TCE exposure (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Radican et al., 2008; Miligi et al., 2006; Zhao et al., 2005; Boice et al., 1999; Persson and Fredrikson, 1999; Morgan et al., 1998; Nordström et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991). Fifteen additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease study power and sensitivity (Clapp and Hoffman, 2008; ATSDR, 2006a; Chang et al., 2005; ATSDR, 2004a; Chang et al., 2003; Morgan and Cassady, 2002; Ritz, 1999a; Henschler et al., 1995; Cohn et al., 1994b; Vartiainen et al., 1993; Sinks et al., 1992; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Wilcosky et al., 1984)The observed lack of association with NHL in these studies likely reflects study design and exposure assessment limitations and is not considered inconsistent with the overall evidence on TCE and NHL.

Consistency of the association between TCE exposure and NHL is further supported by the results of meta-analyses of 17 studies reporting risk estimates for overall TCE exposure that met the meta-analysis inclusion criteria. These meta-analyses found a statistically significant increased RRm estimate for NHL of 1.23 (95% CI: 1.07, 1.42) for overall TCE exposure. The analysis of NHL was robust to the removal of individual studies and the use of alternate RR

estimates from individual studies, and in only one cases was the resulting RRm no longer statistically significant (lower 95% confidence bounds of 1.00). Some evidence heterogeneity was observed, particularly between cohort and case-control studies, but it was not statistically significant ($p = 0.10$); and, in addition, there was some evidence of potential publication bias. Analyzing the cohort and case-control studies separately resolved most of the heterogeneity, but the result for the summary case-control studies was only a 7% increased RR estimate and was not statistically significant. The sources of heterogeneity are uncertain but may be the result of some bias associated with exposure assessment and/or disease classification, or from differences between cohort and case-control studies in average TCE exposure.

Exposure-response relationships are examined in the TCE epidemiologic studies only to a limited extent. Many studies examined only overall "exposed" vs. "unexposed" groups and did not provide exposure information by level of exposure. Others do not have adequate exposure assessments to confidently distinguish between levels of exposure. The NHL case-control study of Purdue et al. (2011) reported a statistically significant trend with TCE exposure ($p = 0.02$ for average-weekly TCE exposure), and NHL risk in Boice et al. (1999) appeared to increase with increasing exposure duration ($p = 0.20$ for routine-intermittent exposed subjects). The borderline statistically significant trend with TCE intensity in the case-control study of Wang et al. (2009 [$p = 0.06$]) and with cumulative TCE exposure in the case-control study of Purdue et al. (2011 [$p = 0.08$] is consistent with that observed with average weekly TCE exposure in Purdue et al. (2011). Further support was provided by meta-analyses using only the highest exposure groups, which yielded a higher RRm estimate (1.43 [95% CI: 1.13, 1.82]) than for overall TCE exposure (1.23 [95% CI: 1.07, 1.42]).

Few risk factors are recognized for NHL, with the exception of viruses, immunosuppression, or smoking, which are associated with specific NHL subtypes (Besson et al., 2006). Associations between NHL and TCE exposure are based on groupings of several subtypes. Two of the seven NHL case-control studies adjusted for age, sex, and smoking in statistical analyses (Wang et al., 2009; Miligi et al., 2006), two others adjusted for age and sex (Purdue et al., 2011; Cocco et al., 2010), and the other three case-control studies presented only unadjusted OR estimates (Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994).

Animal studies describing rates of lymphomas and/or leukemias in relation to TCE exposure (NTP, 1990; Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986; Henschler et al., 1984; Henschler et al., 1980; NCI, 1976) are available. Henschler et al. (1980) reported statistically significant increases in lymphomas in female Han:NMRI mice treated via inhalation. While Henschler et al. (1980) suggested that these lymphomas were of viral origin specific to this strain, subsequent studies reported increased lymphomas in female $B6C3F_1$ mice treated via corn oil gavage (NTP, 1990) and leukemias in male Sprague-Dawley and female August rats (Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986). However, these tumors had relatively

modest increases in incidence with treatment, and were not reported to be increased in other studies.

4.7. RESPIRATORY TRACT TOXICITY AND CANCER

4.7.1. Epidemiologic Evidence

4.7.1.1. Chronic Effects: Inhalation

Two reports of a study of 1,091 gun-manufacturing workers are found on noncancer pulmonary toxicity (Saygun et al., 2007; Cakmak et al., 2004). A subset of these workers (n = 411) had potential exposure to multiple organic solvents including toluene, acetone, butanol, xylene, benzene, and TCE used to clean gun parts; however, both papers lacked information on exposure concentration. Mean exposure duration in Cakmak et al. (2004) was 17 years (SD = 7.9) for nonsmokers and 16 years (SD = 7.1) for smokers. Cakmak et al. (2004) indicated effects of smoking and exposure to solvents, with smoking having the most important effect on asthma-related symptoms (smoking, OR = 2.8, 95% CI: 2.0, 3.8; solvent exposure, OR = 1.4, 95% CI: 1.1, 1.9). Similarly, smoking, but not solvent exposure, was shown as a statistically significant predictor of lung function decrements. Saygun et al. (2007) reported on a 5-year follow-up of 393 of the original 1,091 subjects, 214 of who were exposed to solvents. Of the 393 original subjects, the prevalence of definitive asthma symptoms, a more rigorous definition than used by Cakmak et al. (2004), was 3.3% among exposed and 1.1% among nonexposed subjects, $p > 0.05$. Saygun et al. (2007) presents observations on lung function tests for 697 current workers, a group which includes the 393 original study subjects. Smoking, but not solvent exposure, was a predictor of mean annual forced expiratory volume (FEV_1) decrease.

4.7.1.2. Cancer

Cancers of the respiratory tract including the lung, bronchus, and trachea were examined in 25 cohort, community studies and case-control studies of TCE. Twelve of the 25 studies approached standards of epidemiologic design and analysis identified in the review of the epidemiologic body of literature on TCE and cancer (Radican et al., 2008; Boice et al., 2006b; Zhao et al., 2005; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991). Cancers at other sites besides lung, bronchus, and trachea in the respiratory system are more limitedly reported in these studies. Some information is available on laryngeal cancer; however, only 9 of the 16 occupational cohort studies providing information on lung cancer also reported findings for this site. Case-control studies of lung or laryngeal cancers and occupational title or organic solvent exposure were found in the literature. Two case-control studies of lung cancer, one population-based and the other nested within a cohort, were of TCE exposure specifically. Lung and laryngeal cancer risk ratios reported in cohort, community and case-control studies are found in Table 4-81.

Table 4-81. Selected results from epidemiologic studies of TCE exposure and lung cancer

Exposure group		RR (95% CI)	Number of observable events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al. (2005)
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	1.00[a]	43	
	Medium cumulative TCE score	1.36 (0.86, 2.14)	35	
	High TCE score	1.11 (0.60, 2.06)	14	
	p for trend	0.60		
All employees at electronics factory (Taiwan)		1.07 (0.72, 1.52)	30	Chang et al. (2005)
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. (2003)
	Any exposure, all subjects	1.4 (1.32, 1.55)	632	
	Any exposure, males	1.4 (1.28, 1.51)	559	
	Any exposure, females	1.9 (1.48, 2.35)	73	
	Employment duration			
	<1 yr	1.7 (1.46, 1.93)	209	
	1–4.9 yrs	1.3 (1.16, 1.52)	218	
	≥5 yrs	1.4 (1.23, 1.63)	205	
Biologically-monitored Danish workers				Hansen et al. (2001)
	Any TCE exposure, males	0.8 (0.5, 1.3)	16	
	Any TCE exposure, females	0.7 (0.01, 3.8)	1	
	Cumulative exposure (Ikeda)	Not reported		
	<17 ppm-yr			
	≥17 ppm-yr			
	Mean concentration (Ikeda)	Not reported		
	<4 ppm			
	4+ ppm			
	Employment duration	Not reported		
	<6.25 yr			
	≥6.25 yr			
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
	TCE subcohort	Not reported		
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	1.0 (0.6, 2.0)	24	
	5–25 ppm-yr	0.8 (0.4, 1.6)	11	
	>25 ppm-yr	0.8 (0.4, 1.7)	15	

Table 4-81. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
	Females, cumulative exposure			Blair et al. (1998) (continued)
	0	1.0[a]		
	<5 ppm-yr		1	
	5–25 ppm-yr		1	
	>25 ppm-yr		1	
Biologically-monitored Finnish workers				Anttila et al. (1995)
	All subjects	0.92 (0.59, 1.35)	25	
	Mean air-TCE (Ikeda extrapolation)			
	<6 ppm	1.02 (0.58, 1.66)	16	
	6+ ppm	0.83 (0.33, 1.71)	7	
Biologically-monitored Swedish workers				Axelson et al. (1994)
	Any TCE exposure, males	0.69 (0.31, 1.30)	9	
	Any TCE exposure, females	Not reported		
Cohort and PMR-mortality				
Computer manufacturing workers (IBM), New York				Clapp and Hoffman (2008)
	Males	1.03 (0.71, 1.42)	35	
	Females	0.95 (0.20, 2.77)	3	
Aerospace workers (Rocketdyne)				
	Any TCE (utility or engine flush workers)	1.24 (0.92, 1.63)	51	Boice et al. (2006b)
	Engine flush—duration of exposure			
	Referent	1.0[a]	472	
	0 yr (utility workers with TCE exposure)	0.5 (0.22, 1.00)	7	
	<4 yrs	0.8 (0.50, 1.26)	27	
	≥4 yrs	0.8 (0.46, 1.41)	24	
	Any exposure to TCE	Not reported		Zhao et al. (2005)
	Low cumulative TCE score	1.00[a]	99	
	Medium cumulative TCE score	1.05 (0.76, 1.44)	62	
	High TCE score	1.02 (0.68, 1.53)	33	
	p for trend	0.91		
View-Master employees				ATSDR (2004a)
	Males	0.81 (0.42, 1.42)[b]	12	
	Females	0.99 (0.71, 1.35)[b]	41	

Table 4-81. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
United States uranium-processing workers (Fernald)				Ritz (1999a)
	Any TCE exposure	Not reported		
	Light TCE exposure, >2-yr duration[c]	Not reported		
	Moderate TCE exposure, >2-yr duration[c]	Not reported		
Aerospace workers (Lockheed)				Boice et al. (1999)
	Routine exposure	0.76 (0.60, 0.95)	78	
	Routine-intermittent exposure[a]	Not reported	173	
	Duration of exposure			
	0 yr	1.0	288	
	<1 yr	0.85 (0.65, 1.13)	66	
	1–4 yrs	0.98 (0.74, 1.30)	63	
	≥5 yrs	0.64 (0.46, 0.89)	44	
	Trend test	$p < 0.05$		
Aerospace workers (Hughes)				Morgan et al. (1998)
	TCE subcohort	1.10 (0.89, 1.34)	97	
	Low intensity (<50 ppm)	1.49 (1.09, 1.99)	45	
	High intensity (>50 ppm)	0.90 (0.67, 1.20)	52	
	TCE subcohort (Cox Analysis)[b]			
	Never exposed	1.00[a]	291	
	Ever exposed	1.14 (0.90, 1.44)	97	
	Peak			
	No/Low	1.00[a]	324	
	Medium/High	1.07 (0.82, 1.40)	64	
	Cumulative			
	Referent	1.00[a]	291	
	Low	1.47 (1.07, 2.03)	45	
	High	0.96 (0.72, 1.29)	52	
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
	TCE subcohort			
	Any TCE exposure	0.9 (0.6, 1.3)[a]	109	

Table 4-81. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
	Males, cumulative exposure			Blair et al. (1998) (continued)
	0	1.0[a]	51	
	<5 ppm-yr	1.0 (0.7, 1.6)	43	
	5–25 ppm-yr	0.9 (0.5, 1.6)	23	
	>25 ppm-yr	1.1 (0.7, 1.8)	38	
	Females, Cumulative exp			
	0	1.0[a]	2	
	<5 ppm-yr	0.6 (0.1, 2.4)	2	
	5–25 ppm-yr	0.6 (0.1, 4.7)	11	
	>25 ppm-yr	0.4 (0.1, 1.8)	2	
	TCE subcohort			Radican et al. (2008)
	Any TCE exposure	0.83 (0.63, 1.08)	166	
	Males, cumulative exposure	0.91 (0.67, 1.24)	155	
	0	1.0[a]	66	
	<5 ppm-yr	0.96 (0.67, 1.37)		
	5–25 ppm-yr	0.71 (0.46, 1.11)	31	
	>25 ppm-yr	1.00 (0.69, 1.45)	58	
	Females, cumulative exposure	0.53 (0.27, 1.07)	11	
	0	1.0[a]		
	<5 ppm-yr	0.69 (0.27, 1.77)	5	
	5–25 ppm-yr	0.65 (0.16, 2.73)	2	
	>25 ppm-yr	0.39 (0.14, 1.11)	4	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	TCE-exposed workers	1.38 (0.55, 2.86)	7	
	Unexposed workers	1.06 (0.34, 2.47)	5	
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		1.01 (0.69, 1.47)[d]	139	Greenland et al. (1994)
U.S. Coast Guard employees				Blair et al. (1998)
	Marine inspectors	0.52 (0.31, 0.82)	18	
	Noninspectors	0.81 (0.55, 1.16)	30	
Aircraft manufacturing employees (Italy)				Costa et al. (1989)
	All employees	0.99 (0.73, 1.32)	99	
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)
	All subjects	0.80 (0.68, 0.95)	138	

Table 4-81. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Lamp manufacturing workers (GE)		0.58 (0.27, 1.27)	6	Shannon et al. (1988)
Rubber industry workers (Ohio)		0.64 ($p > 0.05$)[c]	11	Wilcosky et al. (1984)
Case-control studies				
Population of Montreal, Canada				Siemiatycki et al. (1991)
	Any TCE exposure	0.9 (0.6, 1.5)[e]	21	
	Substantial TCE exposure	0.6 (0.3, 1.2)[e]	9	
Geographic-based studies				
Two study areas in Endicott, New York		1.28 (0.99, 1.62)	68	ATSDR (2006a)
Residents of 13 census tracts				Morgan and Cassidy (2002)
	In Redlands, California	0.71 (0.61, 0.81)[f]	356	
Iowa residents with TCE in water supply				Isacson et al. (1985)
	Males			
	<0.15 µg/L	343.1[g]	1,181	
	≥0.15 µg/L	345.7[g]	299	
	Females			
	<0.15 µg/L	58.7[g]	289	
	≥0.15 µg/L	47.8[g]	59	

[a]Internal referents, workers not exposed to TCE.
[b]Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex, and decade (EHS, 1997).
[c]OR from nested case-control study.
[d]OR from nested case-control analysis.
[e]90% CI.
[f]99% CI.
[g]Average annual age-adjusted incidence (per 100,000).

Lung cancer RRs were reported in 11 of 12 cohort studies of aircraft manufacturing, aircraft maintenance, aerospace, and metal workers, with potential exposure to TCE as a degreasing agent, and in occupational cohort studies employing biological markers of TCE exposures. All 11 studies had a high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis (Radican et al., 2008; Boice et al., 2006b; Zhao et al., 2005; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994). Lung cancer risks were not reported for Fernald uranium processing workers with potential TCE exposure (Ritz, 1999a), a study of less weight than the other 11 studies. The incidence study of Raaschou-Nielsen et al. (2003) was the largest cohort, with 40,049 subjects identified as potentially exposed to TCE in several industries (primarily, in the iron/metal and electronic industries), including 14,360 who had presumably higher level exposures to TCE. The study included 632 lung cancer cases and reported a 40%

elevated incidence in TCE exposed males and females combined (95% CI: 1.32, 1.55), with no exposure duration gradient. The 95% CIs in other studies of lung cancer incidence included a risk ratio of 1.0 (Zhao et al., 2005; Hansen et al., 2001; Blair et al., 1998; Anttila et al., 1995; Axelson et al., 1994). Lung cancer mortality risks in studies of TCE exposure to aircraft manufacturing, aircraft maintenance, and aerospace workers included a RR of 1.0 in their 95% CIs (Radican et al., 2008; Boice et al., 2006b; Zhao et al., 2005; Blair et al., 1998; Morgan et al., 1998). Boice et al. (1999) observed a 24% decrement (95% CI: 0.60, 0.95) for subjects with routine TCE exposure. Exposure-response analyses using internal controls (unexposed subjects at the same company) showed a statistically significant decreasing trend between lung cancer risk and routine or intermittent TCE exposure duration. The routine or intermittent category is broader and includes more subjects with potential TCE exposure. Five other studies with internal controls do not provide evidence of either an increasing or decreasing pattern between TCE and lung cancer incidence or mortality (Radican et al., 2008; Boice et al., 2006b; Zhao et al., 2005; Blair et al., 1998; Morgan et al., 1998).

The population studied by Garabrant et al. (1988), ATSDR (2004a) and Chang et al. (2005) are all employees (white- and blue-collar) at a manufacturing facility or plant with potential TCE exposures. Garabrant et al. (1988) observed a 20% deficit in lung cancer mortality (95% CI: 0.68, 0.95) in their study of all employees working for ≥4 years at an aircraft manufacturing company. Blair et al. (1998), a study of Coast Guard marine inspectors with potential for TCE exposure but lacking assessment to individual subjects, observed a 48% deficit in lung cancer mortality (95% CI: 0.31, 0.82). Confidence intervals (95% CI) in Costa et al. (1989), Chang et al. (2005), and ATSDR (2004a) included a risk of 1.0. TCE exposure was not known for individual subjects in these studies. A wide potential for TCE exposure is likely ranging from subjects with little to no TCE exposure potential to those with some TCE exposure potential. Exposure misclassification bias, typically considered as a negative bias, is likely greater in these studies compared to studies adopting more sophisticated exposure assessment approaches, which are able to assign quantitative exposure metrics to individual study subjects. All three studies were of lower likelihood for TCE exposure, in addition to limited statistical power and other design limitations, and these aspects, in addition to potential exposure misclassification bias, were alternative explanations of observed findings.

One population case-control study examined the relationship between lung cancer and TCE exposure (Siemiatycki, 1991) with risk ratios of 0.9 (95% CI: 0.6, 1.5) for any TCE exposure and 0.6 (95% CI: 0.3, 1.2) for substantial TCE exposure after adjustment for cigarette smoking. TCE exposure prevalence in cases in this study was 2.5% for any exposure. Only 1% had ―substantial" (author's term) exposure, limiting the sensitivity of this study. RRs >2.0 could only be detected with sufficient (80%) statistical power. The finding of no association of lung cancer with TCE exposure, therefore, is not surprising. One nested case-control study of rubber

workers observed a smoking unadjusted risk of 0.64 (95% CI: not presented in paper) in those who had >1 year cumulative exposure to TCE (Wilcosky et al., 1984).

Three geographic-based studies reported lung cancer incidence or mortality risks for drinking water contamination with TCE (ATSDR, 2006a; Morgan and Cassady, 2002; Isacson et al., 1985). Morgan and Cassidy (2002) observed a RR of 0.71 (99% CI: 0.61, 0.81) for lung cancer among residents of Redlands (San Bernardino County), California, whose drinking water was contaminated with TCE and perchlorate. However, ATSDR (2006a) reported a 28% increase (95% CI: 0.99, 1.62) in lung cancer incidence among residents living in a area in Endicott, New York, whose drinking water was contaminated with TCE and other solvents. No information on smoking patterns is available for individual lung cancer cases as identified by the New York State Department of Health (NYS DOH) for other cancer cases in this study (ATSDR, 2008b). Isacson et al. (1985) presented lung cancer age-adjusted incidence rates for Iowa residents by TCE level in drinking water supplies and did not observe an exposure-response gradient. Exposure information is inadequate in all three of these studies, with monitoring data, if available, based on few samples and for current periods only, and no information on water distribution, consumption patterns, or temporal changes. Thus, TCE exposure potential to individual subjects was not known with any precision, introducing misclassification bias, and greatly limiting their ability to inform evaluation of TCE and lung cancer.

Laryngeal cancer risks are presented in a limited number of cohort studies involving TCE exposure. No case-control or geographic-based studies of TCE exposure were found in the published literature. All but one of the cohort studies providing information on laryngeal cancer observed less that five incident cases or deaths. Accordingly, these studies are limited for examining the relationship between TCE exposure and laryngeal cancer. Risk ratios for laryngeal cancer are found in Table 4-82.

Table 4-82. Selected results from epidemiologic studies of TCE exposure and laryngeal cancer

Exposure group		RR (95% CI)	Number of observable events	Reference
Cohort studies—incidence				
Aerospace workers with TCE exposure		Not reported		Zhao et al. (2005)
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. (2003)
	Any exposure, males	1.2 (0.87, 1.52)	53	
	Any exposure, females	1.7 (0.33, 4.82)	3	
	Employment duration	Not reported		
	<1 yrs			
	1–4.9 yrs			
	≥5 yrs			
Biologically-monitored Danish workers				Hansen et al. (2001)
	Any TCE exposure, males	1.1 (0.1, 3.9)	2	
	Any TCE exposure, females		0 (0.1 exp)	
	Cumulative exposure (Ikeda)	Not reported		
	<17 ppm-yr			
	≥17 ppm-yr			
	Mean concentration (Ikeda)	Not reported		
	<4 ppm			
	4+ ppm			
	Employment duration	Not reported		
	<6.25 yr			
	≥6.25 yr			
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
	TCE subcohort			
	Any exposure	Not reported		
	Males, cumulative exposure	Not reported		
	0			
	<5 ppm-yr			
	5–25 ppm-yr			
	>25 ppm-yr			
	Females, cumulative exposure	Not reported		
	0			
	<5 ppm-yr			
	5–25 ppm-yr			
	>25 ppm-yr			

Table 4-82. Selected results from epidemiologic studies of TCE exposure and laryngeal cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Biologically-monitored Finnish workers		Not reported		Anttila et al. (1995)
	Mean air-TCE (Ikeda extrapolation from U-TCA)	Not reported		
	<6 ppm			
	6+ ppm			
Biologically-monitored Swedish workers				Axelson et al. (1994)
	Any TCE exposure, males	1.39 (0.17, 5.00)	2	
	Any TCE exposure, females	Not reported		
Cohort and PMR-mortality				
Computer manufacturing workers (IBM), New York		Not reported		Clapp and Hoffman (2008)
Aerospace workers (Rocketdyne)				
	Any TCE (utility or engine flush workers)	1.45 (0.18, 5.25)	2	Boice et al. (2006b)
	Engine flush—duration of exposure	Not reported		
	Referent			
	0 yr (utility workers with TCE exposure)			
	<4 yrs			
	≥4 yrs			
	Any exposure to TCE	Not reported		Zhao et al. (2005)
View-Master employees		Not reported		ATSDR (2004a)
	Males			
	Females			
All employees at electronic factory (Taiwan)				Chang et al. (2003)
	Males		0 (0.90 exp)	
	Females	0	0 (0.23 exp)	
United States uranium-processing workers (Fernald)				Ritz (1999a)
	Any TCE exposure	Not reported		
	Light TCE exposure, >2-yr duration	Not reported		
	Moderate TCE exposure, >2-yr duration	Not reported		
Aerospace workers (Lockheed)				Boice et al. (1999)
	Routine exposure	1.10 (0.30, 2.82)	4	
	Routine-intermittent exposure	Not reported		

Table 4-82. Selected results from epidemiologic studies of TCE exposure and laryngeal cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Aerospace workers (Hughes)				Morgan et al. (1998)
	TCE subcohort	Not reported		
	Low intensity (<50 ppm)			
	High intensity (>50 ppm)			
	Peak	Not reported		
	No/low			
	Medium/high			
	Cumulative	Not reported		
	Referent			
	Low			
	High			
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
	TCE subcohort	Not reported		
	Males, cumulative exposure	Not reported		
	0			
	<5 ppm-yr			
	5–25 ppm-yr			
	>25 ppm-yr			
	Females, cumulative exposure	Not reported		
	0			
	<5 ppm-yr			
	5–25 ppm-yr			
	>25 ppm-yr			
Cardboard manufacturing workers in Arnsburg, Germany		Not reported		Henschler et al. (1995)
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		Not examined		Greenland et al. (1994)
U.S. Coast Guard employees				Blair et al. (1998)
	Marine inspectors	0.57 (0.01, 3.17)	1	
	Noninspectors	0.58 (0.01, 3.20)	1	
Aircraft manufacturing employees (Italy)				Costa et al. (1989)
	All employees	0.27 (0.03, 0.98)	2	
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)
	All subjects		0 (7.41 exp)	

In summary, studies in humans examining lung and laryngeal cancer and TCE exposure are inconclusive and do not support either a positive or a negative association between TCE exposure and lung cancer or laryngeal cancer. Raaschou-Nielsen et al. (2003), with the largest numbers of lung cancer cases of all studies, was the only one to observe a statistically

significantly elevated lung cancer risk with TCE exposure. Raaschou-Nielsen et al. (2003) also noted several factors that may have confounded or biased their results in either a positive or negative direction. This study and other cohort studies, as with almost any occupational study, were not able to control confounding by exposure to chemicals other than TCE (although no such chemical was apparent in the reports). Information available for factors related to SES status (e.g., diet, smoking, alcohol consumption) was also not available. Such information may positively confound smoking-related cancers such as lung cancer, particularly in those studies, which adopted national rates to derive expected numbers of site-specific cancer, if greater smoking rates were over-represented in blue-collar workers or residents of lower SES status. The finding of a larger risk among subjects with shortest exposure also argues against a causal interpretation for the observed association for all subjects (NRC, 2006).

Four studies reported a statistically significant deficit in lung cancer incidence (Morgan and Cassady, 2002; Boice et al., 1999; Blair et al., 1998; Garabrant et al., 1988). Absence of smoking information in these studies would introduce a negative bias if the studied population smoked less than the referent population and may partially explain the lung cancer decrements observed in these studies. Morgan and Cassidy (2002) noted the relatively high education, high income levels, and high access to health care of subjects in this study compared to the averages for the county as a whole, likely leading to a lower smoking rate compared to their referent population. Garabrant et al. (1988) similarly attributed their observations to negative selection bias introduced when comparison is made to national mortality rates, also known as a "healthy worker effect." The statistically significant decreasing trend in Boice et al. (1999) with exposure duration to intermittent or routine exposure may reflect a protective effect between TCE and lung cancer. The use of internal controls in this analysis reduces bias associated with use of an external population who may have different smoking patterns than an employed population. However, the exposure assessment approach in this study is limited due to inclusion of subjects identified with intermittent TCE exposure (i.e., workers who would be exposed only during particular shop runs or when assisting other workers during busy periods) (Boice et al., 1999). The Boice et al. (1999) analysis is based on twice as many lung cancer deaths (i.e., 173 lung cancer deaths) among subjects with routine or intermittent TCE exposure compared to only routinely exposed subjects (78 deaths). Subjects identified as intermittently exposed are considered as having a lower exposure potential than routinely exposed subject and their inclusion in exposure-response analyses may introduce exposure misclassification bias. Such bias is a possible explanation for the decreasing trend observation, particularly if workers with lower potential for TCE exposure have longer exposure (employment) durations.

Thus, a qualitative assessment suggests the epidemiological literature on respiratory cancer and TCE, although limited and of sufficient power to detect only large RRs, does not provide strong evidence for any association between TCE exposure and lung cancer. These

studies can only rule out risks of a magnitude of ≥2.0 for lung cancer and RRs >3.0 or 4.0 for laryngeal cancer for exposures to studied populations.

4.7.2. Laboratory Animal Studies

4.7.2.1. Respiratory Tract Animal Toxicity

Limited studies are available to determine the effects of TCE exposure on the respiratory tract (summarized in Table 4-83). Many of these studies in mice have examined acute effects following i.p. administration at relatively high TCE doses. However, effects on the bronchial epithelium have been noted in mice and rats with TCE administered via gavage, with doses ≥1,000 mg/kg-day reported to cause rales and dyspnea (Narotsky et al., 1995) and pulmonary vasculitis (NTP, 1990) in rats. Mice appear to be more sensitive than rats to histopathological changes in the lung via inhalation; pulmonary effects are also seen in rats with gavage exposure. It is difficult to compare i.p. to oral and inhalation routes of exposure given the risk of peritonitis and paralytic ileus. Any inflammatory response from this route of administration can also affect the pulmonary targets of TCE exposure such as the Clara cells.

This section reviews the existing literature on TCE, and the role of the various TCE metabolites in TCE-induced lung effects. The most prominent toxic effect reported is damage to Clara cells in mouse lung. The nonciliated, columnar Clara cells comprise the majority of the bronchiolar and terminal bronchiolar epithelium in mice, and alveolar Type I and Type II cells constitute the alveolar epithelium. These cells have been proposed as a progenitor of lung adenocarcinomas in both humans and mice (Kim et al., 2005). Long-term studies have not focused on the detection of pulmonary adenoma carcinomas but have shown a consistently positive response in mice but not rats. However, chronic toxicity data on noncancer effects are very limited.

Table 4-83. Animal toxicity studies of TCE

Reference	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Results
Green et al. (1997b)	CD-1 mice (F)	Inhalation	450 ppm, 6 hrs/d, 5 d with 2 d break then 5 more d; sacrificed 18 hrs after 1, 5, 6, or 10 exposures	5/group	Increased vacuolation and proliferation of Clara cells caused by accumulation of chloral.
Forkert and Forkert (1994)	CD-1 mice (M)	i.p. injection	2,000 mg/kg in corn oil (0.01 mL/g body weight); sacrificed 15, 30, 60, and 90 d after single exposure	10/group	Increased fibrotic lesions, with early signs visible at 15 d postexposure.
Villaschi et al. (1991)	BC3F1 mice (M)	Single inhalation	30 min 500, 1,000, 2,000, 3,500, and 7,000 ppm; sacrificed 2 hrs, 24 hrs, 2, 5, or 7 d post exposure	3/group	Increased vacuolation and proliferation of nonciliated bronchial cells. Injury was maximal at 24 hrs with some repair occurring between 24 and 48 hrs.
Odum et al. (1992)	CD-1 mice (F)	Inhalation	6 hrs/d; separate repeated study in mice: 450 ppm for 6 hrs/d, 5 d/wk for 2 wks; sacrificed 24 hrs after exposure; repeat study sacrificed at 2, 5, 6, 8, 9, 12, or 13 d; mice: 20, 100, 200, 450, 1,000, or 2,000 ppm	4/group	Dose-dependent increase in Clara cell vacuolation in mice after a single exposure, resolved after 5 d repeated exposures but recurred following a 2-d break from exposure. Changes accompanied by decrease in CYP activity in mice. Exposure to chloral alone demonstrated similar response as TCE exposure in mice. No changes were seen in rats.
	Alpk APfSD rats (F)	Inhalation	6 hrs/d; repeat study sacrificed at 2, 5, 6, 8, 9, 12, or 13 d; rats: 500, or 1,000 ppm	4/group	
Kurasawa (1988) (translation)	Ethanol-treated (130) and nontreated (110) Wistar rats (M)	Inhalation	500, 1,000, 2,000, 4,000, and 8,000 ppm for 2 hrs; sacrificed 22 hrs after exposure	10/group	TCE exposure resulted in highly selective damage to Clara cells that occurred between 8 and 22 hrs after the highest exposure with repair by 4 wks post exposure.
Forkert et al. (2006)	CD-1 mice (M); wild-type (mixed 129/Sv and C57BL) and CYP2E1-null mice (M)	i.p. injection	500, 750, and 1,000 mg/kg in corn oil; for inhibition studies mice pretreated with 100 mg/kg diallyl sulfone; for immunoblotting, 250, 500, 750, and 1,000 mg/kg; for PNP hydroxylation, 50, 100, 250, 500, 750, and 1,000 mg/kg; sacrificed 4 hrs after exposure	4/group	TCE bioactivation by CYP2E1 and/or 2F2 correlated with bronchiolar cytotoxicity in mice.

Table 4-83. Animal toxicity studies of TCE (continued)

Reference	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Results
Forkert et al. (1985)	CD-1 mice (M)	i.p. injection	2,000, 2,500 or 3,000 mg/kg in mineral oil; sacrificed 24 hrs postexposure for dose response; time course sacrificed 1, 2, 12, and 24 hrs postexposure	10/group	Clara cell injury was increased following exposure at all doses tested; time course demonstrated a rapid and marked reduction in pulmonary microsomal CYP content and aryl hydrocarbon hydroxylase activity. Alveolar Type II cells were also affected.
Forkert and Birch (1989)	CD-1 mice (M)	i.p. injection	2,000 mg/kg in corn oil; sacrificed 1, 2, 4, 8, 12, and 24 hrs postexposure	10/group	Necrotic changes seen in Clara cells as soon as 1 hr postexposure; increased vacuolation was seen by 4 hrs postexposure; covalent binding of TCE to lung macromolecules peaked at 4 hrs and reached a plateau at 12 and 24 hrs post exposure.
Stewart et al. (1979); Le Mesurier et al. (1980)	Wistar Rats (F)	Inhalation (whole-body chamber)	30 min, 48.5 g/m^3 (9,030 ppm); sacrificed at 5 and 15 d postexposure	5/group	Decreased recovery of pulmonary surfactant (dose-dependent).
Lewis et al. (1984)	Mice	Inhalation (Pyrex bell jars)	10,000 ppm, 1–4 hrs daily for 5 consecutive d; sacrificed 24 hrs after last exposure	~28/group	Increased vacuolation and reduced activity of pulmonary mixed function oxidases.
Scott et al. (1988)	CD-1 mice (M)	i.p. injection	single injection of 2,500–3,000 mg/kg, sacrificed 24 hrs postexposure	4/group	Clara cells were damaged and exfoliated from the epithelium of the lung.
NTP (1990)	F344 rats (M,F) $B6C3F_1$ mice (M,F)	Gavage	Male rats: 0, 125, 250, 500, 1,000, and 2,000 mg/kg body weight (corn oil); female rats: 0, 62.5, 125, 250, 500 or 1,000 mg/kg body weight (corn oil); Mice: 0, 375, 750, 1,500, 3,000, and 6,000 mg/kg body weight (corn oil); dosed 5d/w for 13 wks	10/group	Increased pulmonary vasculitis in the high-dose groups of male and female rats (6/10 group as compared to 1/10 in controls). No pulmonary effects described in mice at this time point.

Table 4-83. Animal toxicity studies of TCE (continued)

Reference	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Results
Prendergast et al. (1967)	Sprague-Dawley or Long-Evans rats; Hartley Guinea pigs; New Zealand albino rabbits; beagle dogs; squirrel monkeys (sex not given for any species)	Inhalation	730 ppm for 8 hrs/d, 5 d/w, 6 wks or 35 ppm for 90 d constant	Rats (15); guinea pigs (15); rabbit (3); dog (2); monkey (3)	No histopathological changes observed, although rats were described to show a nasal discharge in the 6-wk study. No quantification was given.
Narotsky et al. (1995)	F344 rats (F)	Gavage	0, 1,125, or 1,500 mg/kg-d	21, 16, or 17 per group	Rales and dyspnea were observed in the TCE high-dose group; two females with dyspnea subsequently died.

4.7.2.1.1. Acute and short-term effects: inhalation

Relatively high-dose single and multiple inhalation exposures to TCE result in dilation of endoplasmic reticulum and vacuolation of nonciliated (Clara) cells throughout the bronchial tree in mice. A single study in rats reported similar findings. In mice, single exposure experiments show vacuolation at all dose levels tested with the extent of damage increasing with dose. Villaschi et al. (1991) reported similar degrees of vacuolation in $B6C3F_1$ mice (3/group) at 24 hours after the start of exposure across all tested doses (500, 1,000, 2,000, 3,500, and 7,000 ppm, 30 minutes), with the percentage of the nonciliated cells remaining vacuolated at 48 hours increasing with dose. Clara cell vacuolation was reported to be resolved 7 days after single 30-minute exposures to TCE. Odum et al. (1992) reported that, when observed 24 hours after the start of 6 hours exposure, the majority of Clara cells in mice were unaffected at the lowest dose of 20 ppm exposures, while marked vacuolation was observed at 200 ppm (no quantitative measures of damage given and only three animals per group were examined).

In rats, Odum et al. (1992) reported no morphological changes in the female Alpk APfSD rat epithelium after 6 hours exposure (500 or 1,000 ppm) when observed 24 hours after the start of exposure (n = 3/group). However, Kurasawa reported pronounced dose-related morphological changes in Clara cells at the highest dose (8,000 ppm) for 2 hours in Wistar rats (n = 10 per group). At 500 and 1,000 ppm, slight dilation of the apical surface was reported, but morphological measurements (the ratio of the lengths of the apical surface to that of the base line of apical cytoplasm) were not statistically-significantly different from controls. From 2,000 to 8,000 ppm, a progressively increasing flattening of the apical surface was observed. In addition, at 2,000 ppm, slight dilation of the smooth endoplasmic reticulum was also observed, with marked dilation and possible necrosis at 8,000 ppm. Kurasawa (1988) also examined the time-course of Clara cell changes following a single 8,000-ppm exposure, reporting the greatest effects at 1 day to 1 week, repair at 2 weeks, and nearly normal morphology at 4 weeks. The only other respiratory effect that has been reported from one study in rats exposed via inhalation is a reduction in pulmonary surfactant yield following 30-minute exposures at 9,030 ppm for 5 or 15 days (Stewart et al., 1979). Therefore, single inhalation experiments (Odum et al., 1992; Villaschi et al., 1991; Kurasawa, 1988) suggest that the Clara cell is the target for TCE exposure in both rats and mice and that mice are more susceptible to these effects. However, the database is limited in its ability to discern quantitative differences in susceptibility or the nature of the dose-response after a single dose of TCE.

Other experiments examined the effects of several days of TCE inhalation exposure in mice and potential recovery. While single exposures require 1–4 weeks for complete recovery, after short-term repeated exposure, the bronchial epithelium in mice appears to either adapt to or become resistant to damage. Odum et al. (1992) and Green et al. (1997b) observed Clara cells in mice to be morphologically normal at the end of exposures 6 hours/day for 4 or 5 days. As with

single-dose experiments, the extent of recovery in multidose exposures may be dose-dependent. Using a very high dose, Lewis et al. (1984) reported vacuolation of bronchial epithelial cells after 4 hours/day, but not 1 hour/day (10,000 ppm), for 5 days in mice. In addition, Odum et al. (1992) reported that the damage to Clara cells that resolved after repeated exposures of 5 days, a sign of adaptation to TCE exposure, returned when exposure was resumed after 2 days.

In rats, only one inhalation study reported in two published articles (Le Mesurier et al., 1980; Stewart et al., 1979) using repeated exposures examined pulmonary histopathology. Interestingly, this study reported vacuolation in Type 1 alveolar cells, but not in Clara cells, after 5 days of exposure to approximately 9,030 ppm for 30 minutes/day (only dose tested). In addition, abnormalities were observed in the endothelium (bulging of thin endothelial segments into the microcirculatory lumen) and minor morphological changes in Type 2 alveolar cells. Although exposures were carried out for 5 consecutive days, histopathology was recorded up to 15 days postexposure, giving cell populations time to recover. Because earlier time points were not examined, it is not possible to discern whether the lack of reported Clara cell damage in rats following repeated exposure is due to recovery or lack of toxicity in this particular experiment.

Although recovery of individual damaged cells may occur, cell proliferation, presumed from labeling index data suggestive of increased DNA synthesis, contributes, at least in part, to the recovery of the bronchial epithelium in mice. Villaschi et al. (1991) observed a dose-dependent increase in labeling index as compared to controls in the mouse lung at 48 hours after a single TCE exposure (30 minutes; 500, 1,000, 2,000, 3,500, or 7,000 ppm), which decreased to baseline values at 7 days postexposure. Morphological analysis of cells was not performed, although the authors stated that the dividing cells had the appearance of Clara cells. Interestingly, Green et al. (1997b) reported no increase in BrdU labeling 24 hours after a single exposure (6 hours, 450 ppm), but did see increased BrdU labeling at the end of multiple exposures (1/day, 5 days) while Villaschi et al. (1991) reported increased [^{3}H]-thymidine labeling 2, 5, and 7 days after single 30-minute exposures to 500–7,000 ppm. Therefore, the data for single exposures at 450–500 ppm may be consistent if increased cell proliferation occurred only for a short period of time around 48 hours postexposure, and was thereby effectively washed-out by the longer "averaging time" in the experiments by Green et al. (1997b). Also, these contradictory results may be due to differences in methodology. Green et al. (1997b) and Villaschi et al. (1991) reported very different control labeling indices (6 and 0%, respectively) while reporting similar absolute labeling indices at 450–500 ppm (6.5 and 5.2%, respectively). The different control values may be a result of substantially different times over which the label was incorporated: the mice in Green et al. (1997b) were given BrdU via a surgically-implanted osmotic pump over 4 days prior to sacrifice, while the mice in Villaschi et al. (1991) were given a single i.p. dose of [^{3}H]-thymidine 1 hour prior to sacrifice. Stewart et al. (1979) observed no stimulation of thymidine incorporation after daily exposure to TCE (9,000 ppm) for up to

15 days. This study did, however, report a nonstatistically significant reduction in orotate incorporation, an indicator of RNA synthesis, after 15 days, although the data were not shown.

At the biochemical level, changes in pulmonary metabolism, particularly with respect to CYP activity, have been reported following TCE exposure via inhalation or i.p. administration in mice. Odum et al. (1992) reported reduced enzyme activity in Clara cell sonicates of ethoxycoumarin *O*-deethylase, aldrin epoxidation, and NADPH cytochrome c reductase after 6 hour exposures to 20–2,000 ppm TCE, although the reduction at 20 ppm was not statistically significant. No reduction of GST activity as determined by chlorodinitrobenzene as a substrate was detected. With repeated exposure at 450 ppm, the results were substrate-dependent, with ethoxycoumarin *O*-deethylase activity remaining reduced, while aldrin epoxidation and NADPH cytochrome c reductase activity showing some eventual recovery by 2 weeks. The results reported by Odum et al. (1992) for NADPH cytochrome c reductase were consistent with those of Lewis et al. (1984), who reported similarly reduced NADPH cytochrome c reductase activity following a much larger dose of 10,000 ppm for 1 and 4 hours/day for 5 days in mice (strain not specified). TCE exposure has also been associated with a decrease in pulmonary surfactant. Repeated exposure of female Wistar rats to TCE (9,000 ppm, 30 minutes/day) for 5 or 15 days resulted in a significant decrease in pulmonary surfactant as compared to unexposed controls (Le Mesurier et al., 1980).

4.7.2.1.1.1. Acute and short-term effects: i.p. injection and gavage exposure

As stated previously, the i.p. route of administration is not a relevant paradigm for human exposure. A number of studies used this route of exposure to study the effects of acute TCE exposure in mice. In general, similar lung targets are seen following inhalation or i.p. treatment in mice (Forkert et al., 2006; Forkert and Birch, 1989; Scott et al., 1988; Forkert et al., 1985). Inhalation studies generally reported the Clara cell as the target in mice. No lung histopathology from i.p. injection studies in rats is available. Forkert et al. (1985) and Forkert and Birch (1989) reported vacuolation of Clara cells as soon as 1 hour following i.p. administration of a single dose of 2,000 mg/kg in mice. At 2,500 mg/kg, both Forkert et al. (1985) and Scott et al. (1988) reported exfoliation of Clara cells and parenchymal changes, with morphological distortion in alveolar Type II cells and inconsistently observed minor swelling in Type I cells at 24 hours postexposure. Furthermore, at 3,000 mg/kg, Scott et al. (1988) also reported a significant (85%) decrease in intracellularly stored surfactant phospholipids at 24 hours postexposure. These data indicate that both Clara cells and alveolar Type I and II cells are targets of TCE toxicity at these doses using this route of administration. Recently, Forkert et al. (2006) reported Clara cell toxicity that showed increased severity with increased dose (pyknotic nuclei, exfoliation) at 500–1,000 mg/kg i.p. doses as soon as 4 hours postexposure in mice. Even at 500 mg/kg, a few Clara cells were reported with pyknotic nuclei that were in the process of exfoliation. Damage to alveolar Type II cells was not observed in this dose range. The study by Scott et al. (1988)

examined surfactant phospholipids and phospholipase A2 activity in male CD-1 mice exposed by i.p. injection of TCE (2,500 or 3,000 mg/kg, 24 hours). The lower concentration led to damage to and exfoliation of Clara cells from the epithelial lining into the airway lumen, while only the higher concentration led to changes in surfactant phospholipids. This study demonstrated an increase in total phospholipid content in the lamellar body fractions in the mouse lung.

The study by Narotsky et al. (1995) exposed F344 timed-pregnant rats to TCE (0, 1,125, and 1,500 mg/kg body weight) by gavage and examined both systemic toxicity and developmental effects at 14 days postexposure. Rales and dyspnea in the dams were observed in the high-dose group, with two of the animals with dyspnea subsequently dying. The developmental effects observed in this study are discussed in more detail in Section 4.8.

4.7.2.1.1.2. Subchronic and chronic effects

There are a few reports of the subchronic and chronic noncancer effects of TCE on the respiratory system from i.p. exposure in mice and from gavage exposure in rats. Forkert and Forkert (1994) reported pulmonary fibrosis in mice 90 days after i.p. administration of a single 2,000 mg/kg dose of TCE. The effects were in the lung parenchyma, not the bronchioles where Clara cell damage has been observed after acute exposure. It is possible that fibrotic responses in the alveolar region occur irrespective of where acute injury occurs. Effects upon Clara cells can also impact other areas of the lung via cytokine regulation (Elizur et al., 2008). Alternatively, the alveolar and/or capillary components of the lung may have been affected by TCE in a manner that was not morphologically apparent in short-term experiments. In addition effects from a single or a few short-term exposures may take longer to manifest. The latter hypothesis is supported by the alveolar damage reported by Odum et al. (1992) after chloral administration by inhalation, and by the adducts reported in alveolar Type II cells by Forkert et al. (2006) after 500–1,000 mg/kg TCE i.p. administration.

As noted previously, rats have responded to short-term inhalation exposures of TCE with Clara cell and alveolar Type I and II effects. After repeated inhalation exposures over 6 weeks (8 hours/day, 5 days/week, 730 ppm) and continuous exposures over 90 days (35 ppm), Prendergast et al. (1967) noted no histopathologic changes in rats, guinea pigs, rabbits, dogs, or monkeys after TCE exposure, but did describe qualitatively observing some nasal discharge in the rats exposed for 6 weeks. The study details in Prendergast et al. (1967) are somewhat limited. Exposed animals are described as ―ytpically" 15 Long-Evans or Sprague-Dawley rats, 15 Hartley guinea pigs, 3 squirrel monkeys, 3 New Zealand albino rabbits, and 2 beagle dogs. Controls were grouped between studies. In a 13-week NTP study in F344/N rats (n = 10/group) exposed to TCE (0–2,000 mg/kg-day 5 days/week) by gavage, pulmonary vasculitis was observed in 6/10 animals of each sex of the highest dose group (2,000 mg/kg-day), in contrast to1/10 in controls of each sex (NTP, 1990).

4.7.2.2. Respiratory Tract Cancer

Limited studies have been performed examining lung cancer following TCE exposure (summarized in Table 4-84). TCE inhalation exposure was reported to cause statistically significant increase in pulmonary tumors (i.e., pulmonary adenocarcinomas) in some studies in mice, but not in studies in rats and hamsters. Oral administration of TCE frequently resulted in elevated lung tumor incidences in mice, but not in any tested species was there a statistically significant increase. This section will describe the data regarding TCE induction of pulmonary tumors in rodent models. The next sections will consider the role of metabolism and potential modes of action for inhalation carcinogenicity, primarily in mice.

4.7.2.2.1. Inhalation

There are three published inhalation studies examining the carcinogenicity of TCE at exposures from 0 to 600 ppm, two of which reported statistically significantly increased lung tumor incidence in mice at the higher concentrations (Maltoni et al., 1988; Maltoni et al., 1986; Fukuda et al., 1983; Henschler et al., 1980). Rats and hamsters did not show an increase in lung tumors following exposure.

The inhalation studies by Fukuda et al. (1983), which involved female ICR mice and Sprague-Dawley rats, observed a threefold increase in lung tumors per mouse in those exposed to the two higher concentrations (150–450 ppm), but reported no increase in lung tumors in the rats. Maltoni et al. (1988; 1986) reported statistically-significantly increased pulmonary tumors in male Swiss and female B6C3F_1 mice at the highest dose of 600 ppm, but no significant increases in any of the other species/strains/sexes tested. Henschler et al. (1980) tested NMRI mice, Wistar rats, and Syrian hamsters of both sexes, and reported no observed increase in pulmonary tumors any of the species tested (see Appendix E for details on the conduct of these studies).

Table 4-84. Animal carcinogenicity studies of TCE

Reference	Animals (sex)	Exposure route	Dose/exposure concentration (stabilizers, if any)	Pulmonary tumor incidences	
				Benign + malignant	Malignant only
Fukuda et al. (1983)	ICR mice (F) Sprague-Dawley rats (F)	Inhalation, 7 hrs/d, 5 d/wk, 104 wks, hold until 107 wks	0, 50, 150, or 450 ppm (epichlorohydrin)	Mice: 6/49, 5/50, 13/50, 11/46 Rats: 0/50, 0/50, 1/47, 1/51	Mice: 1/49, 3/50, 8/50[a], 7/46[a] Rats: none
Maltoni et al. (1988; 1986)	Sprague-Dawley rats (M, F) Swiss mice (M, F) $B6C3F_1$ mice (M, F)	Inhalation, 7 hrs/d, 5 d/wk, 104 wks, hold until death	0, 100, 300, or 600 ppm	Rats: 0/280, 0/260, 0/260, 0/260 Swiss Mice: M: 10/90, 11/90, 23/90[a], 27/90[b];F: 15/90, 15/90, 13/90, 20/90 $B6C3F_1$ Mice: M: 2/90, 2/90, 3/90, 1/90; F: 4/90, 6/90, 7/90, 15/90[a]	Rats: 0/280, 0/260, 0/260, 0/260 Swiss Mice: M: 0/90, 0/90, 0/90, 1/90;F: 2/90, 0/90, 0/90, 2/90 $B6C3F_1$ Mice M: 0/90, 0/90, 0/90, 0/90; F: 0/90, 1/90, 0/90, 0/90
Henschler et al. (1980)	Wistar rats (M, F) Syrian hamsters (M, F) NMRI mice	Inhalation, 6 hrs/d, 5 d/wk, 78 wks, hold until 130 wks (mice and hamsters) or 156 wks (rats)	0, 100, or 500 ppm (triethanolamine)	Rats: M: 1/29, 1/30, 1/30; F: 0/28; 1/30; 0/30 Hamsters: 0/60, 0/59, 0/60 Mice: M: 1/30, 3/29, 1/30; F: 3/29, 0/30,1/28	Rats: M: 1/29, 1/30, 1/30; F: 0/28; 1/30; 0/30 Hamsters: 0/60, 0/59, 0/60 Mice: M: 5/30, 3/29, 1/30; F: 1/29, 3/30,0/28
Henschler et al. (1984)	Swiss mice (M, F)	Gavage, 5/wk, 72 wks hold 104 wks	2.4 g/kg body weight (M), 1.8 g/kg body weight (F) all treatments; (control, triethanolamine, industrial, epichlorohydrin, 1,2-epoxybutane, both)	M: 18/50, 17/50, 14/50, 21/50, 15/50, 18/50; F: 12/50, 20/50, 21/50, 17/50, 18/50, 18/50	M: 8/50, 6/50, 7/50, 5/50, 7/50, 7/50; F: 5/50, 11/50, 8/50, 3/50, 7/50, 7/50
Van Duuren et al. (1979)	Swiss mice (M, F)	Gavage, 1/wk, 89 wks	0 or 0.5 mg (unknown)	0/30 for all groups	0/30 for all groups
NCI (1976)	Osborne-Mendel rats (M, F) $B6C3F_1$ mice (M, F)	Gavage, 5/wk, 78 wks, hold until 110 wks (rats) or 90 wks (mice)	Rats: TWA: 0, 549, or 1,097 mg/kg Mice: TWA: M: 0, 1,169, or 2,339 mg/kg; F: 0, 869, or 1,739 mg/kg (epoxybutane, epichlorohydrin)	Rats: M: 1/20, 0/50, 0/50; F: 0/20, 1/47, 0/50 Mice: M: 0/20, 5/50, 2/48; F: 1/20, 4/50, 7/47	Rats: M: 0/20, 0/50, 0/50; F: 0/20, 1/47, 0/50 Mice: M: 0/20, 0/50, 1/48; F: 0/20, 2/50, 2/47

Table 4-84. Animal carcinogenicity studies of TCE (continued)

Reference	Animals (sex)	Exposure route	Dose/exp conc (stabilizers, if any)	Pulmonary tumor incidences	
				Benign + malignant	Malignant only
NTP (1988)	ACI, August, Marshall, Osborne-Mendel rats	Gavage, 1/d, 5 d/wk, 103 wks	0, 500, or 1,000 mg/kg (diisopropylamine)	ACI M: 1/50, 4/47, 0/46; F: 0/49, 2/47, 2/42 August M: 1/50, 1/50, 0/49; F: 1/50, 1/50, 0/50 Marshall M: 3/49, 2/50, 2/47; F: 3/49, 3/49, 1/46 Osborne-Mendel M: 2/50, 1/50, 1/50; F: 0/50, 3/50, 2/50	ACI M: 1/50, 2/47, 0/46; F: 0/49, 1/47, 2/42 August M: 0/50, 1/50, 0/49; F: 1/50, 0/50, 0/50 Marshall M: 3/49, 2/50, 2/47; F: 3/49, 3/49, 1/46 Osborne-Mendel M: 1/50, 1/50, 0/50; F: 0/50, 3/50, 1/50
NTP (1990)	F344 rats (M, F) $B6C3F_1$ mice (M, F)	Gavage, 1/d, 5 d/wk, 103 wks	Mice: 0 or 1,000 mg/kg Rats: 0, 500, or 1,000 mg/kg	Mice: M: 7/49, 6/50; F: 1/48, 4/49 Rats: M: 4/50, 2/50, 3/49; F: 1/50, 1/49, 4/50	Mice: M: 3/49, 1/50; F: 1/48, 0/49 Rats: M: 3/50, 2/50, 3/49; F: 0/50, 0/49, 2/50
Maltoni et al. (1988; 1986)	Sprague-Dawley rats (M, F)	Gavage, 1/d, 4–5 d/wk, 56 wks; hold until death	0, 50, or 250 mg/kg	M: 0/30, 0/30, 0/30; F: 0/30, 0/30, 0/30	M: 0/30, 0/30, 0/30; F: 0/30, 0/30, 0/30

[a]Statistically-significantly different from controls by Fisher's exact test ($p < 0.05$).
[b]Statistically-significantly different from controls by Fisher's exact test ($p < 0.01$).
M = males, F = females.

4.7.2.2.2. Gavage

None of the six chronic gavage studies (NTP, 1990; Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986; Henschler et al., 1984; Van Duuren et al., 1979; NCI, 1976), which exposed multiple strains of rats and mice to 0–3,000 mg/kg TCE for at least 56 weeks, reported a statistically-significant excess in lung tumors, although nonstatistically significant increases were frequently observed in mice.

The study by Van Duuren et al. (1979) examined TCE along with 14 other halogenated compounds for carcinogenicity in both sexes of Swiss mice. While no excess tumors were observed, the dose rate of 0.5 mg once per week is equivalent to an average dose rate of approximately 2.4 mg/kg-day for a mouse weighing 30 g, which is about 400-fold smaller than that in the other gavage studies. In the NCI (1976) study, the results for Osborne-Mendel rats were considered inconclusive due to significant early mortality, but female $B6C3F_1$ mice (though not males) exhibited a nonstatistically-significant elevation in pulmonary tumor incidence. The NCI study (1976) used technical-grade TCE, which contained two known carcinogenic compounds as stabilizers (epichlorohydrin and 1,2-epoxybutane), but a later study by Henschler et al. (1984) in which mice were given TCE that was either pure, industrial, or stabilized with one or both of these stabilizers found similar pulmonary tumors regardless of the presence of stabilizers. In this study, female mice (n = 50) had elevated, but again not statistically significant, increases in pulmonary tumors. A later gavage study by NTP (1988), which used TCE stabilized with diisopropylamine, observed no pulmonary tumors, but chemical toxicity and early mortality rendered this study inadequate for determining carcinogenicity. The final NTP study (1990) in male and female F344 rats and $B6C3F_1$ mice, using epichlorohydrin-free TCE, again showed early mortality in male rats. Similar to the other gavage studies, a nonstatistically significant elevation in (malignant) pulmonary tumors was observed in mice, in this case in both sexes. These animal studies show that while there is a limited increase in lung tumors following gavage exposure to TCE in mice, the only statistically significant increase in lung tumors occurs following inhalation exposure in mice.

4.7.3. Role of Metabolism in Pulmonary Toxicity

TCE oxidative metabolism has been demonstrated to play a main role in TCE pulmonary toxicity in mice. However, data are not available on the role of specific oxidative metabolites in the lung. The Clara cell is thought to be the cell type responsible for much of the CYP metabolism in the lung. Therefore, damage to this cell type would be expected to also affect metabolism. More direct measures of CYP and isozyme-specific depression following TCE exposure have been reported following i.p. administration in mice. Forkert et al. (1985) reported significant reduction in microsomal aryl hydrocarbon hydroxylase activity as well as CYP content between 1 and 24 hours after exposure (2,000–3,000 mg/kg i.p. TCE). Maximal

depression occurred between 2 and 12 hours, with aryl hydrocarbon hydroxylase activity (a function of CYP) <50% of controls and CYP content <20% of controls. While there was a trend towards recovery from 12 to 24 hours, depression was still significant at 24 hours. Forkert et al. (2005) reported decreases in immunoreactive CYP2E1, CYP2F2, and CYP2B1 in the 4 hours after TCE treatment with 750 mg/kg i.p. injection in mice. The amount and time of maximal reduction was isozyme dependent (CYP2E1: 30% of controls at 2 hours; CYP2F2: abolished at 30 minutes; CYP2B1: 43% of controls at 4 hours). Catalytic markers for CYP2E1, CYP2F2, and CYP2B enzymes showed rapid onset (≤15 minutes after TCE administration) of decreased activity, and continued depression through 4 hours. Decrease in CYP2E1 and CYP2F2 activity (measured by PNP hydroxylase activity) was greater than that of CYP2B (measured by pentoxyresorufin *O*-dealkylase activity). Forkert et al. (2006) reported similar results in which 4 hours after treatment, immunodetectable CYP2E1 protein was virtually abolished at doses of 250–1,000 mg/kg and immunodetectable CYP2F2 protein, while still detectable, was reduced. PNP hydroxylase activity was also reduced 4 hours after treatment to 37% of controls at the lowest dose tested of 50 mg/kg, with further decreases to around 8% of control levels at doses of 500 mg/kg and higher. These results correlate with previously described increases in Clara cell cytotoxicity, as well as dichloroacetyl lysine (DAL) protein adduct formation. DAL adducts were observed in the bronchiolar epithelium of CD-1 mice and most prominent in the cellular apices of Clara cells (Forkert et al., 2006). This study also examined the effect of TCE in vitro exposure on the formation of CH in lung microsomes from male CD-1 mice and CYP2E1 knock-out mice. The rates of CH formation were the same for lysosomes from both CD-1 and CYP2E1 knockout mice from 0.25 mM to 0.75 mM, but the CH formation peaked earlier for in the wild-type lysosomes (0.75 mM) as compared to CYP2E1-null lysosomes (1 mM).

The strongest evidence for the necessary role of TCE oxidation is that pretreatment of mice with diallyl sulfone ($DASO_2$), an inhibitor of CYP2E1 and CYP2F2, protected against TCE-induced pulmonary toxicity. In particular, following an i.p. TCE dose of 750 mg/kg, Clara cells and the bronchiolar epithelium in mice pretreated with the CYP2E1/CYP2F2 inhibitor appeared normal. In naive mice given the same dose, the epithelium was attenuated due to exfoliation and there was clear morphological distortion of Clara cells (Forkert et al., 2005). In addition, the greater susceptibility of mouse lungs relative to rat lungs is consistent with their larger capacity to oxidize TCE, as measured in vitro in lung microsomal preparations (Green et al., 1997b). Analysis by immunolocalization also found considerably higher levels of CYP2E1 in the mouse lung, heavily localized in Clara cells, as compared to rat lungs, with no detectable CYP2E1 in human lung samples (Green et al., 1997b). In addition, both Green et al. (1997b) and Forkert et al. (2006) report substantially lower metabolism of TCE in human lung microsomal preparations than either rats or mice. It is clear that CYP2E1 is not the only CYP enzyme involved in pulmonary metabolism, as lung microsomes from CYP2E1-null mice showed greater or similar rates of CH formation compared to those from wild-type mice. Recent studies have

suggested a role for CYP2F2 in TCE oxidative metabolism, although more work is needed to make definitive conclusions. In addition, there may be substantial variability in human lung oxidative metabolism, as Forkert et al. (2006) reported that in microsomal samples from eight individuals, five exhibited no detectable TCE oxidation (<0.05 pmol/mg protein/20 minutes), while others exhibited levels well above the limit of detection (0.4–0.6 pmol/mg protein/minute).

In terms of direct pulmonary effects of TCE metabolites, Odum et al. (1992) reported that mice exposed to 100 ppm via inhalation of chloral for 6 hours resulted in bronchiolar lesions similar to those seen with TCE, although with a severity equivalent to 1,000 ppm TCE exposures. In addition, some alveolar necrosis, alveolar oedema, and desquamation of the epithelium were evident. In the same study, TCOH (100 and 500 ppm) also produced Clara cell damage, but with lower incidence than TCE, and without alveolar lesions, while TCA treatment produced no observable pulmonary effects. Therefore, it has been proposed that chloral is the active metabolite responsible for TCE pulmonary toxicity, and the localization of damage to Clara cells (rather than to other cell types, as seen with direct exposure to chloral) is due to the localization of oxidative metabolism in that cell type (Green, 2000; Green et al., 1997b; Odum et al., 1992). However, the recent identification by Forkert et al. (2006) of DAL adducts, also localized with Clara cells, suggests that TCE oxidation to DCAC, which is not believed to be derived from chloral, may also contribute to adverse health effects.

Due to the histological similarities between TCE- and chloral-induced pulmonary toxicity, consistent with chloral being the active moiety, it has been proposed that the limited or absent capacity for reduction of chloral (rapidly converted to CH in the presence of water) to TCOH and glucuronidation of TCOH to TCOG in mouse lungs leads to ―accumulation" of chloral in Clara cells. However, the lack of TCOH glucuronidation capacity of Clara cells reported by Odum et al. (1992), while possibly an important determinant of TCOH concentrations, should have no bearing on CH concentrations, which depend on the production and clearance of CH only. While isolated mouse Clara cells form smaller amounts of TCOH relative to CH (Odum et al., 1992), the cell-type distribution of the enzymes metabolizing CH is not clear. Indeed, cytosolic fractions of mouse, rat, and human whole lungs show significant activity for CH conversion to TCOH (Green et al., 1997b). In particular, in mouse lung subcellular fractions, 1 micromole of TCE in a 1.3 mL reactivial was converted to CH at a rate of 1 nmol/minute/mg microsomal protein, while 10 nmol CH in a 1.3 mL reactivial was converted to TCOH at a rate of 0.24 nmol/minute/mg cytosolic protein (Green et al., 1997b). How this fourfold difference in activity would translate in vivo is uncertain given the 100-fold difference in substrate concentrations, lack of information as to the concentration-dependence of activity, and uncertain differences between cytosolic and microsomal protein content in the lung. It is unclear whether local pulmonary metabolism of chloral is the primary clearance process in vivo, as in the presence of water, chloral rapidly converts to CH, which is soluble in water and hence can rapidly diffuse to surrounding tissue and to the blood, which also has the capacity to

metabolize CH (Lipscomb et al., 1996). Nonetheless, experiments with isolated perfused lungs of rats and guinea pigs found rapid appearance of TCOH in blood following TCE inhalation exposure, with no detectable CH or TCOG (Dalbey and Bingham, 1978). Therefore, it appears likely that chloral in the lung either is rapidly metabolized to TCOH, which then diffuses to blood, or diffuses to blood as CH and is rapidly metabolized to TCOH by erythrocytes (Lipscomb et al., 1996).

This hypothesis is further supported by in vivo data. No in vivo data in rats on CH after TCE administration were located, and Fisher et al. (1998) reported CH in blood of volunteers exposed to TCE via inhalation were below detection limits. In mice, however, after both inhalation and gavage exposure to TCE, CH has been reported in whole-lung tissue at concentrations similar to or somewhat greater than that in blood (Greenberg et al., 1999; Abbas and Fisher, 1997). A peak concentration (1.3 µg/g) of pulmonary CH was reported after inhalation exposure to 600 ppm—at or above exposures where Clara cell toxicity was reported in acute studies (Green et al., 1997b; Odum et al., 1992). However, this was fivefold less than the reported pulmonary CH concentration (6.65 µg/g) after gavage exposures of 1,200 mg/kg. Specifically, 600- or 450-ppm exposures reported in the Maltoni et al. (1988; 1986) and Fukuda et al. (1983) studies result in a greater incidence in lung tumors than the 1,000–1,200 mg/kg-day exposures in the NTP (1990) and NCI (1976) bioassays. However, the peak CH levels measured in whole-lung tissues after inhalation exposure to TCE at 600 ppm were reported to be about fivefold *lower* than that at 1,200 mg/kg by gavage, therefore, showing the *opposite* pattern (Greenberg et al., 1999; Abbas and Fisher, 1997). No studies of Clara cell toxicity after gavage exposures were located, but several studies in mice administered TCE via i.p. injection did show Clara cell toxicity at around a dose of 750 mg/kg (Forkert et al., 2006) or above (e.g., Forkert and Forkert, 1994; Forkert and Birch, 1989). However, as noted previously, i.p. exposures are subject to an inflammatory response, confounding direct comparisons of dose via other routes of administration.

Although whole-lung CH concentrations may not precisely reflect the concentrations within specific cell types, as discussed above, the water solubility of CH suggests rapid equilibrium between cell types and between tissues and blood. Both Abbas and Fisher (1997) and Greenberg et al. (1999) were able to fit CH blood and lung levels using a PBPK model that did not include pulmonary metabolism, suggesting that lung CH levels may be derived largely by systemic delivery (i.e., from CH formed in the liver). However, a more detailed PBPK model-based analysis of this hypothesis has not been performed, as CH is not included in the PBPK model developed by Hack et al. (2006) that was updated in Section 3.5.

Two studies have reported formation of reactive metabolites in pulmonary tissues as assessed by macromolecular binding after TCE i.p. administration. Forkert and Birch (1989) reported temporal correlations between the severity of Clara cell necrosis with increased levels of covalent binding macromolecules in the lung of TCE or metabolites with a single 2,000 mg/kg

dose of [^{14}C]-TCE. The amount of bound TCE or metabolites/g of lung tissue, DNA, or protein peaked at 4 hours and decreased progressively at 8, 12, and 24 hours. The fraction of radioactivity in lung tissue macromolecules that was covalently bound reached a plateau of about 20% from 4 to 24 hours, suggesting that clearance of total and covalently bound TCE or metabolites was similar. The amount of covalent binding in the liver was 3–10-fold higher than in the lung, although hepatic cytotoxicity was not apparent. This tissue difference could either be due to greater localization of metabolism in the lung, so that concentrations of reactive metabolites in individual Clara cells are greater than both the lung as a whole and hepatocytes, or because of greater sensitivity of Clara cells as compared to hepatocytes to reactive metabolites. More recently, Forkert et al. (2006) examined DAL adducts resulting from metabolism of TCE to DCAC as an in vivo marker of production of reactive metabolites. Following i.p. administration of 500–1,000 mg/kg TCE in CD-1 mice, the authors found localization of DAL adducts believed to be from oxidative metabolism within Clara cell apices, with dose-dependent increase in labeling with a polyclonal anti-DAL antibody that correlated with increased Clara cell damage. Dose-dependent DAL adducts were also found in alveolar Type II cells, although no morphologic changes in those cells were observed. Both Clara cell damage (as discussed above) and DAL labeling were abolished in mice pretreated with $DASO_2$, an inhibitor of CYP2E1 and CYP2F2. However, Clara cell damage in treated CYP2E1-null mice was more severe than in CD-1 mice. Although DAL labeling was less pronounced in CYP2E1-null mice as compared to CD-1 mice, this was due in part to the greater histopathologic damage leading to attenuation of the epithelium and loss of Clara cells in the null mice. In addition, protein immunoblotting with anti-DAL, anti-CYP2E1, and anti-CYP2F2 antibodies suggested that a reactive TCE metabolite including DCAC was formed that is capable of binding to CYP2E1 and CYP2F2 and changing their protein structures. Follow-up studies are needed in the lung and other target tissues to determine the potential role of the DAL adducts in TCE-induced toxicity.

Finally, although Green (2000) and others have attributed species differences in pulmonary toxicity to differences in the capacity for oxidative metabolism in the lung, it should be noted that the concentration of the active metabolite is determined by both its production and clearance (Clewell et al., 2000). Therefore, while the maximal pulmonary capacity to produce oxidative metabolites is clearly greater in the mouse than in rats or humans, there is little quantitative information as to species differences in clearance, whether by local chemical transformation/metabolism or by diffusion to blood and subsequent systemic clearance. In addition, existing in vitro data on pulmonary metabolism are at millimolar TCE concentrations where metabolism is likely to be approaching saturation, so the relative species differences at lower doses has not been characterized. Studies with recombinant CYP enzymes examined species differences in the catalytic efficiencies of CYP2E1, CYP2F, and CYP2B1, but the relative contributions of each isoform to pulmonary oxidation of TCE in vivo remains unknown (Forkert et al., 2005). Furthermore, systemic delivery of oxidative metabolites to the lung may

contribute, as evidenced by respiratory toxicity reported with i.p. administration. Therefore, while the differences between mice and rats in metabolic capacity are correlated with their pulmonary sensitivity, it is not clear that differences in capacity alone are accurate quantitative predictors of toxic potency. Thus, while it is likely that the human lung is exposed to lower concentrations of oxidative metabolites, quantitative estimates for differential sensitivity made with currently available data and dosimetry models are highly uncertain.

In summary, it appears likely that pulmonary toxicity is dependent on in situ oxidative metabolism; however, the active agent has not been confidently identified. The similarities in histopathologic changes in Clara cells between TCE and chloral inhalation exposure, combined with the wider range of cell types affected by direct chloral administration relative to TCE, led some to hypothesize that chloral is the toxic moiety in both cases, but with that generated in situ from TCE in Clara cells —accumulating" in those cells (Green, 2000). However, chemical and toxicokinetic data suggest that such —accumulation" is unlikely for several reasons. These include the rapid conversion of chloral to CH in the presence of water, the water solubility of CH leading to rapid diffusion to other cell types and blood, the likely rapid metabolism of CH to TCOH either in pulmonary tissue or in blood erythrocytes, and in vivo data showing lack of correlation across routes of exposure between whole-lung CH concentrations and pulmonary carcinogenicity and toxicity. However, additional possibilities for the active moiety exist, such as DCAC, which is derived through a TCE oxidation pathway independent of chloral and appears to result in adducts with lysine localized in Clara cells.

4.7.4. Mode of Action for Pulmonary Carcinogenicity

A number of effects have been hypothesized to be key events in the pulmonary carcinogenicity of TCE, including cytotoxicity leading to increased cell proliferation, formation of DAL protein adducts, and mutagenicity. As stated previously, the target cell for pulmonary adenocarcinoma formation has not been established. Much of the hazard and mode-of-action information has focused on Clara cell effects from TCE, which is a target in both susceptible and nonsusceptible rodent species for lung tumors. However, the role of Clara cell susceptibility to TCE-induced lung toxicity or to other potential targets such as lung stem cells that are activated to repopulate both Clara and Type II alveolar cells after injury, has not been determined for pulmonary carcinogenicity. While all of the events described above may be plausibly involved in the mode of action for TCE pulmonary carcinogenicity, none have been directly shown to be necessary for carcinogenesis.

4.7.4.1. Mutagenicity via Oxidative Metabolism

The hypothesis is that TCE acts by a mutagenic mode of action in TCE-induced lung tumors. According to this hypothesis, the key events leading to TCE-induced lung tumor formation constitute the following: the oxidative metabolism of TCE producing chloral/CH

delivered to pulmonary tissues, causes direct alterations to DNA (e.g., mutation, DNA damage, and/or micronuclei induction). Mutagenicity is a well-established cause of carcinogenicity.

4.7.4.1.1. Experimental support for the hypothesized mode of action

Pulmonary toxicity has been proposed to be dependent on in situ oxidative metabolism; however, the active agent has not been confidently identified. The similarities in histopathologic changes in Clara cells between TCE and chloral inhalation exposure, combined with the wider range of cell types affected by direct chloral administration relative to TCE, led some to hypothesize that chloral is the toxic moiety. Chloral that is formed from the metabolism of TCE is quickly converted to CH upon hydration under physiological conditions. As discussed in Section 4.2.4, CH clearly induces aneuploidy in multiple test systems, including bacterial and fungal assays in vitro (Crebelli et al., 1991; Kappas, 1989; Käfer, 1986), mammalian cells in vitro (Sbrana et al., 1993; Vagnarelli et al., 1990), and mammalian germ-line cells in vivo (Miller and Adler, 1992; Russo et al., 1984). Conflicting results were observed in in vitro and in vivo mammalian studies of micronuclei formation (Beland, 1999; Nesslany and Marzin, 1999; Giller et al., 1995; Russo and Levis, 1992b, a; Degrassi and Tanzarella, 1988) with positive results in germ-line cells (Nutley et al., 1996; Allen et al., 1994). In addition, it is mutagenic in the Ames bacterial mutation assay for some strains (Beland, 1999; Giller et al., 1995; Ni et al., 1994; Haworth et al., 1983). Structurally related chlorinated aldehydes 2-chloroacetyaldehyde and 2,2-dichloroacetaldehyde are both alkylating agents, are both positive in a genotoxic assay (Bignami et al., 1980), and both interact covalently with cellular macromolecules (Guengerich et al., 1979).

As discussed in the section describing the experimental support for the mutagenic mode of action for liver carcinogenesis (see Section 4.5.7.1), it has been argued that CH mutagenicity is unlikely to be the cause of TCE carcinogenicity because the concentrations required to elicit these responses are several orders of magnitude higher that achieved in vivo (Moore and Harrington-Brock, 2000). Similar to the case of the liver, it is not clear how much of a correspondence is to be expected from concentrations in genotoxicity assays in vitro and concentrations in vivo, as reported in vivo CH concentrations are in whole-lung homogenate, while in vitro concentrations are in culture media. None of the available in vivo genotoxicity assays used the inhalation route that elicited the greatest lung tumor response under chronic exposure conditions, so direct in vivo comparisons are not possible. Finally, as discussed in Section 4.5.7.1, the use of i.p. administration in many other in vivo genotoxicity assays complicates the comparison with carcinogenicity data.

As discussed above (see Section 4.7.3), chemical and toxicokinetic data are not supportive of CH being the active agent of TCE-induced pulmonary toxicity, and directly contradict the hypothesis of chloral "accumulation." Nonetheless, CH has been measured in the mouse lung following inhalation and gavage exposures to TCE (Greenberg et al., 1999; Abbas

and Fisher, 1997), possibly the result of both in situ production and systemic delivery. Therefore, in principle, CH could cause direct alterations in DNA in pulmonary tissue. However, as discussed above, the relative amounts of CH measured in whole-lung tissue from inhalation and oral exposures do not appear to correlate with sensitivity to TCE lung tumor induction across exposure routes. While these data cannot rule out a role for mutagenicity mediated by CH due to various uncertainties, such as whether whole-lung CH concentrations accurately reflect cell-type specific concentrations and possible confounding due to strain differences between inhalation and oral chronic bioassays, they do not provide support for this mode of action.

Additional possibilities for the active moiety exist, such as DCAC, which is derived through a TCE oxidation pathway independent of chloral and which appears to result in adducts with lysine localized in Clara cells (Forkert et al., 2006). DCA, which has some genotoxic activity, is, also, presumed to be formed through this pathway (see Section 3.3). Currently, however, there are insufficient data to support a role for these oxidative metabolites in a mutagenic mode of action.

4.7.4.2. Cytotoxicity Leading to Increased Cell Proliferation

The hypothesis is that TCE acts by a cytotoxicity mode of action in TCE-induced pulmonary carcinogenesis. According to this hypothesis, the key events leading to TCE-induced lung tumor formation constitute the following: TCE oxidative metabolism in situ leads to currently unknown reactive metabolites that cause cytotoxicity, leading to compensatory cellular proliferation and subsequently increased mutations and clonal expansion of initiated cells.

4.7.4.2.1. Experimental support for the hypothesized mode of action

Evidence for the hypothesized mode of action consists primarily of: (1) the demonstration of acute cytotoxicity and transient cell proliferation following TCE exposure in laboratory mouse studies; (2) toxicokinetic data supporting oxidative metabolism being necessary for TCE pulmonary toxicity; and (3) the association of lower pulmonary oxidative metabolism and lower potency for TCE-induced cytotoxicity with the lack of observed pulmonary carcinogenicity in laboratory rats. However, there is a lack of experimental support linking TCE acute pulmonary cytotoxicity to sustained cellular proliferation of chronic exposures or clonal expansion of initiated cells.

As discussed above, a number of acute studies have shown that TCE is particularly cytotoxic to Clara cells in mice, which has been suggested to be involved in the development of mouse lung tumors (Kim et al., 2005; Buckpitt et al., 1995; Forkert and Forkert, 1994). In addition, studies examining cell labeling by either BrdU (Green et al., 1997b) or [^{3}H]-thymidine incorporation (Villaschi et al., 1991) suggest increased cellular proliferation in mouse Clara cells following acute inhalation exposures to TCE. Moreover, in short-term studies, Clara cells appear

to become resistant to cytotoxicity with repeated exposure, but regain their susceptibility after 2 days without exposure. This observation led to the hypothesis that the 5 days/week inhalation dosing regime (Maltoni et al., 1988; 1986; Fukuda et al., 1983; Henschler et al., 1980) in the chronic mouse studies leads to periodic cytotoxicity in the mouse lung at the beginning of each week followed by cellular regeneration, and that the increased rate of cell division leads to increased incidence of tumors by increasing the overall mutation rate and by increasing the division rate of already initiated cells (Green, 2000). However, longer-term studies to test this hypothesis have not been carried out.

As discussed above (see Section 4.7.3), there is substantial evidence that pulmonary oxidative metabolism is necessary for TCE-induced pulmonary toxicity, although the active moiety remains unknown. In addition, the lower capacity for pulmonary oxidative metabolism in rats as compared to mice is consistent with studies in rats not reporting pulmonary cytotoxicity until exposures higher than those in the bioassays, and the lack of reported pulmonary carcinogenicity in rats at similar doses to mice. However, rats also have a lower background rate of lung tumors (Green, 2000), and so would be less sensitive to carcinogenic effects in that tissue to the extent that RRs is the important metric across species. In addition, this mode-of-action hypothesis requires a number of additional key assumptions for which there are currently no direct evidence. First, the cycle of cytotoxicity, repair, resistance to toxicity, and loss of resistance after exposure interruption, has not been documented and under the proposed mode of action should continue under chronic exposure conditions. This cycle has, thus far, only been observed in short-term (up to 13-day) studies. In addition, although Clara cells have been identified as the target of toxicity whether they or endogenous stem cells in the lung are the cells responsible for mouse lung tumors has not been established. There are currently no data as to the cell type of origin for TCE-induced lung tumors.

This hypothesized mode of action has been proposed for other compounds that induce mouse lung tumors, such as coumarin, naphthalene, and styrene (e.g., Cruzan et al., 2009). Among these, only for styrene have there been studies of chronic duration linking cytotoxicity with hyperplasia, and no studies appear to provide experimental linkage to clonal expansion of initiated cells.

4.7.4.3. Additional Hypothesized Modes of Action with Limited Evidence or Inadequate Experimental Support

4.7.4.3.1. Role of formation of DAL protein adducts

As discussed above, Forkert et al. (2006) recently observed dose-dependent formation of DAL protein adducts in the Clara cells of mice exposed to TCE via i.p. injection. While adducts were highly localized in Clara cells, they were also found in alveolar Type II cells, though these cells did not show signs of cytotoxicity in this particular experimental paradigm. In terms of the mode of action for TCE-induced pulmonary carcinogenicity, these adducts may either be

causally important in and of themselves, or they may be markers of a different causal effect. For instance, it is possible that these adducts are a cause for the observed Clara cell toxicity, and Forkert et al. (2006) suggested that the lack of toxicity in alveolar Type II cells may indicate that "there may be a threshold in adduct formation and hence bioactivation at which toxicity is manifested." In this case, they are an additional precursor event in the same causal pathway proposed above. Alternatively, these adducts may be indicative of effects related to carcinogenesis but unrelated to cytotoxicity. In this case, the Clara cell need not be the cell type of origin for mouse lung tumors.

Because of their recent discovery, there are little additional data supporting, refuting, or clarifying the potential role for DAL protein adducts in the mode of action for TCE-induced pulmonary carcinogenesis. For instance, the presence and localization of such adducts in rats has not been investigated, and could indicate the extent to which the level of adduct formation is correlated with existing data on species differences in metabolism, cytotoxicity, and carcinogenicity. In addition, the formation of these adducts has only been investigated in a single dose study using i.p. injection. As stated above, i.p. injection may involve the initiation of a systemic inflammatory response that can activate lung macrophages or affect Clara cells. Experiments with repeated exposures over chronic durations and by inhalation or oral of administration would be highly informative. Finally, the biological effects of these adducts, whether cytotoxicity or something else, have not been investigated.

4.7.4.4. Conclusions About the Hypothesized Modes of Action

4.7.4.4.1. Is the hypothesized mode of action sufficiently supported in the test animals?

4.7.4.4.1.1. Mutagenicity

CH is clearly genotoxic, as there are substantial data from multiple in vitro and in vivo assays supporting its ability induce aneuploidy, with more limited data as to other genotoxic effects, such as point mutations. CH is also clearly present in pulmonary tissues of mice following TCE exposures similar to those inducing lung tumors in chronic bioassays. However, chemical and toxicokinetic data are not supportive of CH being the predominant metabolite for TCE carcinogenicity. Such data include the water solubility of CH leading to rapid diffusion to other cell types and blood, it's likely rapid metabolism to TCOH either in pulmonary tissue or in blood erythrocytes, and in vivo data showing lack of correlation across routes of exposure between whole-lung CH concentrations and pulmonary carcinogenicity. Therefore, while a role for mutagenicity via CH in the mode of action of TCE-induced lung tumors cannot be ruled about, available evidence is inadequate to support the conclusion that direct alterations in DNA caused by CH produced in or delivered to the lung after TCE exposure constitute a mode of action for TCE-induced lung tumors.

4.7.4.4.1.2. Cytotoxicity

The mode-of-action hypothesis for TCE-induced lung tumors involving cytotoxicity is supported by relatively consistent and specific evidence for cytotoxicity at tumorigenic doses in mice. However, the majority of cytotoxicity-related key events have been investigated in studies <13 days, and none has been shown to be causally related to TCE-induced lung tumors. In addition, the cell type (or types) of origin for the observed lung tumors in mice has not been determined, so the contribution to carcinogenicity of Clara cell toxicity and subsequent regenerative cell division is not known. Similarly, the relative contribution from recently discovered dichloroacetyl-lysine protein adducts to the tumor response has not been investigated and has currently only been studied in i.p. exposure paradigms of short duration. In summary, while there are no data directly challenging the hypothesized mode of action described above, the existing support for their playing a causal role in TCE-induced lung tumors is largely associative, and based on acute or short term studies. Therefore, there are inadequate data to support a cytotoxic mode of action based on the TCE-induced cytotoxicity in Clara cells in the lungs of test animals.

4.7.4.4.1.3. Additional hypothesis

Inadequate data are available to develop a mode-of-action hypothesis based on recently discovered DAL adducts induced by TCE inhalation and i.p. exposures. It will, therefore, not be considered further in the conclusions below.

Overall, therefore, the mode of action for TCE-induced lung tumors is considered unknown at this time.

4.7.4.4.2. Is the hypothesized mode of action relevant to humans?

4.7.4.4.2.1. Mutagenicity

The evidence discussed above demonstrates that CH is mutagenic in microbial as well as test animal species. There is, therefore, the presumption that they would be mutagenic in humans. Therefore, this mode of action is considered relevant to humans.

4.7.4.4.2.2. Cytotoxicity

No data from human studies are available on the cytotoxicity of TCE and its metabolites in the lung, and no causal link between cytotoxicity and pulmonary carcinogenicity has been demonstrated in animal or human studies. Nonetheless, in terms of human relevance, no data suggest that the proposed key events are not biologically plausible in humans; therefore, qualitatively, TCE-induced lung tumors are considered relevant to humans. This conclusion that this hypothesized mode of action is qualitatively relevant has also been reached for other compounds for which the mode of action has been postulated (Cruzan et al., 2009). Information about the relative pharmacodynamic sensitivity between rodents and humans is absent, but

information on pharmacokinetic differences in lung oxidative metabolism does exist and will be considered in dose-response assessment when extrapolating between species (see Section 5.2.1.2).

4.7.4.4.3. Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?

4.7.4.4.3.1. Mutagenicity

The mutagenic mode of action is considered relevant to all populations and lifestages. According to EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b) and *Supplemental Guidance for Assessing Susceptibility From Early-Life Exposure to Carcinogens* (U.S. EPA, 2005e), there may be increased susceptibility to early-life exposures for carcinogens with a mutagenic mode of action. However, because the weight of evidence is inadequate to support a mutagenic mode of action for TCE pulmonary carcinogenicity, and in the absence of chemical-specific data to evaluate differences in susceptibility, the ADAFs should not be applied, in accordance with the *Supplemental Guidance.*

4.7.4.4.3.2. Cytotoxicity

No information is available as to which populations or lifestages may be particularly susceptible to TCE-induced lung tumors. However, pharmacokinetic differences in lung oxidative metabolism among humans do exist, and because of the association between lung oxidative metabolism and toxicity, these differences will be considered in dose-response assessment when extrapolating within species.

4.7.5. Summary and Conclusions

The studies described here show pulmonary toxicity found mainly in Clara cells in mice (Green et al., 1997b; Odum et al., 1992; Villaschi et al., 1991; Forkert and Birch, 1989; Forkert et al., 1985) and rats (Kurasawa, 1988). The most convincing albeit limited data regarding this type of toxicity were demonstrated predominantly in mice exposed via inhalation, although some toxicity was shown in i.p. injection studies. Increased vacuolation of Clara cells was often seen within the first 24 hours of exposure, depending on dose, but with cellular repair occurring within days or weeks of exposure. Continued exposure led to resistance to TCE-induced Clara cell toxicity, but damage recurred if exposure was stopped after 5 days and then resumed after 2 days without exposure. However, Clara cell toxicity has only been observed in acute and short-term studies, and it is unclear whether they persist with subchronic or chronic exposure, particularly in mice, which are the more sensitive species. With respect to pulmonary carcinogenicity, statistically significantly increased incidence of lung tumors from chronic inhalation exposures to TCE was observed female ICR mice (Fukuda et al., 1983), male Swiss mice, and female B6C3F$_1$ mice (Maltoni et al., 1988; Maltoni et al., 1986), though not in other

sex/strain combinations, or in rats (Maltoni et al., 1988; Maltoni et al., 1986; Henschler et al., 1980). However, lung toxicity and Clara cell effects have also been observed in rats. Overall, the limited carcinogenesis studies described above are consistent with TCE causing mild increases in pulmonary tumor incidence in mice, but not in other species tested such as rats and hamsters.

The epidemiologic studies are quite limited for examining the role of TCE in cancers of the respiratory system, with no studies found on TCE exposure specifically examining toxicity of the respiratory tract. The two studies found on organic solvent exposure which included TCE suggested smoking as a primary factor for observed lung function decreases among exposed workers. Animal studies have demonstrated toxicity in the respiratory tract, particularly damage to the Clara cells (nonciliated bronchial epithelial cells), as well as decreases in pulmonary surfactant following both inhalation and i.p. exposures, especially in mice. Dose-related increases in vacuolation of Clara cells have been observed in mice and rats as early as 24 hours postexposure (2006; Odum et al., 1992; Forkert and Birch, 1989; Kurasawa, 1988; Scott et al., 1988; Forkert et al., 1985). Mice appear to be more sensitive to these changes, but both species show a return to normal cellular morphology at 4 weeks postexposure (Odum et al., 1992). Studies in mice have also shown an adaptation or resistance to this damage after only 4–5 days of repeated exposures (Green et al., 1997b; Odum et al., 1992). The limited epidemiological literature on lung and laryngeal cancer in TCE-exposed groups is inconclusive due to study limitations (low power, null associations, CIs on RRs that include 1.0). These studies can only rule out risks of a magnitude of ≥2.0 for lung cancer and RRs >3.0 or 4.0 for laryngeal cancer for exposures to studied populations and thus, may not detect a level of response consistent with other endpoints. Animal studies demonstrated a statistically significant increase in pulmonary tumors in mice following chronic inhalation exposure to TCE (Maltoni et al., 1988; Maltoni et al., 1986; Fukuda et al., 1983). These results were not seen in other species tested (rats, hamsters; (Maltoni et al., 1988; Maltoni et al., 1986; Fukuda et al., 1983; Henschler et al., 1980). By gavage, elevated, but not statistically significant, incidences of benign and/or malignant pulmonary tumors have been reported in $B6C3F_1$ mice (NTP, 1990; Henschler et al., 1984; NCI, 1976). No increased pulmonary tumor incidences have been reported in rats exposed to TCE by gavage (NTP, 1990, 1988; NCI, 1976), although all of the studies suffered from early mortality in at least one sex of rat.

Although no epidemiologic studies on the role of metabolism of TCE in adverse pulmonary health effects have been published, animal studies have demonstrated the importance of the oxidative metabolism of TCE by CYP2E1 and/or CYP2F2 in pulmonary toxicity. Exposure to $DASO_2$, an inhibitor of both enzymes protects against pulmonary toxicity in mice following exposure to TCE (Forkert et al., 2005). The increased susceptibility in mice correlates with the greater capacity to oxidize TCE based on increased levels of CYP2E1 in mouse lungs relative to lungs of rats and humans (Forkert et al., 2006; Green et al., 1997b), but it is not clear

that these differences in capacity alone are accurate quantitative predictors of sensitivity to toxicity. In addition, available evidence argues against the previously proposed hypothesis (e.g., Green, 2000) that "accumulation" of chloral in Clara cells is responsible for pulmonary toxicity, since chloral is first converted the water-soluble compounds, CH and TCOH, which can rapidly diffuse to surrounding tissue and blood. Furthermore, the observation of DAL protein adducts, likely derived from DCAC and not from chloral, that were localized in Clara cells suggests an alternative to chloral as the active moiety. While CH has shown substantial genotoxic activity, chemical and toxicokinetic data on CH as well as the lack of correlation across routes of exposure between in vivo measurements of CH in lung tissues and reported pulmonary carcinogenicity suggest that evidence is inadequate to conclude that a mutagenic mode of action mediated by CH is operative for TCE-induced lung tumors. Another mode of action for TCE-induced lung tumors has been plausibly hypothesized to involve cytotoxicity leading to increased cell proliferation, but the available evidence is largely associative and based on short-term studies, so a determination of whether this mode of action is operative cannot be made. The recently discovered formation of DAL protein adducts in pulmonary tissues may also play a role in the mode of action of TCE-induced lung tumors, but an adequately defined hypothesis has yet to be developed. Therefore, the mode of action for TCE-induced lung tumors is currently considered unknown, and this endpoint is thus considered relevant to humans. Moreover, none of the available data suggest that any of the currently hypothesized mechanisms would be biologically precluded in humans.

4.8. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

4.8.1. Reproductive Toxicity

An assessment of the human and experimental animal data, taking into consideration the overall weight of evidence, demonstrates a concordance of adverse reproductive outcomes associated with TCE exposures. Effects on male reproductive system integrity and function are particularly notable and are discussed below. Cancers of the reproductive system in both males and females have also been identified and are discussed below.

4.8.1.1. Human Reproductive Outcome Data

A number of human studies have been conducted that examined the effects of TCE on male and female reproduction following occupational and community exposures. These are described below and summarized in Table 4-85. Epidemiological studies of female human reproduction examined infertility and menstrual cycle disturbances related to TCE exposure. Other studies of exposure to pregnant women are discussed in the section on human developmental studies (see Section 4.8.3.1). Epidemiological studies of male human reproduction examined reproductive behavior, altered sperm morphology, altered endocrine function, and infertility related to TCE exposure.

Table 4-85. Human reproductive effects

Subjects	Exposure	Effect	Reference
Female and male combined effects			
Reproductive behavior			
75 men and 71 women living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Medium: ≥5.0–<10.0 ppb High: <10.0 ppb Highest: <15 ppb	Altered libido[a] Low: referent Medium: OR_{adj}: 0.67 (95% CI: 0.18–2.49) High: OR_{adj}: 1.65 (95% CI: 0.54–5.01) Highest: OR_{adj}: 2.46 (95% CI: 0.59–10.28)	ATSDR (2001)
Female effects			
Infertility			
197 women occupationally exposed to solvents in Finland 1973–1983	U-TCA (µmol/L)[b] Median: 48.1 Mean: 96.2 ± 19.2	Reduced incidence of fecundability in the high exposure group[c] as measured by time to pregnancy Low: IDR = 1.21 (95%CI: 0.73–2.00) High: IDR = 0.61 (95%CI: 0.28–1.33)	Sallmén et al. (1995)
71 women living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Medium: ≥5.0–<10.0 ppb High: <10.0 ppb	No effect on lifetime infertility[a] Low: referent Medium: OR_{adj}: 0.45 (95% CI: 0.02–8.92) High: OR_{adj}: 0.88 (95% CI: 0.13–6.22)	ATSDR (2001)
Menstrual cycle disturbance			
71 women living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Medium: ≥5.0–<10.0 ppb High: <10.0 ppb	Increase in abnormal menstrual cycle (defined as <26 d or >30 d) Low: referent Medium: OR_{adj}: 4.17 (95% CI: 0.31–56.65) High: OR_{adj}: 2.39 (95% CI: 0.41–13.97)	ATSDR (2001)
184 women working in a factory assembling small electrical parts in Poland	Mean indoor air TCE: 200 mg/m^3	18% reporting increase in amenorrhea in exposed group (n = 140), compared to 2% increase in unexposed group (n = 44)	Zielinski (1973)
32 women working in dry cleaning or metal degreasing in Czechoslovakia[d]	0.28–3.4 mg/L TCE for 0.5–25 yrs	31% reporting increase in menstrual disturbances[a]	Bardodej and Vyskocil (1956)
20-yr-old woman occupationally exposed to TCE via inhalation	U-TTCs 3.2 ng/mL (21–25 d after exposure)	Amenorrhea, followed by irregular menstruation and lack of ovulation	Sagawa et al. (1973)
Male effects			
Reproductive behavior			
43 men working in dry cleaning or metal degreasing in Czechoslovakia	0.28–3.4 mg/L TCE for 0.5–25 yrs	30% reporting decreased potency[a]	Bardodej and Vyskocil (1956)
30 male workers in a money printing shop in Egypt	38–172 ppm TCE	Decreased libido reported in 10 men (33%), compared to 3 men in the control group (10%)	El Ghawabi et al. (1973)

Table 4-85. Human reproductive effects (continued)

Subjects	Exposure	Effect	Reference
42 yr-old male aircraft mechanic in UK	TCE exposure reported but not measured; exposure for 25 yrs	Gynaecomastia, impotence	Saihan et al. (1978)
Altered sperm quality			
15 men working as metal degreasers in Denmark	TCE exposure reported but not measured	Nonsignificant increase in percentage of two YFF in spermatozoa; no effect on sperm count or morphology	Rasmussen et al. (1988)
85 men of Chinese descent working in an electronics factory	**Mean personal air TCE: 29.6 ppm; Mean U-TCA: 22.4 mg/g creatinine**	**Decreased normal sperm morphology and hyperzoospermia**	**Chia et al. (1996)**
Altered endocrine function			
85 men of Chinese descent working in an electronics factory	Mean personal air TCE: 29.6 ppm; Mean U-TCA: 22.4 mg/g creatinine	Increased DHEAS and decreased FSH, SHBG and testosterone levels; dose-response observed	Chia et al. (1997)
85 men of Chinese descent working in an electronics factory	Mean personal air TCE: 29.6 ppm; Mean U-TCA: 22.4 mg/g creatinine	Decreased serum levels of testosterone and SHBG were significantly correlated with years of exposure to TCE; increased insulin levels for exposure <2 yrs	Goh et al. (1998)
Infertility			
282 men occupationally exposed to solvents in Finland 1973–1983	U-TCA (µmol/L): High exposure:[c] Mean: 45 (SD 42) Median 31 Low exposure:[c] Mean: 41 (SD 88) Median: 15	No effect on fecundability[c] (as measured by time to pregnancy) Low: FDR: 0.99 (95% CI: 0.63–1.56) Intermediate/High: FDR:[c] 1.03 (95% CI: 0.60–1.76)	Sallmén et al. (1998)
8 male mechanics seeking treatment for infertility in Canada	Urine (µmol/): TCA: <0.30–4.22 TCOH: <0.60–0.89 Seminal fluid (pg/extract): TCE: 20.4–5,419.0 Chloral: 61.2–1,739.0 TCOH 2.7–25.5 TCA: <100–5,504 DCA: <100–13,342	Infertility could not be associated with TCE as controls were five men also in treatment for infertility	Forkert et al. (2003)
75 men living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Medium: ≥5.0 to <10.0 ppb High: <10.0 ppb	No effect on lifetime infertility (not defined) Low: referent Medium: NA High: OR_{adj}: 0.83 (95% CI: 0.11–6.37)	ATSDR (2001)

[a]Not defined by the authors.
[b]As reported in Lindbohm et al. (1990).
[c]Low/intermediate exposure indicated use of TCE <1 or 1–4 days/week, and biological measures indicated high exposure. High exposure indicated daily use of TCE, or if biological measures indicated high exposure.
[d]Number inferred from data provided in Tables 2 and 3 in Bardodej and Vyskocil (1956).

Bolded study(ies) carried forward for consideration in dose-response assessment (see Chapter 5).

DHEAS = dehydroepiandrosterone sulphate; FSH = follicle-stimulating hormone; OR_{adj} = adjusted odds ratio; SHBG = sex-hormone binding globulin

4.8.1.1.1. Female and male combined human reproductive effects

Reproductive behavior

A residential study of individuals living near the Rocky Mountain Arsenal in Colorado examined the reproductive outcomes in 75 men and 71 women exposed to TCE in drinking water (ATSDR, 2001). TCE exposure was classified as high (>10.0 ppb), medium (≥5.0–<10.0 ppb), and low (<5.0 ppb). Altered libido for men and women combined was observed in a dose-response fashion, although the results were nonsignificant. The results were not stratified by gender.

4.8.1.1.2. Female human reproductive effects

4.8.1.1.2.1. Infertility

Sallmén et al. (1995) examined maternal occupational exposure to organic solvents and time-to-pregnancy. Cases of spontaneous abortion and controls from a prior study of maternal occupational exposure to organic solvents in Finland during 1973–1983 and pregnancy outcome (Lindbohm et al., 1990) were used to study time-to-pregnancy of 197 couples. Exposure was assessed by questionnaire during the first trimester and confirmed with employment records. Biological measurements of TCA in urine in 64 women who held the same job during pregnancy and measurement (time of measurement not stated) had a median value of 48.1 µmol/L (mean: 96.2 ± 19.2 µmol/L) (Lindbohm et al., 1990). Nineteen women had low exposure to TCE (used <1 or 1–4 times/week), and 9 had high exposure to TCE (daily use). In this follow-up study, an additional questionnaire on time-to-pregnancy was answered by the mothers (Sallmén et al., 1995). The incidence density ratio (IDR) was used in this study to estimate the ratio of average incidence rate of pregnancies for exposed women compared to nonexposed women; therefore, a lower IDR indicates infertility. For TCE, a reduced incidence of fecundability was observed in the high-exposure group (IDR: 0.61, 95% CI: 0.28–1.33) but not in the low-exposure group (IDR: 1.21, 95% CI: 0.73–2.00). A similar study of paternal occupational exposure (Sallmen et al., 1998) is discussed in Section 4.8.1.1.3.4.

The residential study in Colorado discussed above did not observe an effect on lifetime infertility in the medium- (OR_{adj}: 0.45; 95% CI: 0.02–8.92) or high-exposure groups (OR_{adj}: 0.88; 95% CI: 0.13–6.22) (ATSDR, 2001). Curiously, exposed women had more pregnancies and live births than controls.

4.8.1.1.2.2. Menstrual cycle disturbance

The ATSDR (2001) study discussed above also examined effects on the menstrual cycle (ATSDR, 2001). Nonsignificant associations without a dose-response were seen for abnormal menstrual cycle in women (OR_{adj}: 2.23, 95% CI: 0.45–11.18).

Other studies have examined the effect of TCE exposure on the menstrual cycle. One study examined women working in a factory assembling small electrical parts (Zielinski, (1973),

translated). The mean concentration of TCE in indoor air was reported to be 200 mg/m^3. Of the 140 exposed women, 18% suffered from amenorrhea, compared to only 2% of the 44 nonexposed workers. The other study examined 75 men and women working in dry cleaning or metal degreasing (Bardodej and Vyskocil, 1956). Exposures ranged from 0.28 to 3.4 mg/L, and length of exposure ranged from 0.5 to 25 years. This study reported that many women experienced menstrual cycle disturbances, with a trend for increasing air concentrations and increasing duration of exposure.

There is also an additional case study of a 20-year-old woman who was occupationally exposed to TCE via inhalation. The exposure was estimated to be as high as 10 mg/mL or several thousand ppm, based on urine samples 21–25 days after exposure of 3.2 ng/mL of TTCs. The primary effect was neurological, although she also experienced amenorrhea, followed by irregular menstruation and lack of ovulation as measured by basal body temperature curves (Sagawa et al., 1973).

4.8.1.1.3. Male human reproductive effects

4.8.1.1.3.1. Reproductive behavior

One study reported the effect of TCE exposure on the male reproductive behavior in 75 men working in dry cleaning or metal degreasing (Bardodej and Vyskocil, 1956). Exposures ranged from 0.28 to 3.4 mg/L, and length of exposure ranged from 0.5 to 25 years. This study found that men experienced decreased potency or sexual disturbances; the authors speculated that the effects on men could be due to the CNS effects of TCE exposure. This study also measured serial neutral 17-ketosteroid determinations, but they were found to be not statistically significant (Bardodej and Vyskocil, 1956).

In an occupational study, 30 men working in a money printing shop were exposed to TCE for <1–5 years (El Ghawabi et al., 1973). Depending on the job description, the exposures ranged from 38 to 172 ppm TCE. Ten (33%) men suffered from decreased libido, compared to three (10%) of unexposed controls. However, these results were not stratified by exposure level or duration. The authors speculated that decreased libido was likely due to the common symptoms of fatigue and sleepiness.

A case study described a 42-year-old man exposed to TCE who worked as an aircraft mechanic for approximately 25 years (Saihan et al., 1978). He suffered from a number of health complaints including gynaecomastia and impotence, along with neurotoxicity and immunotoxicity. In addition, he drank alcohol daily, which could have increased his response to TCE.

4.8.1.1.3.2. Altered sperm quality

Genotoxic effects on male reproductive function were examined in a study evaluating occupational TCE exposure in 15 male metal degreasers (Rasmussen et al., 1988). No

measurement of TCE exposure was reported. Sperm count, morphology, and spermatozoa Y-chromosomal nondisjunction during spermatogenesis were examined, along with chromosomal aberrations in cultured lymphocytes. A nonsignificant increase in percentage of two fluorescent Y-bodies (YFF) in spermatozoa were seen in the exposed group ($p > 0.10$), and no difference was seen in sperm count or morphology compared to controls.

An occupational study of men using TCE for electronics degreasing (Goh et al., 1998; Chia et al., 1997; Chia et al., 1996) examined subjects (n = 85) who were offered a free medical exam if they had no prior history related to endocrine function, no clinical abnormalities, and normal liver function tests; no controls were used. These participants provided urine, blood, and sperm samples. The mean urine TCA level was 22.4 mg/g creatinine (range: 0.8–136.4 mg/g creatinine). In addition, 12 participants provided personal 8-hour air samples, which resulted in a mean TCE exposure of 29.6 ppm (range: 9–131 ppm). Sperm samples were divided into two exposure groups: low for urine TCA <25 mg/g creatinine and high for urine TCA ≥25 mg/g creatinine. A decreased percentage of normal sperm morphology was observed in the sperm samples in the high-exposure group (n = 48) compared to the low-exposure group (n = 37). However, TCE exposure had no effect on semen volume, sperm density, or sperm motility. There was also an increased prevalence of hyperzoospermia (sperm density of >120 million sperm per mL ejaculate) with increasing urine TCA levels (Chia et al., 1996).

4.8.1.1.3.3. Altered endocrine function

Two studies followed up on the study by Chia et al. (1996) to examine endocrine function (Goh et al., 1998; Chia et al., 1997). The first examined serum testosterone, follicle-stimulating hormone (FSH), dehydroepiandrosterone sulphate (DHEAS), and sex-hormone binding globulin (SHBG) (Chia et al., 1997). With increased number of years of exposure to TCE, increases in DHEAS levels were seen, from 255 ng/mL for <3 years to 717.8 ng/mL ≥7 years of exposure. Also with increased number of years of exposure to TCE, decreased FSH, SHBG, and testosterone levels were seen. The authors speculated that these effects could be due to decreased liver function related to TCE exposure (Chia et al., 1997).

The second follow-up study of this cohort studied the hormonal effects of chronic low-dose TCE exposure in these men (Goh et al., 1998). Because urine TCE measures only indicate short-term exposure, long-term exposure was indicated by years of exposure. Hormone levels examined include androstenedione, cortisol, testosterone, aldosterone, SHBG, and insulin. Results show that a decrease in serum levels of testosterone and SHBG were significantly correlated with years of exposure to TCE, and an increase in insulin levels were seen in those exposed for <2 years. Androstenedione, cortisol, and aldosterone were in normal ranges and did not change with years of exposure to TCE.

4.8.1.1.3.4. Infertility

Sallmén et al. (1998) examined paternal occupational exposure and time-to-pregnancy among their wives. Cases of spontaneous abortion and controls from a prior study of pregnancy outcome (Taskinen et al., 1989) were used to study time-to-pregnancy of 282 couples. Exposure was determined by biological measurements of the father who held the same job during pregnancy and measurement (time of measurement not stated) and questionnaires answered by both the mother and father. An additional questionnaire on time-to-pregnancy was answered by the mother for this study 6 years after the original study (Sallmen et al., 1998). The level of exposure was determined by questionnaire and classified as "low/intermediate" if the chemical was used <1 or 1–4 days/week and biological measures indicated high exposure (defined as above the reference value for the general population), and "high" if used daily or if biological measures indicated high exposure. For 13 men highly exposed, mean levels of urine TCA were 45 µmol/L (SD 42 µmol/L; median 31 µmol/L); for 22 men low/intermediately exposed, mean levels of urine TCA were 41 µmol/L (SD 88 µmol/L; median 15 µmol/L). The terminology IDR was replaced by fecundability density ratio (FDR) in order to reflect that pregnancy is a desired outcome; therefore, a high FDR indicates infertility. No effect was seen on fertility in the low-exposure group (FDR: 0.99, 95% CI: 0.63–1.56) or in the intermediate-/high-exposure group (FDR: 1.03, 95% CI: 0.60–1.76). However, the exposure categories were grouped by low/intermediate vs. high, whereas the outcome categories were grouped by low vs. intermediate/high, making a dose-response association difficult.

A small occupational study reported on eight male mechanics exposed to TCE for at least 2 years who sought medical treatment for infertility (Forkert et al., 2003). The wives were determined to have normal fertility. Samples of urine from two of the eight male mechanics contained TCA and/or TCOH, demonstrating the rapid metabolism in the body. However, samples of seminal fluid taken from all eight individuals detected TCE and the metabolites CH and TCOH, with two samples detecting DCA and one sample detecting TCA. Five unexposed controls also diagnosed with infertility did not have any TCE or metabolites in samples of seminal fluid. There was no control group that did not experience infertility. Increased levels of TCE and its metabolites in the seminal fluid of exposed workers compared to lower levels found in their urine samples was explained by cumulative exposure and mobilization of TCE from adipose tissue, particularly that surrounding the epididymis. In addition, CYP2E1 was detected in the epididymis, demonstrating that metabolism of TCE can occur in the male reproductive tract. However, this study could not directly link TCE to the infertility, as both the exposed and control populations were selected due to their infertility.

The ATSDR (2001) study discussed above on the reproductive effects from TCE in drinking water of individuals living near the Rocky Mountain Arsenal in Colorado did not observe infertility or other adverse reproductive effects for the high exposure group compared to

the low exposure group (OR_{adj}: 0.83; 95% CI: 0.11–6.37). Curiously, exposed men had more pregnancies and live births than controls.

4.8.1.1.4. Summary of human reproductive toxicity

Following exposure to TCE, observed adverse effects on the female reproductive system include reduced incidence of fecundability (as measured by time-to-pregnancy) and menstrual cycle disturbances. Observed adverse effects on the male reproductive system include altered sperm morphology, hyperzoospermia, altered endocrine function, decreased sexual drive and function, and altered fertility. These are summarized in Table 4-85.

4.8.1.2. Animal Reproductive Toxicity Studies

A number of animal studies have been conducted that examined the effects of TCE on reproductive organs and function following either inhalation or oral exposures. These are described below and summarized in Tables 4-86 and 4-87. Other animal studies of offspring exposed during fetal development are discussed in the section on animal developmental studies (see Section 4.8.3.2).

4.8.1.2.1. Inhalation exposures

Studies in rodents exposed to TCE via inhalation are described below and summarized in Table 4-86. These studies focused on various aspects of male reproductive organ integrity, spermatogenesis, or sperm function in rats or mice. In the studies published after the year 2000, the effects of either 376 or 1,000 ppm TCE were studied following exposure durations ranging from 1 to 24 weeks, and adverse effects on male reproductive endpoints were observed.

Table 4-86. Summary of mammalian in vivo reproductive toxicity studies—inhalation exposures

Reference[a]	Species/strain/ sex/number	Exposure level/duration	NOAEL; LOAEL[b]	Effects
Forkert et al. (2002)	**Mouse, CD-1, male, 6/group**	**0 or 1,000 ppm (5,374 mg/m^3)[c]** **6 hrs/d, 5 d/wk, 19 d over 4 wks**	**LOAEL: 1,000 ppm**	**U-TCA and U-TCOH increased by 2nd and 3rd wk, respectively. CYP 2E1 and *p*-nitrophenol hydroxylation in epididymal epithelium > testicular Leydig cells. Choral also generated from TCE in epididymis > testis. Sloughing of epididymal epithelial cells after 4-wk exposure.**

Table 4-86. Summary of mammalian in vivo reproductive toxicity studies—inhalation exposures (continued)

Reference[a]	Species/strain/ sex/number	Exposure level/duration	NOAEL; LOAEL [a]	Effects
Kan et al. (2007)	**Mouse, CD-1, male, 4/group**	**0 or 1,000 ppm** **6 hrs/d,5 d/wk, 1–4 wks**	**LOAEL: 1,000 ppm**	**Light microscopy findings: degeneration and sloughing of epididymal epithelial cells as early as 1 wk into exposure; more severe by 4 wks. Ultrastructional findings: vesiculation in cytoplasm, disintegration of basolateral cell membranes, sloughing of epithelial cells. Sperm found in situ in cytoplasm of degenerated epididymal cells. Abnormalities of the head and tail in sperm located in the epididymal lumen.**
Kumar et al. (2000b)	**Rat, Wistar, male, 12–13/group**	**0 or 376 ppm** **4 hrs/d, 5 d/wk, 2–10 wks exposure, 2–8-wk rest period**	**LOAEL: 376 ppm**	**Alterations in testes histopathology (smaller, necrotic spermatogenic tubules), ↑ sperm abnormalities, and statistically significant ↑ pre- and/or postimplantation loss in litters observed in the groups with 2 or 10 wks of exposure, or 5 wks of exposure with 2-wk rest.**
Kumar et al. (2000a)	Rat, Wistar, males, 12–13/group	0 or 376 ppm 4 hrs/d, 5 d/wk, 12 and 24 wks	LOAEL: 376 ppm	Statistically significant ↓ in total epididymal sperm count and sperm motility, with statistically significant ↓ in serum testosterone, statistically significant ↑ in testes cholesterol, statistically significant ↓ of glucose 6-phosphate dehydrogenase and 17-β-hydroxy steroid dehydrogenase at 12 and 24 wks of exposure.
Kumar et al. (2001b)	**Rat, Wistar, male, 6/group**	**0 or 376 ppm** **4 hrs/d, 5 d/wk, 12 and 24 wks**	**LOAEL: 376 ppm**	**Body weight gain statistically significant ↓. Testis weight, sperm count and motility statistically significant ↓, effect stronger with exposure time. After 12 wks, numbers of spermatogenic cells and spermatids ↓, some of the spermatogenic cells appeared necrotic. After 24 wks, testes were atrophied, tubules were smaller, had Sertoli cells, and were almost devoid of spermatocytes and spermatids. Leydig cells were hyperplastic. SDH, G6PDH statistically significant ↓, GGT and β-glucuronidase statistically significant ↑; effects stronger with exposure time.**
Land et al. (1981)	**Mouse, C57BlxC3H (F1), male, 5 or 10/group**	**0, 0.02%, or 0.2%** **4 hrs/d, 5 d, 23-d rest**	**NOAEL: 0.02%** **LOAEL: 0.2%**	**Statistically significant ↑ percentage morphologically abnormal epididymal sperm.**
Xu et al. (2004)	**Mouse, CD-1, male, 4–27/group**	**0 or 1,000 ppm (5.37 mg/L)[c]** **6 hrs/d, 5 d/wk, 1–6 wks**	**LOAEL: 1,000 ppm**	**Statistically significant ↓ in vitro sperm-oocyte binding and in vivo fertilization**

[a]**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5).
[b]NOAEL and LOAEL are based upon reported study findings.
[c]Dose conversion calculations by study author(s).

G6PDH = glucose 6-p dehydrogenase

Table 4-87. Summary of mammalian in vivo reproductive toxicity studies—oral exposures

Reference[a]	Species/strain/ sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL[b]	Effects
Studies assessing male reproductive outcomes					
DuTeaux et al. (2003)	Rat, Sprague-Dawley, male, 3/group	0, 0.2, or 0.4% (0, 143, or 270 mg/kg-d)	Drinking water; 3% ethoxylated castor oil vehicle	LOEL: 0.2%	TCE metabolite-protein adducts formed by a CYP-mediated pathway were detected by fluorescence imunohistochemistry in the epithelia of corpus epididymis and in efferent ducts.
DuTeaux et al. (2004a)	**Rat, Sprague-Dawley, male, 3/group, or Simonson albino (UC-Davis), male, 3/group**	**0, 0.2, or 0.4%** **(0, 143, or 270 mg/kg-d)** **14 d**	**Drinking water, 3% ethoxylated castor oil vehicle**	**LOAEL: 0.2%**	**Dose-dependent ↓ in ability of sperm to fertilize oocytes collected from untreated ♀s. Oxidative damage to sperm membrane in head and mid-piece was indicated by dose-related ↑ in oxidized proteins and lipid peroxidation.**
Veera-machaneni et al. (2001)	Rabbit, Dutch belted, females and offspring; 7–9 offspring/ group	9.5 or 28.5 ppm TCE[c] GD 20 through lactation, then to offspring thru postnatal wk 15	Drinking water	LOAEL: 9.5 ppm	Decreased copulatory behavior; acrosomal dysgenesis, nuclear malformations; statistically significant ↓ LH and testosterone.
Zenick et al. (1984)	**Rat, Long-Evans, male, 10/group**	**0, 10, 100, or 1,000 mg/kg-d** **6 wk, 5 d/wk; 4 wks recovery**	**Gavage, corn oil vehicle**	**NOAEL: 100 mg/kg-d LOAEL: 1,000 mg/kg-d**	**At 1,000 mg/kg, body weight ↓, liver/body weight ratios↑, and impaired copulatory behavior. Copulatory performance returned to normal by 5th wk of exposure. At wk 6, TCE and metabolites concentrated to a significant extent in male reproductive organs.**
Studies assessing female reproductive outcomes					
Berger and Horner (2003)	Rat, Simonson (Sprague-Dawley derived), female, (5–6); × 3/group	0 or 0.45% 2 wks	Drinking water, 3% Tween vehicle	LOAEL: 0.45%	In vitro fertilization and sperm penetration of oocytes statistically significant ↓ with sperm harvested from untreated males.
Cosby and Dukelow (1992)	Mouse, B6D2F1, female, 7–12/group	0, 24, or 240 mg/kg-d GDs 1–5, 6–10, or 11–15	Gavage, corn oil vehicle	NOAEL: 240 mg/kg-d	No treatment-related effects on in vitro fertilization in dams or offspring.

Table 4-87. Summary of mammalian in vivo reproductive toxicity studies—oral exposures (continued)

Reference[a]	Species/strain/ sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL[a]	Effects
Manson et al. (1984)	**Rat, Long-Evans, female, 23–25/group**	**0, 10, 100, or 1,000 mg/kg-d** **6 wks: 2 wks premating, 1 wk mating period, GDs 1–21**	**Gavage, corn oil vehicle**	**NOAEL: 100 mg/kg-d** **LOAEL: 1,000 mg/kg-d**	**Female fertility and mating success was not affected. At 1,000 mg/kg-d group, 5/23 females died, gestation body weight gain was statistically significant ↓. After subchronic oral TCE exposure, TCE was detected in fat, adrenals, and ovaries; TCA levels in uterine tissue were high.** **At 1,000 mg/kg-d, neonatal deaths (female pups) were ↑ on PNDs 1, 10, and 14. Dose-related ↑ seen in TCA in blood, liver and milk in stomach of ♀ pups, not ♂s.**
Wu and Berger (2007)	Rat, Simonson (Sprague-Dawley derived), female, (number/group not reported)	0 or 0.45% (0.66 g/kg-d)[d] Preovulation d 1–5, 6–10, 11–14, or 1–14	Drinking water, 3% Tween vehicle	LOAEL: 0.45%	In vitro fertilization and sperm penetration of oocytes statistically significant ↓ with sperm harvested from untreated males.
Wu and Berger (2008)	Rat, Simonson (Sprague-Dawley derived), female, (number/group not reported)	0 or 0.45% (0.66 g/kg-d)[d] 1 or 5 d	Drinking water, 3% Tween vehicle	NOEL: 0.45%	Ovarian mRNA expression for ALCAM and Cudz1 protein were not altered.

Table 4-87. Summary of mammalian in vivo reproductive toxicity studies—oral exposures (continued)

Reference[a]	Species/strain/ sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL[a]	Effects
Studies assessing fertility and reproductive outcome in both sexes					
George et al. (1985)	**Mouse, CD-1, male and female, 20 pairs/ treatment group; 40 controls/sex**	**0, 0.15, 0.30, or 0.60%[e] micro-encapsulated TCE (TWA dose estimates: 0, 173, 362, or 737 mg/kg-d)[d] Breeders exposed 1 wk premating, then for 13 wks; pregnant females throughout gestation (i.e., 18 wks total)**	**Dietary**	**Parental systemic toxicity: NOAEL: 0.30% LOAEL: 0.60%**	**At 0.60%, in F0: statistically significant↑ liver weights in both sexes; statistically significant ↓ testis and seminal vesicle weight; histopathology of liver and kidney in both sexes. At 0.60%, in F1: statistically significant ↓ body weight on PND 74, and in postpartum F1 dams; statistically significant ↑ liver, testis, and epididymis weights in males, statistically significant ↑ kidney weights in both sexes; statistically significant ↓ testis and seminal vesicle weight; histopathology of liver and kidney in both sexes.**
				Parental reproductive function: LOAEL: 0.60%[d]	At 0.60%, in F0 and F1 males: statistically significant ↓ sperm motility.
				Offspring toxicity: NOAEL: 0.30% LOAEL: 0.60%	At 0.60%, in F1 pups: statistically significant ↓ live birth weights, statistically significant ↓ PND 4 pup body weight; perinatal mortality ↑ (PNDs 0–21).

Table 4-87. Summary of mammalian in vivo reproductive toxicity studies—oral exposures (continued)

Reference[a]	Species/strain/ sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL[a]	Effects
George et al. (1986)	**Rat, F334, males and female, 20 pairs/ treatment group, 40 controls/sex**	**0, 0.15, 0.30, or 0.60%[e] micro-encapsulated TCE Breeders exposed 1 wk premating, then for 13 wks; pregnant females throughout gestation (i.e., 18 wks total)**	**Dietary**	**Parental systemic toxicity: LOAEL: 0.15%**	**At 0.60%, in F0: statistically significant ↓ postpartum dam body weight; statistically significant ↓ term. body weight in both sexes; statistically significant ↑ liver, and kidney/adrenal weights in both sexes; statistically significant ↑ testis/epididymis weights; in F1: statistically significant ↓ testis weight. At all doses in F1: statistically significant ↓ postpartum dam body weight; statistically significant↓ term. body weight in both sexes, statistically significant ↑ liver weight in both sexes. At 0.30 and 0.60%, in F1: statistically significant ↑ liver weight in females.**
				Parental reproductive function: LOAEL: 0.60%[e]	**At 0.60%, sig ↓ mating in F0 males and females (in cross-over mating trials).**
				Offspring toxicity: LOAEL: 0.15%	**At 0.60%, statistically significant ↓ F1 body weight on PNDs 4 and 14. At all doses, statistically significant ↓ F1 body weight on PNDs 21 and 80. At 0.3 and 0.60%, statistically significant ↓ live F1 pups/litter. Statistically significant trend towards ↓ live litters per pair At 0.15 and 0.60%, trend toward ↓ F1 survival from PNDs 21–80.**

[a]**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5).
[b]NOAEL, LOAEL, NOEL, and LOEL are based upon reported study findings.
[c]Concurrent exposure to several groundwater contaminants; values given are for TCE levels in the mixture.
[d]Dose conversion calculations by study author(s).
[e]Fertility and reproduction assessment of last litter from continuous breeding phase and cross-over mating assessment (rats only) were conducted for 0 or 0.60% dose groups only.

LH = luteinizing hormone

Kumar et al. (2000b) exposed male Wistar rats in whole-body inhalation chambers to 376-ppm TCE for 4 hours/day, 5 days/week over several duration scenarios. These were 2 weeks (to observe the effect on the epididymal sperm maturation phase), 10 weeks (to observe the effect on the entire spermatogenic cycle), 5 weeks with 2 weeks of rest (to observe the effect on primary spermatocytes differentiation to sperm), 8 weeks with 5 weeks of rest (to observe effects on an intermediate stage of spermatogenesis), and 10 weeks with 8 weeks of rest (to observe the effect on spermatogonial differentiation to sperm). Control rats were exposed to ambient air. Weekly mating with untreated females was conducted. At the end of the treatment/rest periods, the animals were sacrificed; testes and cauda epididymes tissues were collected. Alterations in testes histopathology (smaller, necrotic spermatogenic tubules), increased sperm abnormalities, and significantly increased pre- and/or postimplantation loss in litters were observed in the groups with 2 or 10 weeks of exposure, or 5 weeks of exposure with 2 of weeks rest. It was hypothesized that postmeiotic cells of spermatogenesis and epididymal sperm were affected by TCE exposure, leading to reproductive impairment.

To test the hypothesis that TCE exposure adversely affects sperm function and fertilization, Xu et al. (2004) conducted a study in which male CD-1 mice were exposed by inhalation to atmospheres containing 1,000 ppm (5.37 mg/L) TCE for 1–6 weeks (6 hours/day, 5 days/week). After each TCE exposure, body weights were recorded. Following termination, the right testis and epididymis of each treated male were weighed, and sperm was collected from the left epididymis and vas deferens for assessment of the number of total sperm and motile sperm. Sperm function was evaluated in the following experiments: (1) suspensions of capacitated vas deferens/cauda epididymal sperm were examined for spontaneous acrosome reaction; (2) in vitro binding of capacitated sperm to mature eggs from female CF-1 mice (expressed as the number of sperm bound per egg) was assessed; and (3) in vivo fertilization was evaluated via mating of male mice to superovulated female CF-1 mice immediately following inhalation exposure; cumulus masses containing mature eggs were collected from the oviducts of the females, and the percentage of eggs fertilized was examined. Inhalation exposure to TCE did not result in altered body weight, testis and epididymis weights, sperm count, or sperm morphology or motility. Percentages of acrosome-intact sperm populations were similar between treated and control animals. Nevertheless, for males treated with TCE for ≥2 weeks decreases were observed in the number of sperm bound to the oocytes in vitro (significant at 2 and 6 weeks, $p < 0.001$). In a follow-up assessment, control sperm were incubated for 30 minutes in buffered solutions of TCE or metabolites (CH or TCOH); while TCE-incubation had no effect on sperm-oocyte binding, decreased binding capacity was noted for the metabolite-incubated sperm. The ability for sperm from TCE-exposed males to bind to and fertilize oocytes in vivo was also found to be significantly impaired ($p < 0.05$).

A study designed to investigate the role of testosterone, and of cholesterol and ascorbic acid (which are primary precursors of testosterone) in TCE-exposed rats with compromised reproductive function was conducted by Kumar et al. (2000a). Male Wistar rats (12–13/group) were exposed (whole body) to 376 ppm TCE by inhalation for 4 hours/day, 5 days/week, for either 12 or 24 weeks and then terminated. Separate ambient-air control groups were conducted for the 12- and 24-week exposure studies. Epididymal sperm count and motility were evaluated, and measures of 17-β-hydroxy steroid dehydrogenase (17-β-HSD), testicular total cholesterol and ascorbic acid, serum testosterone, and glucose 6-p dehydrogenase (G6PDH) in testicular homogenate were assayed. In rats exposed to TCE for either 12 or 24 weeks, total epididymal sperm count and motility, serum testosterone concentration, and specific activities of both 17-β-HSD and G6PDH were significantly decreased ($p < 0.05$), while total cholesterol content was significantly ($p < 0.05$) increased. Ascorbic acid levels were not affected.

In another study, Kumar et al. (2001b) utilized the same exposure paradigm to examine cauda epididymal sperm count and motility, testicular histopathology, and testicular marker enzymes: sorbitol dehydrogenase (SDH), G6PDH, glutamyl transferase (GT), and glucuronidase, in Wistar rats (6/group). After 24 weeks of exposure, testes weights and epididymal sperm count and motility were significantly decreased ($p < 0.05$). After 12 weeks of TCE exposure, histopathological examination of the testes revealed a reduced number of spermatogenic cells in the seminiferous tubules, fewer spermatids as compared to controls, and the presence of necrotic spermatogenic cells. Testicular atrophy, smaller tubules, hyperplastic Leydig cells, and a lack of spermatocytes and spermatids in the tubules were observed after 24 weeks of TCE exposure. After both 12 and 24 weeks of exposure, SDH and G6PDH were significantly ($p < 0.05$) reduced, while GT and β-glucuronidase were significantly ($p < 0.05$) increased.

In a study by Land et al. (1981), 8–10-week-old male mice (C57BlxC3H)F1 (5 or 10/group) were exposed (whole body) by inhalation to a number of anesthetic agents for 5 consecutive days at 4 hours/day and sacrificed 28 days after the first day of exposure. Chamber concentration levels for the TCE groups were 0.02 and 0.2%. The control group received ambient air. Epididymal sperm were evaluated for morphological abnormalities. At 0.2% TCE, the percentage of abnormal sperm in a sample of 1,000 was significantly ($p < 0.01$) increased as compared to control mice; no treatment-related effect on sperm morphology was observed at 0.02% TCE.

Forkert et al. (2002) exposed male CD-1 mice by inhalation to 1,000-ppm TCE (6 hours/day, 5 days/week) for 4 consecutive weeks and observed sloughing of portions of the epithelium upon histopathological evaluation of testicular and epididymal tissues.

Kan et al. (2007) also demonstrated that damage to the epididymal epithelium and sperm of CD-1 mice (4/group) resulted from exposure to 0 or 1,000 ppm TCE by inhalation for 6 hours/day, 5 days/week, for 1–4 weeks. Segments of the epididymis (caput, corpus, and cauda) were examined by light and electron microscope. As early as 1 week after TCE exposure,

degeneration and sloughing of epithelial cells from all three epididymal areas were observed by light microscopy; these findings became more pronounced by 4 weeks of exposure. Vesiculation in the cytoplasm, disintegration of basolateral cell membranes, and epithelial cell sloughing were observed with electron microscopy. Sperm were found in situ in the cytoplasm of degenerated epididymal cells. A large number of sperm in the lumen of the epididymis were abnormal, including head and tail abnormalities.

4.8.1.2.2. Oral exposures

A variety of studies were conducted to assess various aspects of male and/or female reproductive capacity in laboratory animal species following oral exposures to TCE. These are described below and summarized in Table 4-87. They include studies that focused on male reproductive outcomes in rats or rabbits following gavage or drinking water exposures (DuTeaux et al., 2004a; DuTeaux et al., 2003; Veeramachaneni et al., 2001; Zenick et al., 1984), studies that focused on female reproductive outcomes in rats following gavage or drinking water exposures (Wu and Berger, 2008, 2007; Berger and Horner, 2003; Cosby and Dukelow, 1992; Manson et al., 1984), and studies that assessed fertility and reproductive outcome in both sexes following dietary exposures to CD-1 mice or F344 rats (George et al., 1986; George et al., 1985).

4.8.1.2.2.1. Studies assessing male reproductive outcomes

Zenick et al. (1984) conducted a study in which sexually experienced Long-Evans hooded male rats were administered 0, 10, 100, or 1,000 mg/kg-day TCE by gavage in corn oil for 6 weeks. A 4-week recovery phase was also incorporated into the study design. Endpoints assessed on weeks 1 and 5 of treatment included copulatory behavior, ejaculatory plug weights, and ejaculated or epididymal sperm measures (count, motility, and morphology). Sperm measures and plug weights were not affected by treatment, nor were Week 6 plasma testosterone levels found to be altered. TCE effects on copulatory behavior (ejaculation latency, number of mounts, and number of intromissions) were observed at 1,000 mg/kg-day; these effects were recovered by 1–4 weeks posttreatment. Although the effects on male sexual behavior in this study were believed to be unrelated to narcotic effects of TCE, a later study by Nelson and Zenick (1986) showed that naltrexone (an opioid receptor antagonist, 2.0 mg/kg, i.p., administered 15 minutes prior to testing) could block the effect. Thus, it was hypothesized that the adverse effects of TCE on male copulatory behavior in the rat at 1,000 ppm may, in fact, be mediated by the endogenous opioid system at the CNS level.

In a series of experiments by DuTeaux et al. (2004a; 2003), adult male rats were administered 0, 0.2, or 0.4% TCE (v/v) (equivalent to 0, 2.73, or 5.46 mg/L) in a solution of 3% ethoxylated castor oil in drinking water for 14 days. These concentrations were within the range of measurements obtained in formerly contaminated drinking water wells, as reported by ATSDR (1997b). The average ingested doses of TCE (based upon animal body weight and

average daily water consumption of 28 mL) were calculated to be 143 or 270 mg/kg-day for the low- and high-dose groups, respectively (DuTeaux et al., 2003). Cauda epididymal and vas deferens sperm from treated males were incubated in culture medium with oviductal cumulus masses from untreated females to assess in vitro fertilization capability. Treatment with TCE resulted in a dose-dependent decrease in the ability of sperm to fertilize oocytes. Terminal body weights and testis/epididymal weights were similar between control and treated groups. Evaluation of sperm concentration or motility parameters did not reveal any treatment-related alterations; acrosomal stability and mitochondrial membrane potential were not affected by treatment. Although no histopathological changes were observed in the testis or in the caput, corpus, or cauda epididymis, exposure to 0.2 and 0.4% TCE resulted in slight cellular alterations in the efferent ductule epithelium.

Veeramachaneni et al. (2001) evaluated the effects of drinking water containing chemicals typical of groundwater near hazardous waste sites (including 9.5 or 28.5 ppm TCE) on male reproduction. In this study, pregnant Dutch-belted rabbits were administered treated drinking water starting on GD 20; treatment continued through the lactation period and to weaned offspring (7–9/group) through postnatal week 15. Deionized water was administered from postnatal weeks 16–61, at which time the animals were terminated. At 57–61 weeks of age, ejaculatory capability, and seminal, testicular, epididymal, and endocrine characteristics were evaluated. In both treated groups, long-term effects consisted of decreased copulatory behavior (interest, erection, and/or ejaculation), significant increases in acrosomal dysgenesis and nuclear malformations ($p < 0.03$), and significant decreases in serum concentration of luteinizing hormone (LH) ($p < 0.05$) and testosterone secretion after human chorionic gonadotropin administration ($p < 0.04$). There were no effects on total spermatozoa per ejaculate or on daily sperm production. The contribution of individual drinking water contaminants to adverse male reproductive outcome could not be discerned in this study. Additionally, it was not designed to distinguish between adverse effects that may have resulted from exposures in late gestation (i.e., during critical period of male reproductive system development) vs. postnatal life.

4.8.1.2.2.2. Studies assessing female reproductive outcomes

In a study that evaluated postnatal growth following gestational exposures, female B6D2F1 mice (7–12/group) were administered TCE at doses of 0, 1% LD_{50} (24 mg/kg-day), and 10% LD_{50} (240 mg/kg-day) by gavage in corn oil on GDs 1–5, 6–10, or 11–15 (day of mating was defined as GD 1) (Cosby and Dukelow, 1992). Litters were examined for pup count, sex, weight, and crown-rump measurement until GD 21. Some offspring were retained to 6 weeks of age, at which time they were killed and the gonads were removed, weighed, and preserved. No treatment-related effects were observed in the dams or offspring. In a second series of studies conducted by (Cosby and Dukelow) and reported in the same paper, TCE and its metabolites, DCA, TCA, and TCOH, were added to culture media with capacitated sperm and cumulus

masses from B6D2F1 mice to assess effects on in vitro fertilization. Dose-related decreases in fertilization were observed for DCA, TCA, and TCOH at 100 and 1,000 ppm, but not with TCE. Synergystic effects were not observed with TCA and TCOH.

A study was conducted by Manson et al. (1984) to determine if subchronic oral exposure to TCE affected female reproductive performance, and if TCE or its metabolites, TCA or TCOH, accumulated in female reproductive organs or neonatal tissues. Female Long-Evans hooded rats (22–23/group) were administered 0 (corn oil vehicle), 10, 100, or 1,000 mg/kg-day of TCE by gavage for 2 weeks prior to mating, throughout mating, and to GD 21. Delivered pups were examined for gross anomalies, and body weight and survival were monitored for 31 days. Three maternal animals per group and 8–10 neonates per group (killed on GDs 3 and 31) were analyzed for TCE and metabolite levels in tissues. TCE exposure resulted in five deaths and decreased maternal body weight gain at 1,000 mg/kg-day, but did not affect estrous cycle length or female fertility at any dose level. There were no evident developmental anomalies observed at any treatment level; however, at 1,000 mg/kg-day, there was a significant increase in the number of pups (mostly female) born dead, and the cumulative neonatal survival count through PND 18 was significantly decreased as compared to control. TCE levels were uniformly high in fat, adrenal glands, and ovaries across treatment groups, and TCA levels were high in uterine tissue. TCE levels in the blood, liver, and milk contents of the stomach increased in female PND-3 neonates across treatment groups. These findings suggest that increased metabolite levels did not influence fertility, mating success, or pregnancy outcome.

In another study that examined the potential effect of TCE on female reproductive function, Berger and Horner (2003) conducted 2-week exposures of Sprague-Dawley derived female Simonson rats to tetrachloroethylene, TCE, several ethers, and 4-vinylcyclohexene diepoxide in separate groups. The TCE-treated group received 0.45% TCE in drinking water containing 3% Tween vehicle; control groups were administered either untreated water, or water containing the 3% Tween vehicle. There were 5–6 females/group, and three replicates were conducted for each group. At the end of exposure, ovulation was induced, the rats were killed, and the ovaries were removed. The zona pellucida was removed from dissected oocytes, which were then placed into culture medium and inseminated with sperm from untreated males. TCE treatment did not affect female body weight gain, the percentage of females ovulating, or the number of oocytes per ovulating female. Fertilizability of the oocytes from treated females was reduced significantly (46% for TCE-treated females vs. 56% for vehicle controls). Oocytes from TCE-treated females had reduced ability to bind sperm plasma membrane proteins compared with vehicle controls.

In subsequent studies, Wu and Berger (Wu and Berger, 2008, 2007) examined the effect of TCE on oocyte fertilizibility and ovarian gene expression. TCE was administered to female Simonson rats (number of subjects not reported) in the drinking water at 0 or 0.45% (in 3% Tween vehicle); daily doses were estimated to be 0.66 g TCE/kg body weight/day. In the oocyte

fertilizibility study (Wu and Berger, 2007), the female rats were treated on days 1–5, 6–10, 11–14, or 1–14 of the 2-week period preceding ovulation (on day 15). Oocytes were extracted and fertilized in vitro with sperm from a single male donor rat. With any duration of TCE exposure, fertilization (as assessed by the presence of decondensed sperm heads) was significantly ($p < 0.05$) decreased as compared to controls. After exposure on days 6–10, 11–14, or 1–14, the oocytes from TCE-treated females had a significantly decreased ability to bind sperm ($p < 0.05$) in comparison to oocytes from vehicle controls. Increased protein carbonyls (an indicator of oxidatively modified proteins) were detected in the granulosa cells of ovaries from females exposed to TCE for 2 weeks. The presence of oxidized protein was confirmed by Western blot analysis. Microsomal preparations demonstrated the localization of CYP 2E1 and GST (TCE-metabolizing enzymes) in the ovary. Ovarian mRNA transcription for ALCAM and Cuzd1 protein was not found to be altered after 1 or 5 days of exposure (Wu and Berger, 2008), suggesting that the posttranslational modification of proteins within the ovary may partially explain the observed reductions in oocyte fertilization.

4.8.1.2.2.3. Studies assessing fertility and reproductive outcomes in both sexes

Assessments of reproduction and fertility with continuous breeding were conducted in NTP studies in CD-1 mice (George et al., 1985) and F344 rats (George et al., 1986). TCE was administered to the mice and rats at dietary levels of 0, 0.15, 0.30, or 0.60%, based upon the results of preliminary 14-day dose-range finding toxicity studies. Actual daily intake levels for the study in mice were calculated from the results of dietary formulation analyses and body weight/food consumption data at several time points during study conduct; the most conservative were from the second week of the continuous breeding study: 0, 52.5, 266.3, and 615.0 mg/kg-day. No intake calculations were presented for the rat study. In these studies, which were designed as described by Chapin and Sloane (1997), the continuous breeding phase in F0 adults consisted of a 7-day premating exposure, 98-day cohabitation period, and 28-day segregation period. In rats, a crossover mating trial (i.e., control males × control females; 0.60% TCE males × control females; control males × 0.60% TCE females) was conducted to further elucidate treatment-related adverse reproductive trends observed in the continuous breeding phase. The last litter of the continuous breeding phase was raised to sexual maturity for an assessment of fertility and reproduction in control and high-dose groups; for the rats, this included an open field behavioral assessment of F1 pups. The study protocol included terminal studies in both generations, including sperm evaluation (count, morphology, and motility) in 10 selected males per dose level, macroscopic pathology, organ weights, and histopathology of selected organs.

In the continuous breeding phase of the CD-1 mouse study (George et al., 1985), no clinical signs of toxicity were observed in the parental (F0) animals, and there were no treatment-related effects on the proportion of breeding pairs able to produce a litter, number of live pups per litter, percentage born live, proportion of pups born live, sex of pups born live, absolute live

pup weights, or adjusted female pup weights. At the high-dose level of 0.60%, a number of adverse outcomes were observed. In the parental animals, absolute and body-weight-adjusted male and female liver weight values were significantly increased ($p < 0.01$), and right testis and seminal vesicle weights were decreased ($p < 0.05$), but kidney/adrenal weights were not affected. Sperm motility was significantly ($p < 0.01$) decreased by 45% in treated males as compared to controls. Histopathology examination revealed lesions in the liver (hypertrophy of the centrilobular liver cells) and kidneys (tubular degeneration and karyomegaly of the corticomedullary renal tubular epithelium) of F0 males and females. In the pups at 0.60%, adjusted live birth weights for males and both sexes combined were significantly decreased ($p < 0.01$) as compared to control. The last control and high-dose litters of the continuous breeding assessment were raised to the age of sexual maturity for a further assessment of reproductive performance. In these F1 pups, body weights (both sexes) were significantly decreased at PND 4, and male offspring body weights were significantly ($p < 0.05$) less than controls at PND 74 (±10). It was reported that perinatal mortality (PNDs 0–21) was increased, with a 61.3% mortality rate for TCE-treated pups vs. a 28.3% mortality rate for control pups. Reproductive performance was not affected by treatment, and postmortem evaluations of the F1 adult mice revealed significant findings at 0.60% TCE that were consistent with those seen in the F0 adults and additionally demonstrated renal toxicity (i.e., elevated liver and kidney/adrenal weights and hepatic and renal histopathological lesions in both sexes) elevated testis and epididymis weights in males, and decreased sperm motility (18% less than control).

The F344 rat study continuous breeding phase demonstrated no evidence of treatment-related effects on the proportion of breeding pairs able to produce a litter, percentage of pups born alive, the sex of pups born alive, or absolute or adjusted pup weights (George et al., 1986). However, the number of live pups per litter was significantly ($p < 0.05$) decreased at 0.30 and 0.60% TCE, and a significant ($p < 0.01$) trend toward a dose-related decrease in the number of live litters per pair was observed; individual data were reported to indicate a progressive decrease in the number of breeding pairs in each treatment group producing third, fourth, and fifth litters. The crossover mating trial conducted in order to pursue this outcome demonstrated that the proportion of detected matings was significantly depressed ($p < 0.05$) in the mating pairs with TCE-treated partners compared to the control pairs. In the F0 adults at 0.60% TCE, postpartum dam body weights were significantly decreased ($p < 0.01$ or 0.05) in the continuous breeding phase and the crossover mating trials, and terminal body weights were significantly decreased ($p < 0.01$) for both male and female rats. Postmortem findings for F0 adults in the high-dose group included significantly increased absolute and body-weight-adjusted liver and kidney/adrenal weights in males, increased adjusted liver and kidney/adrenal weights in females, and significantly increased adjusted left testis/epididymal weights. Sperm assessment did not identify any effects on motility, concentration, or morphology, and histopathological examination was negative. The last control and high-dose litters of the continuous breeding

assessment were raised to the age of sexual maturity for assessment of open field behavior and reproductive performance. In these F1 pups at 0.60% TCE, body weights of male and females were significantly ($p < 0.05$ or 0.01, respectively) decreased at PNDs 4 and 14. By PND 21, pup weights in both sexes were significantly reduced in all treated groups, and this continued until termination (approximately PND 80). A tendency toward decreased postweaning survival (i.e., from PND 21 to PND 81 ± 10) was reported for F1 pups at the 0.15 and 0.60% levels. Open field testing revealed a significant ($p < 0.05$) dose-related trend toward an increase in the time required for male and female F1 weanling pups to cross the first grid in the testing device, suggesting an effect on the ability to react to a novel environment. Reproductive performance assessments conducted in this study phase were not affected by treatment. Postpartum F1 dam body weights were significantly decreased ($p < 0.05$ or 0.01) in all of the TCE-treated groups as compared to controls, as were terminal body weights for both adult F1 males and females. Postmortem evaluations of the F1 adult rats revealed significantly ($p < 0.01$) decreased left testis/epididymis weight at 0.60% TCE, and significantly ($p < 0.05$ or 0.01) increased adjusted mean liver weight in all treated groups for males and at 0.30 and 0.60% for females. Sperm assessments for F1 males revealed a significant increase ($p < 0.05$) in the percentage of abnormal sperm in the 0.30% TCE group, but no other adverse effects on sperm motility, concentration, or morphology were observed. As with the F0 adults, there were no adverse treatment-related findings revealed at histopathological assessment. The study authors concluded that the observed effects to TCE exposure in this study were primarily due to generalized toxicity and not to a specific effect on the reproductive system; however, based upon the overall toxicological profile for TCE, which demonstrates that the male reproductive system is a target for TCE exposures, this conclusion is not supported.

4.8.1.3. Discussion/Synthesis of Noncancer Reproductive Toxicity Findings

The human epidemiological findings and animal study evidence consistently indicate that TCE exposures can result in adverse reproductive outcomes. Although the epidemiological data may not always be robust or unequivocal, they demonstrate the potential for a wide range of exposure-related adverse outcomes on female and male reproduction. In animal studies, there is some evidence for female-specific reproductive toxicity; but there is strong and compelling evidence for adverse effects of TCE exposure on male reproductive system and function.

4.8.1.3.1. Female reproductive toxicity

Although few epidemiological studies have examined TCE exposure in relation to female reproductive function (see Table 4-88), the available studies provide evidence of decreased fertility, as measured by time to pregnancy (Sallmén et al., 1995) and effects on menstrual cycle patterns, including abnormal cycle length (ATSDR, 2001), amenorrhea (Sagawa et al., 1973; Zielinski, 1973), and menstrual ―disturbanc̈e (Bardodej and Vyskocil, 1956). In experimental

animals, the effects on female reproduction include evidence of reduced in vitro oocyte fertilizability in rats (Wu and Berger, 2007; Berger and Horner, 2003). However, in other studies that assessed reproductive outcome in female rodents (Cosby and Dukelow, 1992; George et al., 1986; George et al., 1985; Manson et al., 1984), there was no evidence of adverse effects of TCE exposure on female reproductive function. Overall, although the data are suggestive, there are inadequate data to make conclusions as to whether adverse effects on human female reproduction are caused by TCE.

Table 4-88. Summary of adverse female reproductive outcomes associated with TCE exposures

Finding	Species	References
Menstrual cycle disturbance	Human	ATSDR (2001)[a]
		Bardodej and Vyskocil (1956)
		Sagawa et al. (1973)
		Zielinski (1973)
Reduced fertility	Human[a]	Sallmén et al. (1995)
	Rat[b]	Berger and Horner (2003)
		Wu and Berger (2007)

[a]Not significant.
[b]In vitro oocyte fertilizability.

4.8.1.3.2. Male reproductive toxicity

Notably, the results of a number of studies in both humans and experimental animals have suggested that exposure to TCE can result in targeted male reproductive toxicity (see Table 4-89). The adverse effects that have been observed in both male humans and male animal models include altered sperm count, morphology, or motility (Kumar et al., 2001b; Veeramachaneni et al., 2001; Kumar et al., 2000a; Kumar et al., 2000b; Chia et al., 1996; Rasmussen et al., 1988; George et al., 1985; Land et al., 1981); decreased libido or copulatory behavior (Veeramachaneni et al., 2001; George et al., 1986; Zenick et al., 1984; Saihan et al., 1978; El Ghawabi et al., 1973; Bardodej and Vyskocil, 1956); alterations in serum hormone levels (Veeramachaneni et al., 2001; Kumar et al., 2000a; Goh et al., 1998; Chia et al., 1997); and reduced fertility (George et al., 1986). However, other studies in humans did not see evidence of altered sperm count or morphology (Rasmussen et al., 1988) or reduced fertility (Forkert et al., 2003; Sallmen et al., 1998), and some animal studies also did not identify altered sperm measures (Xu et al., 2004; Cosby and Dukelow, 1992; George et al., 1986; Zenick et al., 1984). Additional adverse effects observed in animals include histopathological lesions of the testes (Kumar et al., 2001b; Kumar et al., 2000b; George et al., 1986) or epididymides (Kan et al., 2007; Forkert et al., 2002) and altered in vitro sperm-oocyte binding and/or in vivo fertilization for TCE and/or its metabolites (DuTeaux et al., 2004a; Xu et al., 2004).

Table 4-89. Summary of adverse male reproductive outcomes associated with TCE exposures

Finding	Species	References
Testicular toxicity/pathology	Rat	George et al. (1986)
		Kumar et al. (2000b)
		Kumar et al. (2001b)
	Mouse	Kan et al. (2007)
Epididymal toxicity/pathology	Mouse	Forkert et al. (2002)
Decreased sperm quantity/quality	Human	Chia et al. (1996)
		Rasmussen et al. (1988)[a]
	Rat	Kumar et al. (2001b; 2000a; 2000b)
	Mouse	George et al. (1985)
		Land et al. (1981)
	Rabbit	Veeramachaneni et al. (2001)
Altered in vitro sperm-oocyte binding or in vivo fertilization	Rat	DuTeaux et al. (2004a)
	Mouse	Cosby and Dukelow (1992)[b]
		Xu et al. (2004)[b]
Altered sexual drive or function	Human	El Ghawabi et al. (1973)
		Saihan et al. (1978)[c]
		Bardodej and Vyskocil (1956)
	Rat	George et al. (1986)
		Zenick et al. (1984)
	Rabbit	Veeramachaneni et al. (2001)
Altered serum testosterone levels	Human	Chia et al. (1997)[d]
		Goh et al. (1998)[e]
	Rat	Kumar et al. (2000a)
	Rabbit	Veeramachaneni et al. (2001)
Reduced fertility	Rat	George et al. (1986)
Gynaecomastia	Human	Saihan et al. (1978)[c]

[a]Nonsignificant increase in percentage of two YFF in spermatozoa; no effect on sperm count or morphology.
[b]Observed with metabolite(s) of TCE only.
[c]Case study of one individual.
[d]Also observed altered levels of DHEAS, FSH, and SHBG.
[e]Also observed altered levels of SHBG.

In spite of the preponderance of studies demonstrating effects on sperm parameters, there is an absence of overwhelming evidence in the database of adverse effects of TCE on overall fertility in the rodent studies. That is not surprising, however, given the redundancy and efficiency of rodent reproductive capabilities. Nevertheless, the continuous breeding reproductive toxicity study in rats (George et al., 1986) did demonstrate a trend towards reproductive compromise (i.e., a progressive decrease in the number of breeding pairs producing third, fourth, and fifth litters).

It is noted that in the studies by George et al. (1986; George et al., 1985), adverse reproductive outcomes in male rats and mice were observed at the highest dose level tested (0.060% TCE in diet), which was also systemically toxic (i.e., demonstrating kidney toxicity and liver enzyme induction and toxicity, sometimes in conjunction with body weight deficits). Because of this, the study authors concluded that the observed reproductive toxicity was a secondary effect of generalized systemic toxicity; however, this conclusion is not supported by the overall toxicological profile of TCE, which provides significant evidence indicating that TCE is a reproductive toxicant.

4.8.1.3.2.1. The role of metabolism in male reproductive toxicity

There has been particular focus on evidence of exposure to male reproductive organs by TCE and/or its metabolites, as well as the role of TCE metabolites in the observed toxic effects.

In humans, a few studies demonstrating male reproductive toxicity have measured levels of TCE in the body. U-TCA was measured in men employed in an electronics factory, and adverse effects observed included abnormal sperm morphology and hyperzoospermia and altered serum hormone levels (Goh et al., 1998; Chia et al., 1997; Chia et al., 1996). U-TCA was also measured as a marker of exposure to TCE in men occupationally exposed to solvents, although this study did not report any adverse effects on fertility (Sallmen et al., 1998).

In the study in Long-Evans male rats by Zenick et al. (1984), blood and tissue levels of TCE, TCA, and TCOH were measured in three rats/group following 6 weeks of gavage treatment at 0, 10, 100, and 1,000 mg/kg-day. Additionally, the levels of TCE and metabolites were measured in seminal plugs recovered following copulation at week 5. Marked increases in TCE levels were observed only at 1,000 mg/kg-day, in blood, muscle, adrenals, and seminal plugs. It was reported that dose-related increases in TCA and TCOH concentrations were observed in the organs evaluated, notably including the reproductive organs (epididymis, vas deferens, testis, prostate, and seminal vesicle), thus creating a potential for interference with reproductive function.

This potential was explored further in a study by Forkert et al. (2002), in which male CD-1 mice were exposed by inhalation to 1,000 ppm TCE (6 hours/day, 5 days/week) for 4 consecutive weeks. Urine was obtained on days 4, 9, 14, and 19 of exposure and analyzed for concentrations of TCE and TCOH. Microsomal preparations from the liver, testis, and

epididymis were used for immunoblotting, determining *p*-nitrophenol hydroxylase and CYP2E1 activities, and evaluating the microsomal metabolism of TCE.

Subsequent studies conducted by the same laboratory (Forkert et al., 2003) evaluated the potential of the male reproductive tract to accumulate TCE and its metabolites including chloral, TCOH, TCA, and DCA. Human seminal fluid and urine samples from eight mechanics diagnosed with clinical infertility and exposed to TCE occupationally were analyzed. Urine samples from two of the eight subjects contained TCA and/or TCOH, suggesting that TCE exposure and/or metabolism was low during the time just prior to sample collection. TCE, chloral, and TCOH were detected in seminal fluid samples from all eight subjects, while TCA was found in one subject, and DCA was found in two subjects. Additionally, TCE and its metabolites were assessed in the epididymis and testis of CD-1 mice (4/group) exposed by inhalation (6 hours/day, 5 days/week) to 1,000 ppm TCE for 1, 2, and 4 weeks. TCE, chloral, and TCOH were found in the epididymis at all timepoints, although TCOH levels were increased significantly (tripled) at 4 weeks of exposure. This study showed that the metabolic disposition of TCE in humans is similar to that in mice, indicating that the murine model is appropriate for investigating the effects of TCE-induced toxicity in the male reproductive system. These studies provide support for the premise that TCE is metabolized in the human reproductive tract, mainly in the epididymis, resulting in the production of metabolites that cause damage to the epididymal epithelium and affect the normal development of sperm.

Immunohistochemical experiments (Forkert et al., 2002) confirmed the presence of CYP2E1 in the epididymis and testis of mice; it was found to be localized in the testicular Leydig cells and the epididymal epithelium. Similar results were obtained with the immunohistochemical evaluation of human and primate tissue samples. CYP2E1 has been previously shown by Lipscomb et al. (1998a) to be the predominant CYP enzyme catalyzing the hepatic metabolism of TCE in both animals and rodents. These findings support the role of CYP2E1 in TCE metabolism in the male reproductive tract of humans, primates, and mice.

4.8.1.3.2.2. Mode of action for male reproductive toxicity

A number of studies have been conducted to attempt to characterize various aspects of the mode of action for observed male reproductive outcomes.

Studies by Kumar et al. (2001b; 2000a) suggest that perturbation of testosterone biosynthesis may have some role in testicular toxicity and altered sperm measures. Significant decreases in the activity of G6PDH and accumulation of cholesterol are suggestive of an alteration in testicular steroid biosynthesis. Increased testicular lipids, including cholesterol, have been noted for other testicular toxicants such as lead (Saxena et al., 1987), triethylenemelamine (Johnson et al., 1967), and quinalphos (Ray et al., 1987), in association with testicular degeneration and impaired spermatogenesis. Since testosterone has been shown to be essential for the progression of spermatogenesis (O'Donnell et al., 1994), alterations in

testosterone production could be a key event in male reproductive dysfunction following TCE exposure. Additionally, the observed TCE-related reduction of 17-β-HSD, which is involved in the conversion of androstenedione to testosterone, has also been associated with male reproductive insufficiency following exposure to phthalate esters (Srivastava, 1991), quinalphos (Ray et al., 1987), and lead (Saxena et al., 1987). Reductions in SDH, which are primarily associated with the pachytene spermatocyte maturation of germinal epithelium, have been shown to be associated with depletion of germ cells (Chapin et al., 1982; Mills and Means, 1972), and the activity of G6PDH is greatest in premeiotic germ cells and Leydig cells of the interstitium (Blackshaw, 1970). The increased GT and glucuronidase observed following TCE exposures appear to be indicative of impaired Sertoli cell function (Sherins and Hodgen, 1976; Hodgen and Sherins, 1973). Based upon the conclusions of these studies, Kumar et al. (2001b) hypothesized that the reduced activity of G6PDH and SDH in testes of TCE-exposed male rats is indicative of the depletion of germ cells, spermatogenic arrest, and impaired function of the Sertoli cells and Leydig cells of the interstitium.

In the series of experiments by DuTeaux et al. (2004a; 2003), protein dichloroacetyl adducts were found in the corpus epididymis and in the efferent ducts of rats administered TCE; this effect was also demonstrated following in vitro exposure of reproductive tissues to TCE. Oxidized proteins were detected on the surface of spermatozoa from TCE-treated rats in a dose-response pattern; this was confirmed using a Western blotting technique. Soluble (but not mitochondrial) cysteine-conjugate β-lyase was detected in the epididymis and efferent ducts of treated rats. Following a single i.p. injection of DCVC, no dichloroacetylated protein adducts were detected in the epididymis and efferent ducts. The presence of CYP2E1 was found in epididymis and efferent ducts, suggesting a role of CYP–dependent metabolism in adduct formation. An in vitro assay was used to demonstrate that epididymal and efferent duct microsomes are capable of metabolizing TCE; TCE metabolism in the efferent ducts was found to be inhibited by anti-CYP2E1 antibody. Lipid peroxidation in sperm, presumably initiated by free radicals, was increased in a significant ($p < 0.005$) dose-dependent manner after TCE exposure.

Overall, it has been suggested (DuTeaux et al., 2004a) that reproductive organ toxicities observed following TCE exposure are initiated by metabolic bioactivation, leading to subsequent protein adduct formation. It has been hypothesized that epoxide hydrolases in the rat epididymis may play a role in the biological activation of metabolites (DuTeaux et al., 2004b). Disruption of colony stimulating factor and of macrophage development may also play a role in sperm production (Cohen et al., 1999), and thus, may be another route through which immune-related effects of TCE may operate. In addition, the potential for epigenetic changes, through which heritable changes in gene mutations occur without changes in DNA sequencing, should also be considered in the evaluation of transgenerational effects (Guerrero-Bosagna and Skinner, 2009).

4.8.1.3.3. Summary of noncancer reproductive toxicity

The toxicological database for TCE includes a number of studies that demonstrate adverse effects on the integrity and function of the reproductive system in females and males. Both the epidemiological and animal toxicology databases provide suggestive, but limited, evidence of adverse outcomes to female reproductive outcomes. However, much more extensive evidence exists in support of an association between TCE exposures and male reproductive toxicity. The available epidemiological data and case reports that associate TCE with adverse effects on male reproductive function are limited in size and provide little quantitative dose data (Lamb and Hentz, 2006). However, the animal data provide extensive evidence of TCE-related male reproductive toxicity. Strengths of the database include the presence of both functional and structural outcomes, similarities in adverse treatment-related effects observed in multiple species, and evidence that metabolism of TCE in male reproductive tract tissues is associated with adverse effects on sperm measures in both humans and animals (suggesting that the murine model is appropriate for extrapolation to human health risk assessment). Additionally, some aspects of a putative mode of action (e.g., perturbations in testosterone biosynthesis) appear to have some commonalities between humans and animals.

4.8.2. Cancers of the Reproductive System

The effects of TCE on cancers of the reproductive system have been examined for males and females in both epidemiological and experimental animal studies. The epidemiological literature includes data on prostate in males and cancers of the breast and cervix in females. The experimental animal literature includes data on prostate and testes in male rodents; and uterus, ovary, mammary gland, vulva, and genital tract in female rodents. The evidence for these cancers is generally not robust.

4.8.2.1. Human Data

The epidemiologic evidence on TCE and cancer of the prostate, breast, and cervix is from cohort and geographic-based studies. Two additional case-control studies of prostate cancer in males are nested within cohorts (Krishnadasan et al., 2007; Greenland et al., 1994). The nested case-control studies are identified in Tables 4-90 through 4-92 with cohort studies given their source population for case and control identification. One population-based, case-control study examined on TCE exposure and prostate (Siemiatycki, 1991); however, no population case-control studies on breast or cervical cancers and TCE exposure were found in the peer-reviewed literature.

Table 4-90. Summary of human studies on TCE exposure and prostate cancer

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Krishnadasan et al. (2007)
	Low/moderate TCE score	1.3 (0.81, 2.1)[a,b]	90	
	High TCE score	2.1 (1.2, 3.9)[a,b]	45	
	p for trend	0.02		
	Low/moderate TCE score	1.3 (0.81, 2.1)[a,c]		
	High TCE score	2.4 (1.3, 4.4)[a,c]		
	p for trend	0.01		
All employees at electronics factory (Taiwan)		0.14 (0.00, 0.76)[d]	1	Chang et al. (2005)
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. (2003)
	Any exposure	0.9 (0.79, 1.08)	163	
Biologically-monitored Danish workers				Hansen et al. (2001)
	Any TCE exposure, females	0.6 (0,2, 1.3)	6	
Aircraft maintenance workers (Hill Air Force Base, Utah)				
	TCE subcohort	Not reported	158	Blair et al. (1998)
	Cumulative exposure			
	0	1.0[e]		
	<5 ppm-yr	1.1 (0.7, 1.6)	64	
	5–25 ppm-yr	1.0 (0.6, 1.6)	38	
	>25 ppm-yr	1.2 (0.8, 1.8)	56	
	TCE subcohort	1.2 (0.92, 1.76)	116	Radican et al. (2008)
	Cumulative exposure			
	0	1.0[e]		
	<5 ppm-yr	1.03 (0.65, 1.62)	41	
	5–25 ppm-yr	1.33 (0.82, 2.15)	42	
	>25 ppm-yr	1.31 (0.84, 2.06)	43	
Biologically-monitored Finnish workers		1.38 (0.73, 2.35)	13	Anttila et al. (1995)
	Mean air-TCE (Ikeda extrapolation			
	<6 ppm	1.43 (0.62, 2.82)	8	
	6+ ppm	0.68 (0.08, 2.44)	2	

Table 4-90. Summary of human studies on TCE exposure and prostate cancer (continued)

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	Exposed workers	Not reported		
Biologically-monitored Swedish workers		1.25 (0.84, 1.84)	26	Axelson et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported		Sinks et al. (1992)
Cohort and PMR-mortality				
Aerospace workers (Rocketdyne)				Boice et al. (2006b)
	Any TCE (utility/eng flush)	0.82 (0.36, 1.62)	8	
View-Master employees		1.69 (0.68, 3.48)[f]	8	ATSDR (2004a)
All employees at electronics factory (Taiwan)		Not reported	0	Chang et al. (2003)
Fernald workers				Ritz (1999a)
	Any TCE exposure	Not reported		
	Light TCE exposure, >2-yr duration	0.91 (0.38, 2.18)[e,g]	10	
	Moderate TCE exposure, >2-yr duration	1.44 (0.19, 11.4)[e,g]	1	
Aerospace workers (Lockheed)				Boice et al. (1999)
	Routine exposure to TCE	1.31 (0.52, 2.69)	7	
	Routine-intermittent	Not reported		
Aerospace workers (Hughes)				Morgan et al. (2000, 1998)
	TCE subcohort	1.18 (0.73, 1.80)	21	
	Low intensity (<50 ppm)	1.03 (0.51, 1.84)	7	
	High intensity (>50 ppm)	0.47 (0.15, 1.11)	14	
	TCE subcohort (Cox Analysis)			
	Never exposed	1.00[e]		
	Ever exposed	1.58 (0.96, 2.62)[h]		
	Peak			
	No/low	1.00[e]		
	Medium/high	1.39 (0.80, 2.41)[h]		
	Cumulative			
	Referent	1.00[e]		
	Low	1.72 (0.78, 3.80)[h]		
	High	1.53 (0.85, 2.75)[h]		

Table 4-90. Summary of human studies on TCE exposure and prostate cancer (continued)

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
	TCE subcohort	1.1 (0.6, 1.8)	54	
	Cumulative exposure			
	0	1.0[e]		
	<5 ppm-yr	0.9 (0.5, 1.8)	19	
	5–25 ppm-yr	1.0 (0.5, 2.1)	13	
	>25 ppm-yr	1.3 (0.7, 2.4)	22	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	TCE exposed workers	Not reported		
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		0.82 (0.46, 1.46)[a]	58	Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported	0	Sinks et al. (1992)
U.S. Coast Guard employee				Blair et al. (1989)
	Marine inspectors	1.06 (0.51, 1.95)	10	
	Noninspectors	0.57 (0.15, 1.45)	7	
Aircraft manufacturing plant employees (Italy)				Costa et al. (1989)
Aircraft manufacturing plant employees (San Diego, California)		0.93 (0.60, 1.37)	25	Garabrant et al. (1988)
Lamp manufacturing workers (GE)		1.56 (0.63, 3.22)	7	Shannon et al. (1988)
Rubber workers				Wilcosky et al. (1984)
	Any TCE exposure	0.62 (not reported)	3	
Case-control studies				
Population of Montreal, Canada				Siemiatycki (1991)
	Any TCE exposure	1.1 (0.6, 2.1)[i]	11	
	Substantial TCE exposure	1.8 (0.8, 4.0)[i]	7	
Geographic-based studies				
Residents in two study areas in Endicott, New York		1.05 (0.75, 1.43)	40	ATSDR (2006a)
Residents of 13 census tracts in Redlands, California		1.11 (0.98, 1.25)[j]	483	Morgan and Cassady (2002)
Finnish residents				Vartiainen et al. (1993)
	Residents of Hausjarvi	Not reported		
	Residents of Huttula	Not reported		

[a]OR from nested case-control study.
[b]OR, zero lag.
[c]OR, 20-year lag.
[d]Chang et al. (2005) presents SIRs for a category site of all cancers of male genital organs.
[e]Internal referents, workers without TCE exposure.
[f]PMR.
[g]Analysis for >2 years exposure duration and a lagged TCE exposure period of 15 years.
[h]Risk ratio from Cox Proportional Hazard Analysis, stratified by age and sex, from Environmental Health Strategies (1997) Final Report to Hughes Corporation (Communication from Paul A. Cammer, President, Trichloroethylene Issues Group to Cheryl Siegel Scott, U.S. EPA, December 22, 1997).
[i]90% CI.
[j]99% CI.

Table 4-91. Summary of human studies on TCE exposure and breast cancer

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al. (2005)
	Any TCE exposure	Not reported		
	Low cumulative TCE score			
	Medium cumulative TCE score			
	High TCE score			
	p for trend			
All employees at electronics factory (Taiwan)				
	Females	1.09 (0.96, 1.22)[a]	286	Sung et al. (2007)
	Females	1.19 (1.03, 1.36)	215	Chang et al. (2005)
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. (2003)
	Any exposure, males	0.5 (0.06, 1.90)	2	
	Any exposure, females	1.1 (0.89, 1.24)	145	
Biologically-monitored Danish workers				Hansen et al. (2001)
	Any TCE exposure, males		0 (0.2 exp)	
	Any TCE exposure, females	0.9 (0.2, 2.3)	4	
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
	TCE subcohort	Not reported	34	
	Females, cumulative exposure			
	0	1.0[b]		
	<5 ppm-yr	0.3 (0.1, 1.4)	20	
	5–25 ppm-yr	0.4 (0.1, 2.9)	11	
	>25 ppm-yr	0.4 (0.4, 1.2)	3	
Biologically-monitored Finnish workers		Not reported		Anttila et al. (1995)
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	Exposed workers	Not reported		
Biologically-monitored Swedish workers		Not reported		Axelson et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported		Sinks et al. (1992)

Table 4-91. Summary of human studies on TCE exposure and breast cancer (continued)

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
Cohort and PMR-mortality				
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	Not reported		Boice et al. (2006b)
	Any exposure to TCE	Not reported		Zhao et al. (2005)
	Low cumulative TCE score	Not reported		
	Medium cumulative TCE score	Not reported		
	High TCE score	Not reported		
	p for trend			
View-Master employees				ATSDR (2004a)
	Males		0 (0.05 exp)	
	Females	1.02 (0.67, 1.49)[c]	27	
Fernald workers				Ritz (1999a)
	Any TCE exposure	Not reported		
	Light TCE exposure, >2-yr duration	Not reported		
	Moderate TCE exposure, >2-yr duration	Not reported		
Aerospace workers (Lockheed)				Boice et al. (1999)
	Routine exposure to TCE	1.31 (0.52, 2.69)[d]	7	
	Routine-intermittent[a]	Not reported		
Aerospace workers (Hughes)				Morgan et al. (1998)
	TCE subcohort	0.75 (0.43, 1.22)[d]	16	
	Low intensity (<50 ppm)	1.03 (0.51, 1.84)[d]	11	
	High intensity (>50 ppm)	0.47 (0.15, 1.11)[d]	5	
	TCE subcohort (Cox Analysis)			
	Never exposed	1.00[d]	NR	
	Ever exposed	0.94 (0.51, 1.75)[d,e]	NR	
	Peak			
	No/low	1.00[d]		
	Medium/high	1.14 (0.48, 2.70)[d,e]	NR	
	Cumulative			
	Referent	1.00[b]		
	Low	1.20 (0.60, 2.40)[d,e]	NR	
	High	0.65 (0.25, 1.69)[d,e]	NR	

Table 4-91. Summary of human studies on TCE exposure and breast cancer (continued)

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
Aircraft maintenance workers (Hill Air Force Base, Utah)				
	TCE subcohort (females)	2.0 (0.9, 4.6)	20	Blair et al. (1998)
	Females, cumulative exposure			
	0	1.0[b]		
	<5 ppm-yr	2.4 (1.1, 5.2)	10	
	5–25 ppm-yr	1.2 (0.3, 5.4)	21	
	>25 ppm-yr	1.4 (0.6, 3.2)	8	
	Low level intermittent exposure	3.1 (1.5, 6.2)	15	
	Low level continuous exposure	3.4 (1.4, 8.0)	8	
	Frequent peaks	1.4 (0.7, 3.2)	10	
	TCE subcohort (females)	1.23 (0.73, 2.06)	26	Radican et al. (2008)
	Females, cumulative exposure			
	0	1.0[b]		
	<5 ppm-yr	1.57 (0.81, 3.04)	12	
	5–25 ppm-yr	1.01 (0.31, 3.30)	3	
	>25 ppm-yr	1.05 (0.53, 2.07)	11	
	Low level intermittent exposure	1.92 (1.08, 3.43)	18	
	Low level continuous exposure	1.71 (0.79, 3.71)	8	
	Frequent peaks	1.08 (0.57, 2.02)	14	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	TCE exposed workers	Not examined		
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		Not reported		Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported	0	Sinks et al. (1992)
U.S. Coast Guard employees				Blair et al. (1989)
	Marine inspectors	Not reported		
	Noninspectors	Not reported		
Aircraft manufacturing plant employees (Italy)		Not reported[f]		Costa et al. (1989)
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)
	All subjects, females	0.81 (0.52, 1.48)[d]	16	
Lamp manufacturing workers (GE)				Shannon et al. (1988)
	Coil/wire drawing	2.04 (0.88, 4.02)	8	
	Other areas	0.97 (0.57, 1.66)	13	
Case-control studies				
Population of Montreal, Canada				Siemiatycki (1991)
	Any TCE exposure	Not reported		
	Substantial TCE exposure	Not reported		

Table 4-91. Summary of human studies on TCE exposure and breast cancer (continued)

Studies	Exposure group	RR (95% CI)	Number of observable events	Reference
Geographic-based studies				
Residents in two study areas in Endicott, New York		0.88 (0.65, 1.18)	46	ATSDR (2006a)
Residents of 13 census tracts in Redlands, California		1.09 (0.97, 1.21)	536	Morgan and Cassady (2002)
Finnish residents				Vartiainen et al. (1993)
	Residents of Hausjarvi	Not reported		
	Residents of Huttula	Not reported		

[a]15-year lag.
[b]Internal referents, workers not exposed to TCE.
[c]PMR.
[d]In Garabrant et al. (1988), Morgan et al. (1998), and Boice et al. (1999), breast cancer risk is for males and females combined (ICD-9, 174, 175).
[e]Risk ratio from Cox Proportional Hazard Analysis, stratified by age and sex, from Environmental Health Strategies (1997) Final Report to Hughes Corporation c,c.CEa.
[f]The cohort of Blair et al. (1989) and Costa et al. (1989) are composed of males only.

NR = not reported

Table 4-92. Summary of human studies on TCE exposure and cervical cancer

Exposure group		RR (95% CI)	Number of observable events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al. (2005)
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	Not reported		
	Medium cumulative TCE score			
	High TCE score			
	p for trend			
All employees at electronics factory (Taiwan)		0.96 (0.86, 1.22)[a]	337	Sung et al. (2007)
Danish blue-collar worker w/TCE exposure				Raaschou-Nielsen et al. (2003)
	Any exposure	1.9 (1.42, 2.37)	62	
	Exposure lag time			
	20 yrs	1.5 (0.7, 2.9)	9	
	Employment duration			
	<1 yr	2.5 (1.7, 3.5)	30	
	1–4.9 yrs	1.6 (1.0, 2.4)	22	
	≥5 yrs	1.3 (0.6, 2.4)	10	
Biologically-monitored Danish workers				Hansen et al. (2001)
	Any TCE exposure	3.8 (1.0, 9.8)	4	
	Cumulative exposure (Ikeda)			
	<17 ppm-yr	2.9 (0.04, 16)	1	
	≥17 ppm-yr	2.6 (0.03, 14)	1	
	Mean concentration (Ikeda)			
	<4 ppm	3.4 (0.4, 12)	2	
	4+ ppm	4.3 (0.5, 16)	2	
	Employment duration			
	<6.25 yrs	3.8 (0.1, 21)	1	
	≥6.25 yrs	2.1 (0.03, 12)	1	
Aircraft maintenance workers from Hill Air Force Base, Utah				Blair et al. (1998)
	TCE subcohort	Not reported		
	Cumulative exposure	Not reported		

Table 4-92. Summary of human studies on TCE exposure and cervical cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Biologically-monitored Finnish workers				Anttila et al. (1995)
	All subjects	2.42 (1.05, 4.77)	8	
	Mean air-TCE (Ikeda extrapolation)			
	<6 ppm	1.86 (0.38, 5.45)	3	
	6+ ppm	4.35 (1.41, 10.1)	5	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	Exposed workers	Not reported		
Biologically-monitored Swedish workers				Axelson et al. (1994)
	Any TCE exposure	Not reported		
Cardboard manufacturing workers, Atlanta area, Georgia				Sinks et al. (1992)
	All subjects	Not reported		
Cohort studies-mortality				
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	Not reported		Boice et al. (2006b)
	Any exposure to TCE	Not reported		Zhao et al. (2005)
View-Master employees				ATSDR (2004a)
	Females	1.77 (0.57, 4.12)[b]	5	
United States uranium-processing workers (Fernald, Ohio)				Ritz (1999a)
	Any TCE exposure	Not reported		
	Light TCE exposure, >2-yr duration	Not reported		
	Moderate TCE exposure, >2-yr duration	Not reported		
Aerospace workers (Lockheed)				Boice et al. (1999)
	Routine exposure	-- (0.00, 5.47)	0	
	Routine-intermittent	Not reported		
Aerospace workers (Hughes)				Morgan et al. (1998)
	TCE subcohort	(0.00, 1.07)	0 (3.5 exp)	
	Low intensity (<50 ppm)		0 (1.91 exp)	
	High intensity (>50 ppm)		0 (1.54 exp)	

Table 4-92. Summary of human studies on TCE exposure and cervical cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Aircraft maintenance workers (Hill Air Force Base, Utah)				
	TCE subcohort	1.8 (0.5, 6.5)[c]	5	Blair et al. (1998)
	Cumulative exposure			
	0	1.0[c]		
	<5 ppm-yr	0.9 (0.1, 8.3)	1	
	5–25 ppm-yr		0	
	>25 ppm-yr	3.0 (0.8, 11.7)	4	
	TCE subcohort	1.67 (0.54, 5.22)	6	Radican et al. (2008)
	Cumulative exposure			
	0	1.0[c]		
	<5 ppm-yr	0.76 (0.09, 6.35)	1	
	5–25 ppm-yr		0	
	>25 ppm-yr	2.83 (0.86, 9.33)	5	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	TCE exposed workers	Not reported		
	Unexposed workers	Not reported		
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		Not examined[d]		Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia		Not reported		Sinks et al. (1992)
U.S. Coast Guard employees		Not reported[e]		Blair et al. (1989)
Aircraft manufacturing plant employees (Italy)		Not reported[e]		Costa et al. (1989)
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)
	All subjects	0.61 (0.25, 1.26)[f]	7	
Lamp manufacturing workers (GE)				Shannon et al. (1988)
	Coil/wire drawing	1.05 (0.03, 5.86)	1	
	Other areas	1.16 (0.32, 2.97)	4	

Table 4-92. Summary of human studies on TCE exposure and cervical cancer (continued)

Exposure group	RR (95% CI)	Number of observable events	Reference
Case-control studies			
Geographic-based studies			
Residents in two study areas in Endicott, New York	1.06 (0.29, 2.71)	<6	ATSDR (2006a)
Residents in Texas			Coyle et al. (2005)
Counties reporting any air TCE release	66.4[g]		
Countries not reporting any air TCE release	60.8[g]		
Residents of 13 census tracts in Redlands, California	0.65 (0.38, 1.02)	29	Morgan and Cassady (2002)
Finnish residents			Vartiainen et al. (1993)
Residents of Hausjarvi	Not reported		
Residents of Huttula	Not reported		

[a]SIR for females in Sung et al. (2007) reflects a 15-year lag period.
[b]PMR.
[c]Internal referents, workers not exposed to TCE.
[d]Nested case-control analysis.
[e]Males only in cohort.
[f]SMR is for cancer of the genital organs (cervix, uterus, endometrium, etc.).
[g]Median annual age-adjusted breast cancer rate (1995–2000).

4.8.2.1.1. Prostate cancer

Sixteen cohort or PMR studies, two nested case-control, one population case-control, and two geographic-based studies present RR estimates for prostate cancer (Radican et al., 2008; Krishnadasan et al., 2007; ATSDR, 2006a; Boice et al., 2006b; Chang et al., 2005; ATSDR, 2004a; Chang et al., 2003; Raaschou-Nielsen et al., 2003; Morgan and Cassady, 2002; Hansen et al., 2001; Boice et al., 1999; Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991; Blair et al., 1989; Garabrant et al., 1988; Shannon et al., 1988; Wilcosky et al., 1984). Three small cohort studies (Henschler et al., 1995; Sinks et al., 1992; Costa et al., 1989), one multiple-site population case-control (Siemiatycki, 1991), and one geographic-based study (Vartiainen et al., 1993) do not report estimates for prostate cancer in their published papers. Twelve of the 19 studies with prostate cancer RR estimates had high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis (Radican et al., 2008; Krishnadasan et al., 2007; Boice et al., 2006b; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Morgan et al., 2000; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991). Krishnadasan et al. (2007), in their nested case-control study of prostate cancer, observed a twofold OR estimate with high cumulative TCE exposure score (2.4, 95% CI: 1.3, 4.4, 20-year lagged exposure) and an increasing positive relationship between prostate

cancer incidence and TCE cumulative exposure score ($p = 0.02$). TCE exposure was positively correlated with several other occupational exposures, and Krishnadasan et al. (2007) adjusted for possible confounding from all other chemical exposures as well as age at diagnosis, occupational physical activity, and SES status in statistical analyses. RR estimates in studies other than Krishnadasan et al. (2007) were >1.0 for overall TCE exposure [1.8, 95% CI: 0.8, 4.0 (Siemiatycki, 1991); 1.1, 95% CI: 0.6, 1.8 (Blair et al., 1998) and 1.20, 95% CI: 0.92, 1.76, with an additional 10-year follow-up (Radican et al., 2008); 1.58, 95% CI: 0.96, 2.62 (Morgan et al., 2000, 1998; EHS, 1997); 1.3, 95% CI: 0.52, 2.69 (Boice et al., 1999); 1.38, 95% CI: 0.73, 2.35 (Anttila et al., 1995)] and prostate cancer risks did not appear to increase with increasing exposure. Four studies observed RR estimates below 1.0 for overall TCE exposure (0.93, 95% CI: 0.60, 1.37 (Garabrant et al., 1988); 0.6, 95% CI: 0.2, 1.3 (Hansen et al., 2001); 0.9, 95% CI: 0.79, 1.08 (Raaschou-Nielsen et al., 2003); 0.82, 95% CI: 0.36, 1.62 (Boice et al., 2006b), and are not considered inconsistent because alternative explanations are possible and included observations are based on few subjects, lowering statistical power, or to poorer exposure assessment approaches that may result in a higher likelihood of exposure misclassification.

Seven other cohort, PMR, and geographic-based studies were given less weight in the analysis because of their lesser likelihood of TCE exposure and other study design limitations that would decrease statistical power and study sensitivity (ATSDR, 2006a; Chang et al., 2005; ATSDR, 2004a; Morgan and Cassady, 2002; Blair et al., 1989; Shannon et al., 1988; Wilcosky et al., 1984). Chang et al. (2005) observed a statistically significant deficit in prostate cancer risk, based on one case, and an insensitive exposure assessment (0.14, 95% CI: 0.00, 0.76). Relative risks in the other five studies ranged from 0.62 (CI not presented in paper) (Wilcosky et al., 1984) to 1.11 (95% CI: 0.98, 1.25) (Morgan and Cassady, 2002).

Risk factors for prostate cancer include age, family history of prostate cancer, and ethnicity as causal with inadequate evidence for a relationship with smoking or alcohol (Wigle et al., 2008). All studies except Krishnadasan et al. (2007) were not able to adjust for possible confounding from other chemical exposures in the work environment. None of the studies including Krishnadasan et al. (2007) accounted for other well-established nonoccupational risk factors for prostate cancer such as race, prostate cancer screening, and family history. There is limited evidence that physical activity may provide a protective effect for prostate cancer (Wigle et al., 2008). Krishnadasan et al. (2008) examined the effect of physical activity in the Rocketdyne aerospace cohort (Krishnadasan et al., 2007; Zhao et al., 2005). Their finding of a protective effect with high physical activity (0.55, 95% CI: 0.32, 0.95, p trend = 0.04) after control for TCE exposure provides additional evidence (Krishnadasan et al., 2008) and suggests that underlying risk may be obscured in studies lacking adjustment for physical activity.

4.8.2.1.2. Breast cancer

Fifteen studies of TCE exposure reported findings on breast cancer in males and females combined (Boice et al., 1999; Greenland et al., 1994; Garabrant et al., 1988), in males and females, separately (Clapp and Hoffman, 2008; ATSDR, 2004a; Raaschou-Nielsen et al., 2003; Hansen et al., 2001), or in females only (Radican et al., 2008; Sung et al., 2007; ATSDR, 2006a; Chang et al., 2005; Coyle et al., 2005; Blair et al., 1998; Morgan et al., 1998; Shannon et al., 1988). Six studies have high likelihood of TCE exposure in individual study subjects and met, to a sufficient degree, the standards of epidemiologic design and analysis (Radican et al., 2008; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998). Four studies with risk estimates for other cancer sites did not report risk estimates for breast cancer (Boice et al., 2006b; Anttila et al., 1995; Axelson et al., 1994; Siemiatycki, 1991). No case-control studies were found on TCE exposure, although several studies examined occupational title or organic solvent as a class (Ji et al., 2008; Rennix et al., 2005; Band et al., 2000; Weiderpass et al., 1999). While association is seen with occupational title or industry and breast cancer [employment in aircraft and aircraft part industry, 2.48, 95% CI: 1.14, 5.39 (Band et al., 2000); solvent user: 1.48, 95% CI: 1.03, 2.12 (Rennix et al., 2005)], TCE exposure is not uniquely identified. The two studies suggest that an association between organic solvents and female breast cancer needs further investigation of possible risk factors.

Relative risk estimates in the five studies in which there is a high likelihood of TCE exposure in individual study subjects and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review ranged from 0.75 (0.43, 1.22) (females and males; (Morgan et al., 1998)) to 2.0 (0.9, 4.6) (mortality in females; (Blair et al., 1998)). Blair et al. (1998) additionally observed stronger risk estimates for breast cancer mortality among females with low-level, intermittent (3.1, 95% CI: 1.5, 6.2) and low-level, continuous (3.4, 95% CI: 1.4, 8.0) TCE exposures, but not with frequent peaks (1.4, 95% CI: 0.7, 3.2). A similar pattern of risks was also observed by Radican et al. (2008) who studied mortality in this cohort and adding 10 years of follow-up, although the magnitude of breast cancer risk in females was lower than that observed in Blair et al. (1998). Risk estimates did not appear to increase with increasing cumulative exposure in the two studies that included exposure-response analyses (Blair et al., 1998; Morgan et al., 1998). None of these five studies reported a statistically significant deficit in breast cancer and CIs on RRs estimates included 1.0 (no risk). Few female subjects in these studies appear to have high TCE exposure. For example, Blair et al. (1998) identified 8 of the 28 breast cancer deaths and 3 of the 34 breast cancer cases with high cumulative exposure.

Relative risk estimates in six studies of lower likelihood TCE exposure and other design deficiencies ranged from 0.81 (95% CI: 0.52, 1.48) (Garabrant et al., 1988) to 1.19 (1.03, 1.36) (Chang et al., 2005). These studies lack a quantitative surrogate for TCE exposure to individual subjects and instead classify all subjects as —potentially exposed," with resulting large dilution of

actual risk and decreased sensitivity (Sung et al., 2007; ATSDR, 2006a; NRC, 2006; Chang et al., 2005; Morgan and Cassady, 2002; Garabrant et al., 1988; Shannon et al., 1988).

Four studies reported on male breast cancer separately (Clapp and Hoffman, 2008; ATSDR, 2004a; Raaschou-Nielsen et al., 2003; Hansen et al., 2001) and a total of three cases were observed. Breast cancer in men is a rare disease and is best studied using a case-control approach (Weiss et al., 2005). Reports exist of male breast cancer among former residents of Camp Lejuene (ATSDR, 2010, 2007b). Further assessment of TCE exposure and male breast cancer is warranted.

Overall, the epidemiologic studies on TCE exposure and breast cancer are quite limited in statistical power; observations are based on few breast cancer cases or on inferior TCE exposure assessment in studies with large numbers of observed cases. Additionally, adjustment for nonoccupational breast cancer risk factors is less likely in cohort and geographic-based studies given their use of employment and public records. Breast cancer mortality observations in Blair et al. (1998) and further follow-up of this cohort by Radican et al. (2008) of an elevated risk with overall TCE exposure, particularly low-level intermittent and continuous TCE exposure, provide evidence of an association with TCE. No other study with high likelihood of TCE exposure in individual study subjects reported a statistically significant association with breast cancer, although few observed cases leading to lower statistical power or examination of risk for males and females combined are alternative explanations for the null observations in these studies. Both Chang et al. (2005) and Sung et al. (2007), two overlapping studies of female electronics workers exposed to TCE, perchloroethylene, and mixed solvents, reported association with breast cancer incidence, with breast cancer risk in Chang et al. (2005) appearing to increase with employment duration. Both studies, in addition to association provided by studies of exposure to broader category of organic solvents (Rennix et al., 2005; Band et al., 2000), support Blair et al. (1998) and Radican et al. (2008), although the lack of exposure assessment is an uncertainty. The epidemiologic evidence is limited for examining TCE and breast cancer, and while these studies do not provide any strong evidence for association with TCE exposure, they in turn do not provide evidence of an absence of association.

4.8.2.1.3. Cervical cancer

Eleven cohort or PMR studies and two geographic-based studies present RR estimates (Radican et al., 2008; Sung et al., 2007; ATSDR, 2006a, 2004a; Raaschou-Nielsen et al., 2003; Morgan and Cassady, 2002; Hansen et al., 2001; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Garabrant et al., 1988; Shannon et al., 1988). Seven of these studies had high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis (Radican et al., 2008; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995). Three small cohort studies (Henschler et al.,

1995; Sinks et al., 1992; Costa et al., 1989) as well as three studies with high likelihood of TCE exposure in individual study subjects (Boice et al., 2006b; Zhao et al., 2005; Axelson et al., 1994) did not present RR estimates for cervical cancer. Additionally, one population case-control and one geographic study of several site-specific cancers did not present information on cervical cancer (Vartiainen et al., 1993; Siemiatycki, 1991).

Five studies with high likelihood of TCE exposure in individual study subjects and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review observed elevated risk for cervical cancer and overall TCE exposure [2.42, 95% CI: 1.05, 4.77 (Anttila et al., 1995); 1.8, 95% CI: 0.5, 6.5 (Blair et al., 1998) that changed little with an additional 10 years follow-up, 1.67, 95% CI: 0.54, 5.22 (Radican et al., 2008); 3.8, 95% CI: 1.0, 9.8 (Hansen et al., 2001); 1.9, 95% CI: 1.42, 2.37 (Raaschou-Nielsen et al., 2003)]. The observations of a three- to fourfold elevated cervical cancer risk with high mean TCE exposure compared to subjects in the low exposure category [6+ ppm: 4.35, 95% CI: 1.41, 10.1 (Anttila et al., 1995); 4+ ppm: 4.3, 95% CI: 0.5, 16 (Hansen et al., 2001)] or with high cumulative TCE exposure (0.25-ppm year: 3.0, 95% CI: 0.8, 11.7 (Blair et al., 1998), 2.83, 95% CI: 0.86, 9.33 (Radican et al., 2008)) provide additional support for association with TCE. Cervical cancer risk was lowest for subjects in the high-exposure duration category (Raaschou-Nielsen et al., 2003; Hansen et al., 2001); however, duration of employment is a poor exposure metric given that subjects may have differing exposure intensity with similar exposure duration (NRC, 2006). No deaths due to cervical cancer were observed in two other studies (Boice et al., 1999; Morgan et al., 1998); less than four deaths were expected, suggesting that these cohorts contained few female subjects with TCE exposure.

Human papilloma virus and low SES status are known risk factors for cervical cancer (American Cancer Society, 2008). Subjects in Raaschou-Nielsen et al. (2003) are blue-collar workers and low SES status likely explains observed associations in this and the other studies. The use of internal controls in Blair et al. (1998) who are similar in SES status as TCE subjects is believed to partly account for possible confounder related to SES status; however, direct information on individual subjects is lacking.

Six other cohort, PMR, and geographic-based studies were given less weight in the analysis because of their lesser likelihood of TCE exposure and other study design limitations that would decrease statistical power and study sensitivity (Sung et al., 2007; ATSDR, 2006a, 2004a; Morgan and Cassady, 2002; Garabrant et al., 1988; Shannon et al., 1988). Cervical cancer risk estimates in these studies ranged between 0.65 (95% CI: 0.38, 1.02) (Morgan and Cassady, 2002) and 1.77 [PMR; 95% CI: 0.57, 4.12 (ATSDR, 2004a)]. No study reported a statistically significant deficit in cervical cancer risk.

4.8.2.2. Animal Studies

Histopathology findings have been noted in reproductive organs in various cancer bioassay studies conducted with TCE. A number of these findings (summarized in Table 4-93) do not demonstrate a treatment-related profile.

Table 4-93. Histopathology findings in reproductive organs

Tumor incidence in mice after 18 mo inhalation exposure[a]								
	Tissue	**Finding**	**Control**	**100 ppm**	**500 ppm**			
Males	Number examined:		30	29	30			
	Prostate	Myoma	1	0	0			
	Testis	Carcinoma	0	0	1			
		Cyst	0	0	1			
Females	Number examined:		29	30	28			
	Uterus	Adenocarcinoma	1	0	0			
	Ovary	Adenocarcinoma	1	0	0			
		Adenoma	3	1	3			
		Carcinoma	0	2	2			
		Granulosa cell tumor	4	0	2			
Tumor incidence in rats after 18 mo inhalation exposure[a]								
	Tissue	**Finding**	**Control**	**100 ppm**	**500 ppm**			
Males	Number examined:		29	30	30			
	Testis	Interstitial cell tumors	4	0	3			
Females	Number examined:		28	30	30			
	Mammary	Fibroadenoma	2	0	0			
		Adenocarcinoma	3	2	2			
	Uterus	Adenocarcinoma	3	1	4			
	Ovary	Carcinoma	4	0	1			
		Granulosa cell tumor	1	0	0			
	Genital tract	Squamous cell carcinoma	0	2	0			
Tumor incidence in hamsters after 18 mo inhalation exposure[a]								
	Tissue	**Finding**	**Control**	**100 ppm**	**500 ppm**			
Females	Number examined:		30	29	30			
	Ovary	Cystadenoma	1	0	0			
Tumor incidence in mice after 18 mo gavage administration[b]								
	Tissue	**Finding**	**Con-trol**	**TCE Pure**	**TCE Industrial**	**TCE + EPC**	**TCE + BO**	**TCE + EPC + BO**
Females	Number examined:		50	50	50	50	48	50
	Mammary	Carcinoma	1	2	0	0	0	0
	Ovary	Granulosa cell tumor	0	1	0	0	0	0
	Vulva	Squamous cell carcinoma	0	0	0	0	1	1

[a]Henschler et al. (1980).
[b]Henschler et al. (1984).

EPC = epichlorohydrin; BO = 1,2-epoxybutane

Cancers of the reproductive system that are associated with TCE exposure and observed in animal studies are comprised of testicular tumors (interstitial cell and Leydig cell). A summary of the incidences of testicular tumors observed in male rats is presented in Table 4-94.

Table 4-94. Testicular tumors in male rats exposed to TCE, adjusted for reduced survival[a]

Interstitial cell tumors after 103-wk gavage exposure, beginning at 6.5–8 wks of age (NTP, 1988, 1990)				
Administered dose (mg/kg-d)	**Untreated control**	**Vehicle control**	**500**	**1,000**
Male ACI rats	38/45 (84%)	36/44 (82%)	23/26 (88%)	17/19 (89%)
Male August rats	36/46 (78%)	34/46 (74%)	30/34 (88%)	26/30 (87%)
Male Marshall rats[b]	16/46 (35%)	17/46 (37%)	21/33 (64%)	32/39 (82%)
Male Osborne-Mendel rats	1/30 (3%)	0/28 (0%)	0/25 (0%)	1/19 (5%)
Male F344/N rats	44/47 (94%)	47/48 (98%)	47/48 (98%)	32/44 (73%)
Leydig cell tumors after 104-wk inhalation exposure, beginning at 12 wks of age (Maltoni et al., 1986)				
Administered daily concentration (mg/m^3)[c]	**Control**	**112.5**	**337.5**	**675**
Male Sprague-Dawley rats[b]	6/114 (5%)	16/105 (15%)	30/107 (28%)	31/113 (27%)

[a]ACI rats alive at week 70, August rats at week 65, Marshall rats at week 32, Osborne-Mendel rats at week 97, F344/N rats at week 32, Sprague-Dawley rats at week 81 (except BT304) or week 62 (except BT304 bis).
[b]Equivalent to 100, 300, or 600 ppm (100 ppm = 540 mg/m^3), adjusted for 7 hours/day, 5 days/week exposure.
[c]Statistically significant by Cochran-Armitage trend test ($p < 0.05$).

Sources: NTP (1988) Tables A2, C2, E2, G2; NTP (1990) Table A3; Maltoni et al. (1986) IV/IV Table 21, IV/V Table 21.

4.8.2.3. Mode of Action for Testicular Tumors

The database for TCE does not include an extensive characterization of the mode of action for Leydig cell tumorigenesis in the rat, although data exist that are suggestive of hormonal disruption in male rats. A study by Kumar et al. (2000a) found significant decreases in serum testosterone concentration and in 17-β-HSD, G6PDH, and total cholesterol and ascorbic acid levels in testicular homogenate from male rats that had been exposed via inhalation to 376 ppm TCE for 12 or 24 weeks. In a follow-up study, Kumar et al. (2001b) also identified decreases in SDH in the testes of TCE-treated rats. These changes are markers of disruption to testosterone biosynthesis. Evidence of testicular atrophy, observed in the 2001 study by Kumar et al., as well as the multiple in vivo and in vitro studies that observed alterations in spermatogenesis and/or sperm function, could also be consistent with alterations in testosterone levels. Therefore, while the available data are suggestive of a mode of action involving hormonal disruption for TCE-induced testicular tumors, the evidence is inadequate to specify and test a hypothesized sequence of key events.

Leydig cell tumors can be chemically induced by alterations of steroid hormone levels, through mechanisms such as agonism of estrogen, gonadotropin releasing hormone, or dopamine receptors; antagonism of androgen receptors; and inhibition of 5α-reductase, testosterone biosynthesis, or aromatase (Cook et al., 1999). For those plausible mechanisms that involve disruption of the hypothalamic-pituitary-testis (HPT) axis, decreased testosterone or estradiol levels or recognition is involved, and increased LH levels are commonly observed. Although there is evidence to suggest that humans are quantitatively less sensitive than rats in their proliferative response to LH, evidence of treatment-related Leydig cell tumors in rats that are induced via HPT disruption is considered to represent a potential risk to humans (with the possible exception of GnRh or dopamine agonists), since the pathways for regulation of the HPT axis are similar in rats and humans (Clegg et al., 1997).

4.8.3. Developmental Toxicity

An evaluation of the human and experimental animal data for developmental toxicity, considering the overall weight and strength of the evidence, suggests a potential for adverse outcomes associated with pre- and/or postnatal TCE exposures.

4.8.3.1. Human Developmental Data

Epidemiological developmental studies (summarized in Table 4-95) examined the relationship between TCE exposure and prenatal developmental outcomes including spontaneous abortion and perinatal death; decreased birth weight, small for gestational age (SGA), and postnatal growth; congenital malformations; and other adverse birth outcomes. Postnatal developmental outcomes examined include developmental neurotoxicity, developmental immunotoxicity, other developmental outcomes, and childhood cancer.

4.8.3.1.1. Adverse fetal/birth outcomes

4.8.3.1.1.1. Spontaneous abortion and perinatal death

Spontaneous abortion or miscarriage is defined as nonmedically induced premature delivery of a fetus prior to 20 weeks of gestation. Perinatal death is defined as stillbirths and deaths before 7 days after birth. Available data comes from several studies of occupational exposures in Finland and Santa Clara, California, and by geographic-based studies in areas with known contamination of water supplies in Woburn, Massachusetts; Tucson Valley, Arizona; Rocky Mountain Arsenal, Colorado; Endicott, New York; and New Jersey.

Table 4-95. Developmental studies in humans

Subjects	Exposure	Effect	Reference
Adverse fetal/birth outcomes			
Spontaneous abortion and perinatal death			
371 men occupationally exposed to solvents in Finland 1973–1983	Questionnaire Low/rare used <1 d/wk; Intermediate used 1–4 d/wk or intermediate/low TCA urine levels; High/frequent used daily or high TCA urine levels	No risk of spontaneous abortion after paternal exposure, based on 17 cases and 35 controls exposed to TCE OR: 1.0, 95% CI: 0.6–2.0	Taskinen et al. (1989)
535 women occupationally exposed to solvents in Finland 1973–1986	Questionnaire Rare used 1–2 d/wk; Frequent used ≥3 d/wk	Increased risk of spontaneous abortion among frequently-exposed women, based on 7 cases and 9 controls exposed to TCE OR: 1.6, 95% CI: 0.5–4.8	Taskinen et al. (1994)
3,265 women occupationally exposed to organic solvents in Finland 1973–1983	Questionnaire U-TCA: median: 48.1 µmol/L; mean 96.2 ± 19.2 µmol/L	No increased risk of spontaneous abortion based on 3 cases and 13 controls exposed to TCE OR: 0.6, 95% CI: 0.2–2.3	Lindbohm et al. (1990)
361 women occupationally and residentially exposed to solvents in Santa Clara County, California June 1986–February 1987 (735 controls)	Questionnaire	Increased risk of spontaneous abortion based on 6 cases and 4 controls exposed to TCE[a] OR: 3.1, 95% CI: 0.92–10.4	Windham et al. (1991)
4,396 pregnancies among residents of Woburn, Massachusetts 1960–1982	TCE: 267 µg/L Tetrachloroethylene: 21 µg/L Chloroform: 12 µg/L	Increased risk of perinatal death (n = 67) after 1970 (p = 0.55) but not before 1970 (OR: 10, p = 0.003) No increased risk of spontaneous abortion (n = 520; p = 0.66)	Lagakos et al. (1986)
707 parents of children with congenital heart disease in Tucson Valley, Arizona 1969–1987	6–239 ppb TCE, along with DCA and chromium	No increased risk of fetal death (not quantified) based on 246 exposed and 461 unexposed cases	Goldberg et al. (1990)
75 men and 71 women living near Rocky Mountain Arsenal, Colorado 1981–1986	Low: <5.0 ppb Medium: ≥5.0–<10.0 ppb High: <10.0 ppb	Increased risk of miscarriage OR_{adj}: 4.44, 95% CI: 0.76–26.12 Increased risk of no live birth OR_{adj}: 2.46, 95% CI: 0.24–24.95	ATSDR (2001)

Table 4-95. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
1,440 pregnancies among residents of Endicott, New York 1978–2002	Indoor air from soil vapor: 0.18–140 mg/m^3	No increase in spontaneous fetal death SIR: 0.66, 95% CI: 0.22–1.55	ATSDR (2008b, 2006a)
81,532 pregnancies among residents of 75 New Jersey towns 1985–1988 (3 control groups)	55 ppb TCE, along with many other compounds	No increased risk of fetal death for >10 ppb OR: 1.12	Bove (1996); Bove et al. (1995)
Decreased birth weight, SGA, and postnatal growth			
361 women occupationally and residentially exposed to solvents in Santa Clara County, California June 1986–February 1987 (735 controls)	Questionnaire	Increased risk of IUGR based on one case exposed to both TCE and tetrachloroethylene OR: 12.5	Windham et al. (1991)
3,462 births in Woburn, Massachusetts 1960–1982	267 µg/L TCE in drinking water, along with tetrachloroethylene and chloroform	No increase in low birth weight (p = 0.77)	Lagakos et al. (1986)
1,099 singleton births[b] to residents of three census tracts near Tucson International Airport 1979–1981 (877 controls)	<5–107 µg/L	No increase in full-term low birth weight (OR: 0.81) No increase in low birth weight (OR: 0.9) Increase in very low birth weight OR: 3.3, 95% CI: 0.53–20.6	Rodenbeck et al. (2000)
1,440 births[c] to residents of Endicott, New York 1978–2002	Indoor air from soil vapor: 0.18–140 mg/m^3	Small increase in low birth weight OR: 1.26, 95% CI: 1.00–1.59 Small increase in SGA OR: 1.22, 95% CI: 1.02–1.45 Increase in full-term low birth weight OR: 1.41, 95% CI: 1.01–1.95	ATSDR (2008b, 2006a)
6,289 pregnancies among women residing at Camp Lejeune, North Carolina 1968–1985 (141 short-term and 31 long-term TCE-exposed, 5,681 unexposed controls)[d]	Tarrawa Terrace: TCE: 8 ppb 1,2-DCE: 12 ppb Perchloroethylene: 215 ppb Hadnot Point: TCE: 1,400 ppb 1,2-DCE: 407 ppb	Change in mean birth weight Long-term total: –139 g, 90% CI: –277, –1 Long-term males: –312 g, 90% CI: –540, –85 Short term total: +70g, 90% CI: –6, 146 Increase in SGA Long-term total: OR: 1.5, 90% CI: 0.5, 3.8 Long-term males: OR: 3.9, 90% CI: 1.1–11.9 Short term total: OR: 1.1, 90% CI: 0.2–1.1	ATSDR (1998a)

Table 4-95. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
81,532 pregnancies[e] among residents of 75 New Jersey towns 1985–1988	55 ppb TCE, along with many other compounds	Decreased birth weight at >5 ppb by 17.9g No increase in prematurity at >10 ppb: OR: 1.02 Increase in low birth weight, term >10 ppb: OR: 1.23, 50% CI: 1.09–1.39 No risk for very low birth weight	Bove (1996); Bove et al. (1995)
Congenital malformations			
1,148 men and 969 women occupationally exposed to TCE in Finland 1963–1976	U-TCA: <10 to >500 mg/L	No congenital malformations reported	Tola et al. (1980)
371 men occupationally exposed to solvents in Finland 1973–1983	Low/rare used <1 d/wk; Intermediate used 1–4 d/wk or if biological measures indicated high exposure; High/frequent used daily or if biological measures indicated high exposure	No increase in congenital malformations based on 17 cases and 35 controls exposed to TCE OR: 0.6, 95% CI: 0.2–2.0	Taskinen et al. (1989)
100 babies with oral cleft defects born to women occupationally exposed in Europe 1989–1992	Questionnaire	Increase in cleft lip based on 2 of 4 TCE-exposed women OR_{adj}: 3.21, 95% CI: 0.49–20.9 Increase in cleft palate based on 2 of 4 TCE-exposed women OR_{adj}: 4.47, 95% CI: 1.02–40.9	Lorente et al. (2000)
4,396 pregnancies among residents of Woburn, Massachusetts 1960–1982	TCE: 267 µg/L Tetrachloroethylene: 21 µg/L Chloroform: 12 µg/L	Increase in eye/ear birth anomalies: OR: 14.9, $p < 0.0001$ Increase in CNS/chromosomal/oral cleft anomalies: OR: 4.5, $p = 0.01$ Increase in kidney/urinary tract disorders: OR: 1.35, $p = 0.02$ Small increase in lung/respiratory tract disorders: OR: 1.16, $p = 0.05$ No increase in cardiovascular anomalies (n = 5): $p = 0.91$	Lagakos et al. (1986)
707 children with congenital heart disease in Tucson Valley, Arizona 1969–1987 (246 exposed, 461 unexposed)	Wells contaminated with TCE (range: 6–239 ppb), along with DCA and chromium	Increase in congenital heart disease <1981: OR: ≈3 ($p < 0.005$) >1981: OR: ≈1 Increased prevalence after maternal exposure during first trimester ($p < 0.001$, 95% CI: 1.14–4.14)	Goldberg et al. (1990)
75 men, 71 women living near Rocky Mountain Arsenal, Colorado 1981–1986	Low: <5.0 ppb Medium: ≥5.0–<10.0 ppb High: <10.0 ppb	Increase in total birth defects (n = 9) OR: 5.87, 95% CI: 0.59–58.81	ATSDR (2001)

Table 4-95. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
Births to residents of Endicott, New York 1983–2000[f]	Indoor air from soil vapor: 0.18–140 mg/m^3	No increase in total birth defects: RR: 1.08, 95% CI: 0.82–1.42 Increase in total cardiac defects: RR: 1.94, 95% CI: 1.21–3.12 Increase in major cardiac defects: RR: 2.52, 95% CI: 1.2–5.29 Increase in conotruncal heart defects: RR: 4.83, 95% CI: 1.81–12.89	ATSDR (2008b, 2006a)
81,532 pregnancies among residents of 75 New Jersey towns 1985–1988	55 ppb TCE, along with many other compounds	No increase in total birth defects: >10 ppb: OR: 1.12 Increase in total CNS defects at high dose >1–5 ppb: OR: 0.93, 90% CI: 0.47–1.77 >10 ppb: OR: 1.68, 90% CI: 0.76–3.52 Increase in neural tube defects >1–5 ppb: OR: 1.58, 90% CI: 0.69–3.40 >10 ppb: OR: 2.53, 90% CI: 0.91–6.37 Increase in oral clefts: >5 ppb: OR: 2.24, 95% CI: 1.16–4.20 Increase in major cardiac defects: >10 ppb: OR: 1.24, 50% CI: 0.75–1.94 Increase in ventrical septal defects >5ppb: OR: 1.30, 95% CI: 0.88–1.87	Bove (1996); Bove et al. (1995)
1,623 children <20 yrs old dying from congenital anomalies in Maricopa County, Arizona 1966–1986	8.9 and 29 ppb TCE in drinking water	Increase in deaths due to congenital anomalies in East Central Phoenix 1966–1969: RR: 1.4, 95% CI: 1.1–1.7 1970–1981: RR: 1.5, 95% CI: 1.3–1.7 1982–1986: RR: 2.0, 95% CI: 1.5–2.5	AZ DHS (Flood, 1988)
4,025 infants born with congenital heart defects in Milwaukee, Wisconsin 1997–1999	Maternal residence within 1.32 miles from at least one TCE emissions source	Increase in congenital heart defects for mothers ≥38 yrs old Exposed: OR: 6.2, 95% CI: 2.6–14.5 Unexposed: OR: 1.9, 95% CI: 1.1–3.5 No increase in congenital heart defects for exposed mothers <38 yrs old: OR: 0.9, 95% CI: 0.6–1.2	Yauck et al. (2004)
12 children exposed to TCE in well water in Michigan	5–10 yrs to 8–14 ppm	One born with multiple birth defects	Bernad et al. (1987), abstract
Other adverse birth outcomes			
34 live births for which inhalation of TCE for anesthesia was used in Japan 1962–1967	2–8 mL (mean 4.3 mL) for 2–98 min (mean: 34.7 min)	One case of asphyxia; 3 ~~-~~sleepy babies" with Apgar scores of 5–9; delayed appearance of newborn reflexes	Beppu (1968)

Table 4-95. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
51 U.K. women whose fetus was considered to be at risk for hypoxia during labor administered TCE as an analgesic (50 controls)	Amount and route of exposure not reported	TCE caused fetal pH to fall more, base deficit increased more, and PO_2 fell more than the control group by fourfold or more compared to other analgesics used	Phillips and Macdonald (1971)
Postnatal developmental outcomes			
Developmental neurotoxicity			
54 individuals from 3 residential cohorts in the United States exposed to TCE in drinking water	Woburn, Massachusetts 63–400 ppb for <1–12 yrs Alpha, Ohio 3.3–330 ppb for 5–17 yrs Twin Cities, Minnesota 261–2,440 ppb for 0.25–25 yrs	Woburn, Massachusetts Verbal naming/language impairment in 6/13 children (46%) Alpha, Ohio Verbal naming/language impairment in 1/2 children (50%) Twin Cities, Minnesota Verbal naming/language impairment in 4/4 children (100%) Memory impairment in 4/4 children (100%) Academic impairment in 4/4 children (100%) Moderate encephalopathy in 4/4 children (100%) Poor performance on reading/spelling test in 3/4 children (75%) Poor performance on information test in 3/4 children (75%)	White et al. (1997)
284 cases of ASD diagnosed <9 yrs old and 657 controls born in the San Francisco Bay Area 1994	Births geocoded to census tracts, and linked to hazardous air pollutants data	Increase in ASD upper 3rd quartile: OR: 1.37, 95% CI: 0.96–1.95 upper 4th quartile: OR: 1.47, 95% CI: 1.03–2.08	Windham et al. (2006)
948 children (<18 yrs) in the TCE Subregistry	0.4–>5,000 ppb TCE	Increase in speech impairment: 0–9 yrs old: RR: 2.45, 99% CI: 1.31–4.58 10–17 yrs old: RR: 1.14, 99% CI: 0.46–2.85 Increase in hearing impairment: 0–9 yrs old: RR: 2.13, 99% CI: 1.12–4.07 10–17 yrs old: RR: 1.12, 99% CI: 0.52–2.24	ATSDR (2002); Burg et al. (1995); Burg and Gist (1999)
12 children exposed to TCE in well water in Michigan	5–10 yrs to 8–14 ppm	9 of 12 children (75%) had poor learning ability, aggressive behavior, and low attention span	Bernad et al. (1987), abstract

Table 4-95. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
Developmental immunotoxicity			
200 children aged 36 mo old born prematurely[g] and at risk of atopy[h] in Lepzig, Germany 1995–1996	Median air level in child's bedroom: 0.42 µg/m^3	No association with allergic sensitization to egg white and milk, or to cytokine producing peripheral T-cells	Lehmann et al. (2001)
85 healthy[i] full-term neonates born in Lepzig, Germany 1997–1999	Median air level in child's bedroom 3–4 wks after birth: 0.6 µg/m^3	Significant reduction of Th1 IL-2 producing T-cells	Lehmann et al. (2002)
Other developmental outcomes			
55 children (6 mo to 10 yrs old) were anesthetized for operations to repair developmental defects of the jaw and face in Poland 1964	≥10 mL TCE	Reports of bradycardia, accelerated heart rate, and respiratory acceleration observed; no arrhythmia was observed	Jasinka (1965), translation
Childhood cancer			
98 children (<10 yrs old) diagnosed with brain tumors in Los Angeles County 1972–1977	Questionnaire of parental occupational exposures	Two cases were reported for TCE exposure, one with methyl ethyl ketone	Peters and Preston-Martin (1981)
22 children (<19 yrs old) diagnosed with neuroblastoma in United States and Canada 1992–1994 (12 controls)	Questionnaire of parental occupational exposures	Increase in neuroblastoma after paternal exposure OR: 1.4, 95% CI: 0.7–2.9 Maternal exposure not reported	De Roos et al. (2001)
61 boys and 62 girls (<10 yrs old) diagnosed with leukemia and 123 controls in Los Angeles County 1980–1984	Questionnaire of parents for occupational exposure	Increase in leukemia after paternal exposure Preconception (1 yr): OR: 2.0, $p = 0.16$ Prenatal: OR: 2.0, $p = 0.16$ Postnatal: OR: 2.7, $p = 0.7$ Maternal exposure not reported	Lowengart et al. (1987)
1,842 children (<15 yrs old) diagnosed with ALL in United States and Canada 1989–1993 (1986 controls)	Questionnaire of parents for occupational exposure	Increase in ALL after maternal exposure Preconception: OR: 1.8, 95% CI: 0.6–5.2 Pregnancy: OR: 1.8, 95% CI: 0.5–6.4 Postnatal: OR: 1.4, 95% CI: 0.5–4.1 Anytime: OR: 1.8, 95% CI: 0.8–4.1 No increase in ALL after paternal exposure Anytime: OR: 1.1, 95% CI: 0.8–1.5	Shu et al. (1999)

Table 4-95. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
109 children (<15 yrs old) born in the U.K. 1974–1988 (218 controls)	Questionnaire of parents for occupational exposure	Increase in leukemia and NHL after paternal exposure Preconception: OR: 2.27, 95% CI: 0.84–6.16 Prenatal: OR: 4.40, 95% CI: 1.15–21.01 Postnatal: OR: 2.66, 95% CI: 0.82–9.19 No increase in leukemia and NHL after maternal exposure Preconception: OR: 1.16, 95% CI: 0.13–7.91	McKinney et al. (1991)
22 children (<15 yrs old) diagnosed with childhood cancer in California 1988–1998	0.09–97 ppb TCE in drinking water	No increase in total cancer: SIR: 0.83, 99% CI: 0.44–1.40 No increase in CNS cancer: SIR: 1.05, 99% CI: 0.24–2.70 No increase in leukemia: SIR: 1.09, 99% CI: 0.38–2.31	Morgan and Cassady (2002)
1,190 children (<20 yrs old) diagnosed with leukemia in 4 counties in New Jersey 1979–1987	0–67 ppb TCE in drinking water	Increase in ALL in girls with >5 ppb exposure <20 yrs old: RR: 3.36, 95% CI: 1.29–8.28 <5 yrs old: RR: 4.54, 95% CI: 1.47–10.6	Cohn et al. (1994b)
24 children (<15 yrs old) diagnosed with leukemia in Woburn, Massachusetts 1969–1997	267 µg/L TCE in drinking water, along with tetrachloroethylene, arsenic, and chloroform	Increase in childhood leukemia Preconception: OR_{adj}: 2.61, 95% CI: 0.47–14.97 Pregnancy: OR_{adj}: 8.33, 95% CI: 0.73–94.67 Postnatal: OR_{adj}: 1.18, 95% CI: 0.28–5.05 Ever: OR_{adj}: 2.39, 95% CI: 0.54–10.59	Costas et al. (2002); Cutler et al. (1986); Lagakos et al. (1986); MDPH (1997c)[j]
347 children (<20 yrs old) diagnosed with cancer in Endicott, New York 1980–2001	indoor air from soil vapor: 0.18–140 mg/m^3	No increase in cancer (<6 cases, similar to expected)	ATSDR (2008b, 2006a)
189 children (<20 yrs old) diagnosed with cancer in Maricopa County, Arizona 1965–1990	8.9 and 29 ppb TCE in drinking water	Increase in leukemia: 1965–1986: SIR: 1.67, 95% CI: 1.20–2.27 1982–1986: SIR: 1.91, 95% CI: 1.11–3.12 No increase in total childhood cancers, lymphoma, brain/CNS, or other cancers	AZ DHS (Flood, 1997a; Flood, 1988) (1990)[k]
16 children (<20 yrs old) diagnosed with cancer in East Phoenix, Arizona 1965–1986	TCE, TCA, and other contaminants in drinking water	No increase in leukemia: SIR: 0.85, 95% CI: 0.50–1.35	AZ DHS (Kioski et al., 1990b)

Table 4-95. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
37 children (<20 yrs old) diagnosed with cancer in Pima County, Arizona 1970–1986	1.1–239 ppb TCE, along with 1,1-DCE, chloroform and chromium in drinking water	Increase in leukemia (n = 11): SIR: 1.50, 95% CI: 0.76–2.70 No increase in testicular cancer (n = 6): SIR: 0.78, 95% CI: 0.32–1.59 No increase in lymphoma (n = 2): SIR: 0.63, 95% CI: 0.13–1.80 No increase in CNS/brain cancer (n = 3): SIR: 0.84, 95% CI: 0.23–2.16 Increase in other cancer (n = 15): SIR: 1.40, 95% CI: 0.79–2.30	AZ DHS (Kioski et al., 1990a)

[a]Of those exposed to TCE, four were also exposed to tetrachloroethylene and one was also exposed to paint strippers and thinners.
[b]Full term defined as between 35 and 46 weeks gestation, low birth weight as <2,501 g, and very low birth weight as <1,501 g.
[c]Low birth weight defined as <2,500, moderately low birth weight (1,500–<2,500 g), term low birth weight (≥37 weeks gestation and <25,000 g).
[d]Unexposed residents resided at locations not classified for long-term or short-term TCE exposure. Long-term TCE exposed mothers resided at Hospital Point during 1968–1985 for at least 1 week prior to birth. Short-term TCE exposed mothers resided at Berkeley Manor, Midway Park, Paradise Point, and Wakins Village at the time of birth and at least 1 week during January 27 to February 7, 1985. In addition, the mother's last menstrual period occurred on or before January 31, 1985 and the birth occurred after February 2, 1985.
[e]Low birth weight defined as <2,500 g, very low birth weight as <1,500 g.
[f]1,440 births reported for years 1978–2002, but number not reported for years 1983–2000.
[g]Premature defined as 1,500–2,500 g at birth.
[h]Risk of atopy defined as cord blood IgE >0.9 kU/L; double positive family atopy history.
[i]Healthy birth defined as ≥2,500 g and ≥37 weeks gestation.
[j]Only results from Costas et al. (2002) are reported in the table.
[k]Only results from AZ DHS (1990) are reported in the table.

ALL = acute lymphoblastic leukemia; IUGR = intrauterine growth restriction

4.8.3.1.1.1.1. Occupational studies

The risks of spontaneous abortion and congenital malformations among offspring of men occupationally exposed to TCE and other organic solvents were examined by Taskinen et al. (1989). This nested case-control study was conducted in Finland from 1973 to 1983. Exposure was determined by biological measurements of the father and questionnaires answered by both the mother and father. The level of exposure was classified as "low/rare" if the chemical was used <1 day/week, "intermediate" if used 1–4 days/week or if TCA urine measurements indicated intermediate/low exposure, and "high/frequent" if used daily or if TCA urine measurements indicated clear occupational exposure (defined as above the reference value for the general population). There was no risk of spontaneous abortion from paternal TCE exposure (OR: 1.0, 95% CI: 0.6–2.0), although there was a significant increase for paternal organic solvent exposure (OR: 2.7, 95% CI: 1.3–5.6) and a nonsignificant increase for maternal organic solvent exposure (OR: 1.4, 95% CI: 0.6–3.0). (Also see section below for results from this study for congenital malformations.)

Another case-control study in Finland examined pregnancy outcomes in 1973–1986 among female laboratory technicians aged 20–34 years (Taskinen et al., 1994). Exposure was reported via questionnaire, and was classified as "rare" if the chemical was used 1–2 days/week, and "frequent" if used at least 3 days/week. Cases of spontaneous abortion (n = 206) were compared with controls who had delivered a baby and did not report prior spontaneous abortions (n = 329). A nonstatistically significant increased risk was seen between spontaneous abortion and TCE use at least 3 days/week (OR: 1.6, 95% CI: 0.5–4.8).

The association between maternal exposure to organic solvents and spontaneous abortion was examined in Finland for births 1973–1983 (Lindbohm et al., 1990). Exposure was assessed by questionnaire and confirmed with employment records, and the level of exposure was either high, low, or none based on the frequency of use and known information about typical levels of exposure for job type. Biological measurements of TCA in urine were also taken on 64 women, with a median value of 48.1 µmol/L (mean: 96.2 ± 19.2 µmol/L). Three cases and 13 controls were exposed to TCE, with no increased risk seen for spontaneous abortion (OR: 0.6, 95% CI: 0.2–2.3, $p = 0.45$).

A case-control study in Santa Clara County, California, examined the association between solvents and adverse pregnancy outcomes in women ≥18 years old (Windham et al., 1991). For pregnancies occurring between June 1986 and February 1987, 361 cases of spontaneous abortion were compared to 735 women who had a live birth during this time period. Telephone interviews included detailed questions on occupational solvent exposure, as well as additional questions on residential solvent use. For TCE exposure, 6 cases of spontaneous abortion were compared to 4 controls of live births; of these 10 TCE-exposed individuals, 4 reported exposure to tetrachloroethylene, and 1 reported exposure to paint strippers and thinners. An increased risk of spontaneous abortions was seen with TCE exposure (OR: 3.1,

95% CI: 0.92–10.4), with a statistically significant increased risk for those exposed ≥0.5 hours/week (OR: 7.7, 95% CI: 1.3–47.4). An increased risk for spontaneous abortion was also seen for those reporting a more "intense" exposure based primarily on odor, as well as skin contact or other symptoms (OR: 3.9, $p = 0.04$). (Also see section below from this study on low birth weight.)

4.8.3.1.1.1.2. Geographic-based studies

A community in Woburn, Massachusetts with contaminated well water experienced an increased incidence of adverse birth outcomes and childhood leukemia (Lagakos et al., 1986). In 1979, the wells supplying drinking water were found to be contaminated with 267 ppb TCE, 21 ppb tetrachloroethylene, and 12 ppb chloroform, and were subsequently closed. Pregnancy and childhood outcomes were examined from 4,396 pregnancies among residents (Lagakos et al., 1986). No association between water access and incidence of spontaneous abortion (n = 520) was observed ($p = 0.66$). The town's water distribution system was divided into five zones, which was reorganized in 1970. Prior to 1970, no association was observed between water access and incidence of perinatal deaths (n = 46 stillbirths and 21 deaths before 7 days) ($p = 0.55$). However, after 1970, a statistically significant positive association between access to contaminated water and perinatal deaths was observed (OR: 10.0, $p = 0.003$). The authors could not explain why this discrepancy was observed, but speculated that contaminants were either not present prior to 1970, or were increased after 1970. (Also see sections below on decreased birth weight, congenital malformations, and childhood cancer for additional results from this cohort.)

A community in Tucson Valley, Arizona with contaminated well water had a number of reported cases of congenital heart disease. The wells were found to be contaminated with TCE (range = 6–239 ppb), along with DCE and chromium (Goldberg et al., 1990). This study identified 707 children born with congenital heart disease during the years 1969–1987. Of the study participants, 246 families had parental residential and occupational exposure during 1 month prior to conception and during the first trimester of pregnancy, and 461 families had no exposure before the end of the first trimester. In addition to this control group, two others were used: (1) those that had contact with the contaminated water area, and (2) those that had contact with the contaminated water area and matched with cases for education, ethnicity, and occupation. Among these cases of congenital heart disease, no significant difference was seen for fetal death (not quantified) for exposed cases compared to unexposed cases. (Also see section below on congenital malformations for additional results from this cohort.)

A residential study of individuals living near the Rocky Mountain Arsenal in Colorado examined the outcomes in offspring of 75 men and 71 women exposed to TCE in drinking water (ATSDR, 2001). TCE exposure was stratified by high (>10.0 ppb), medium (≥5.0–<10.0 ppb), and low (<5.0 ppb). Among women with >5 ppb exposure experiencing miscarriage (n = 22/57) compared to unexposed women experiencing miscarriage (n = 2/13) an elevated nonsignificant

association was observed (OR_{adj}: 4.44, 95% CI: 0.76–26.12). For lifetime number of miscarriages reported by men and women, results were increased but without dose-response for women (medium: OR_{adj}: 8.56, 95% CI: 0.69–105.99; high: OR_{adj}: 4.16, 95% CI: 0.61–25.99), but less for men (medium: OR_{adj}: 1.68, 95% CI: 0.26–10.77; high: OR_{adj}: 0.65, 95% CI: 0.12–3.48). Among women with >5 ppb exposure experiencing no live birth (n = 9/57) compared to unexposed women experiencing no live birth (n = 1/13) an elevated nonsignificant association was observed (OR_{adj}: 2.46, 95% CI: 0.24–24.95). (Also see below for results from this study on birth defects.)

NYS DOH and ATSDR conducted a study in Endicott, New York to examine childhood cancer and birth outcomes in an area contaminated by a number of VOCs, including ~~"t~~housands of gallons" of TCE (ATSDR, 2006a). Soil vapor levels tested ranged from 0.18 to 140 mg/m^3 in indoor air. A follow-up study by ATSDR (2008b) reported that during the years 1978–1993 only five spontaneous fetal deaths occurring ≥20 weeks gestation were reported when 7.5 were expected (SIR: 0.66, 95% CI: 0.22–1.55). (See sections on low birth weight, congenital malformations, and childhood cancer for additional results from this cohort.)

Women were exposed to contaminated drinking water while pregnant and living in 75 New Jersey towns during the years 1985–1988 (Bove, 1996; Bove et al., 1995). The water contained multiple trihalomethanes, including an average of 55 ppb TCE, along with tetrachloroethylene, 1,1,1-trichloroethane, carbon tetrachloride, 1,2-dichloroethane, and benzene. A number of birth outcomes were examined for 81,532 pregnancies, which resulted in 80,938 live births and 594 fetal deaths. No association was seen for exposure to >10 ppb TCE and fetal death (OR_{adj}: 1.12). (See below for results from this study on decreased birth weight and congenital malformations.)

4.8.3.1.1.2. Decreased birth weight, SGA, and postnatal growth

Available data pertaining to birth weight and other growth-related outcomes come from the case-control study in Santa Clara, California (discussed above), and by geographic-based studies as well as geographic areas with known contamination of water supplies areas in Woburn, Massachusetts; Tucson, Arizona, Endicott, New York; Camp Lejeune, North Carolina; and New Jersey.

4.8.3.1.1.2.1. Occupational studies

The case-control study of the relationship between solvents and adverse pregnancy outcomes discussed above (Windham et al., 1991) also examined intrauterine growth restriction (IUGR). Telephone interviews included detailed questions on occupational solvent exposure, as well as additional questions on residential solvent use. An increased risk of IUGR was observed (OR: 12.5), although this was based only on one case that was exposed to both TCE and tetrachloroethylene (also see section above on spontaneous abortion).

4.8.3.1.1.2.2. Geographic-based studies

The study of Woburn, Massachusetts with contaminated well water discussed above (Lagakos et al., 1986) examined birth weight. Of 3,462 live births surviving to 7 days, 220 were <6 pounds at birth (6.4%). No association was observed between water access and low birth weight ($p = 0.77$). (See section on spontaneous abortion for study details, and see sections on spontaneous abortion, congenital malformations, and childhood cancer for additional results from this cohort.)

An ecological analysis of well water contaminated with TCE in Tucson and birth-weight was conducted by Rodenbeck et al. (2000). The source of the exposure was a U.S. Air Force plant and the Tucson International Airport. The wells were taken out of service in 1981 after concentrations of TCE were measured in the range of <5–107 µg/L. The study population consisted of 1,099 babies born within census tracts between 1979 and 1981, and the comparison population consisted of 877 babies from nearby unexposed census tracts. There was a nonsignificant increased risk for maternal exposure to TCE in drinking water and very-low-birth-weight (<1,501 g) (OR: 3.3, 95% CI: 0.53–20.6). No increases were observed in the low-birth-weight (<2,501 g) (OR: 0.9) or full-term (>35–<46-week gestation) low-birth-weight (OR: 0.81).

The study of VOC exposure in Endicott, New York reported data on low birth weight and SGA (ATSDR, 2006a), see section on spontaneous abortion for study details). For births occurring during the years 1978–2002, low birth weight was slightly but statistically elevated (OR: 1.26, 95% CI: 1.00–1.59), as was SGA (OR: 1.22, 95% CI: 1.02–1.45), and full-term low birth weight (OR: 1.41, 95% CI: 1.01–1.95). (Also see sections on spontaneous abortion, congenital malformations, and childhood cancer for additional results from this cohort.)

Well water at the U.S. Marine Corps Base in Camp Lejeune, North Carolina was identified to be contaminated with TCE, tetrachloroethylene, and 1,2-dichloroethane in April, 1982 and the wells were closed in December, 1984. ATSDR examined pregnancy outcomes among women living on the base during the years 1968–1985 (ATSDR, 1998a). Compared to unexposed residents[12] (n = 5,681), babies exposed to TCE long-term[13] (n = 31) had a lower mean birth weight after adjustment for gestational age (-139 g, 90% CL = -277, -1), and babies exposed short-term[14] (n = 141) had a slightly higher mean birth weight (+70 g, 90% CL = -6, 146). For the long-term group, no effect was seen for very low birth weight (<1,500 g) or prematurity (>5 ppb, OR: 1.05). No preterm births were reported in the long-term group and those (n = 8) in the short-term group did not have an increased risk (OR: 0.7, 90% CI: 0.3–1.2).

[12]Unexposed residents resided at locations not classified for long- or short-term TCE exposure.
[13]Long-term TCE exposed mothers resided at Hospital Point during 1968–1985 for at least 1 week prior to birth.
[14]Short-term TCE exposed mothers resided at Berkeley Manor, Midway Park, Paradise Point, and Wakins Village at the time of birth and at least 1 week during January 27 to February 7, 1985. In addition, the mother's last menstrual period occurred on or before January 31, 1985 and the birth occurred after February 2, 1985.

A higher prevalence of SGA[15] was seen in the long-term exposed group (n = 3; OR 1.5, 90% CL: 0.5, 3.8) compared to the short-term exposed group (OR: 1.1, 90% CI: 0.2–1.1). When the long-term group was stratified by gender, male offspring were at more risk for both reduced birth weight (-312 g, 90% CL = -632, -102) and SGA (OR: 3.9, 90% CL: 1.1–11.8). This study is limited due the mixture of chemicals in the water, as well as it small sample size. ATSDR is currently reanalyzing the findings because of an error in the exposure assessment related to the start-up date of a water treatment plant (ATSDR, 2009, 2007a; U.S. GAO, 2007a, b)

Pregnancy outcomes among women were exposed to contaminated drinking water while pregnant and living in 75 New Jersey towns during the years 1985–1988 were examined by Bove et al. (Bove, 1996; Bove et al., 1995). The water contained multiple trihalomethanes, including an average of 55 ppb TCE, along with tetrachloroethylene, 1,1,1-trichloroethane, carbon tetrachloride, 1,2-dichloroethane, and benzene. A number of birth outcomes were examined for 81,532 pregnancies, which resulted in 80,938 live births and 594 fetal deaths. A slight decrease of 17.9 g in birth weight was seen for exposure >5 ppb, with a slight increase in risk for exposure >10 ppb (OR: 1.23), but no effect was seen for very low birth weight or SGA/prematurity (>5 ppb, OR: 1.05). However, due to the multiple contaminants in the water, it is difficult to attribute the results solely to TCE exposure. (See below for results from this study on congenital malformations.)

4.8.3.1.1.3. Congenital malformations

Three studies focusing on occupational solvent exposure and congenital malformations from Europe provide data pertaining to TCE. Analyses of risk of congenital malformations were also included in the studies in the four geographic areas described above (Woburn, Massachusetts; Tucson, Arizona; Rocky Mountain Arsenal, Colorado; Endicott, New York; and New Jersey), as well as additional sites in Phoenix, Arizona; and Milwaukee, WI. Specific categories of malformations examined include cardiac defects, as well as cleft lip or cleft palate.

4.8.3.1.1.3.1. Occupational studies

A study of 1,148 men and 969 women occupationally exposed to TCE in Finland from 1963 to 1976 examined congenital malformations of offspring (Tola et al., 1980). U-TCA measurements available for 2,004 employees ranged from <10 to >500 mg/L, although 91% of the samples were <100 mg/L. No congenital malformations were seen in the offspring of women between the ages of 15–49 years, although 3 were expected based on the national incidence. Expected number of cases for the cohort could not be estimated because the number of pregnancies was unknown.

[15]The criteria for SGA being singleton births less than the 10th percentile of published sex-specific growth curves.

Men from Finland occupationally exposed to organic solvents including TCE did not observe a risk of congenital malformations from paternal organic solvent exposure based on 17 cases and 35 controls exposed to TCE (OR: 0.6, 95% CI: 0.2–2.0) (Taskinen et al., 1989). (Also see section above on spontaneous abortion for study details and additional results from this cohort.)

An occupational study of 100 women who gave birth to babies born with oral cleft defects and 751 control women with normal births were examined for exposure to a number of agents including TCE during the first trimester of pregnancy (Lorente et al., 2000). All women were participants in a multicenter European case-referent study whose children were born between 1989 and 1992. Four women were exposed to TCE, resulting in two cases of cleft lip (OR_{adj}: 3.21, 95% CI: 0.49–20.9), and two cases of cleft palate (OR_{adj}: 4.47, 95% CI: 1.02–40.9). Using logistic regression, the increased risk of cleft palate remained high (OR: 6.7, 95% CI: 0.9–49.7), even when controlling for tobacco and alcohol consumption (OR: 7.8, 95% CI: 0.8–71.8). However, the number of cases was small, and exposure levels were not known.

4.8.3.1.1.3.2. Geographic-based studies

A community in Woburn, Massachusetts with contaminated well water experienced an increased incidence of adverse birth outcomes and childhood leukemia (Lagakos et al., 1986, see section on spontaneous abortion for study details). Statistically significant positive association between access to contaminated water and eye/ear birth anomalies (OR: 14.9, $p < 0.0001$), CNS/chromosomal/oral cleft anomalies (OR: 4.5, $p = 0.01$), kidney/urinary tract disorders (OR: 1.35, $p = 0.02$) and lung/respiratory tract disorders (OR: 1.16, $p = 0.05$) were observed. There were also five cases of cardiovascular anomalies, but there was not a significant association with TCE ($p = 0.91$). However, since organogenesis occurs during gestational weeks 3–5 in humans, some of these effects could have been missed if fetal loss occurred. (Also see sections on spontaneous abortion, perinatal death, decreased birth weight, and childhood cancer for additional results from this cohort.)

A high prevalence of congenital heart disease was found within an area of Tucson Valley, Arizona (Goldberg et al., 1990, see section on spontaneous abortion for study details and additional results). Of the total 707 case families included, 246 (35%) were exposed to wells providing drinking water found to be contaminated with TCE (range = 6–239 ppb), along with DCE and chromium. Before the wells were closed after the contamination was discovered in 1981, the OR of congenital heart disease was 3 times higher for those exposed to contaminated drinking water compared to those not exposed; after the wells were closed, there was no difference seen. This study observed 18 exposed cases of congenital heart disease when 16.4 would be expected (RR: 1.1). Prevalence of congenital heart disease in offspring after maternal exposure during the first trimester (6.8 in 1,000 live births) was significantly increased compared to nonexposed families (2.64 in 1,000 live births) ($p < 0.001$, 95% CI: 1.14–4.14). No

difference in prevalence was seen if paternal data were included, and there was no difference in prevalence by ethnicity. In addition, no significant difference was seen for cardiac lesions.

A residential study of individuals living near the Rocky Mountain Arsenal in Colorado examined the outcomes in offspring of 75 men and 71 women exposed to TCE in drinking water (ATSDR, 2001). The risk was elevated for the nine birth defects observed (OR: 5.87, 95% CI: 0.59–58.81), including one nervous system defect, one heart defect, and one incidence of cerebral palsy. The remaining cases were classified as "other," and the authors speculate these may be based on inaccurate reports. (See above for study details and results on spontaneous abortion.)

The study of VOC exposure in Endicott, New York examined a number of birth defects during the years 1983–2000 (ATSDR, 2006a), see section on spontaneous for study details). These include total reportable birth defects, structural birth defects, surveillance birth defects, total cardiac defects, major cardiac defects, cleft lip/cleft palate, neural tube defects, and choanal atresia (blocked nasal cavities). There were 56 expected cases of all birth defects and 61 were observed resulting in no elevation of risk (rate ratio: 1.08, 95% CI: 0.82–1.42). There were no cases of cleft lip/cleft palate, neural tube defects, or choanal atresia. Both total cardiac defects (n = 15; rate ratio: 1.94, 95% CI: 1.21–3.12) and major cardiac defects (n = 6; rate ratio: 2.52, 95% CI: 1.2–5.29) were statistically increased. A follow-up study by ATSDR (2008b) reported that conotruncal heart malformations were particularly elevated (n = 4; rate ratio: 4.83, 95% CI: 1.81–12.89). The results remained significantly elevated (rate ratio: 3.74; 95% CI: 1.21–11.62) when infants with Down syndrome were excluded from the analysis. (Also see sections on spontaneous abortion, decreased birth weight, and childhood cancer for additional results from this cohort.)

In the New Jersey study described previously, the prevalence of birth defects reported by surveillance systems was examined among the women exposed to TCE and other contaminants in water while pregnant between 1985 and 1988 (Bove, 1996; Bove et al., 1995). For exposure >10 ppb (n = 1,372), an increased risk, with relatively wide CIs, was seen for all birth defects (OR: 2.53, 95% CI: 0.77–7.34). An increased risk was also seen for CNS defects (>10 ppb: OR: 1.68), specifically 56 cases of neural tube defects (<1–5 ppb: 1.58, 95% CI: 0.61–3.85; >10 ppb: OR: 2.53, 95% CI: 0.77–7.34). A slight increase was seen in major cardiac defects (>10 ppb: OR: 1.24, 50% CI: 0.75–1.94), including ventrical septal defects (>5 ppb: OR: 1.30, 95% CI: 0.88–1.87). An elevated risk was seen for nine cases of oral clefts (<5 ppb: OR: 2.24, 95% CI: 1.04–4.66), although no dose-response was seen (>10 ppb, OR: 1.30). However, due to the multiple contaminants in the water, it is difficult to attribute the results solely to TCE exposure. (See above for results from this study on fetal death and decreased birth weight.)

Arizona Department of Health Services (AZ DHS) conducted studies of contaminated drinking water and congenital malformations (<20 years old) in Maricopa County, which encompasses Phoenix and the surrounding area (Flood, 1988). TCE contamination was

associated with elevated levels of deaths in children <20 years old due to total congenital anomalies in East Central Phoenix from 1966 to 1969 (RR: 1.4, 95% CI: 1.1–1.7), from 1970 to 1981 (RR: 1.5, 95% CI: 1.3–1.7), and from 1982 to 1986 (RR: 2.0, 95% CI: 1.5–2.5), as well as in other areas of the county. (See below for results from this study on childhood leukemia.)

A study was conducted of children born in 1997–1999 with congenital heart defects in Milwaukee, Wisconsin (Yauck et al., 2004). TCE emissions data were ascertained from state and EPA databases, and distance between maternal residence and the emission source was determined using a GIS. Exposure was defined as those within 1.32 miles from at least one site. Results showed that an increased risk of congenital heart defects was seen for the offspring of exposed mothers ≥38 years old (OR: 6.2, 95% CI: 2.6–14.5), although an increased risk was also seen for offspring of unexposed mothers ≥38 years old (OR: 1.9, 95% CI: 1.1–3.5), and no risk was seen for offspring of exposed mothers <38 years old (OR: 0.9, 95% CI: 0.6–1.2). The authors speculate that studies that did not find a risk only examined younger mothers. The authors also note that statistically significant increased risk was seen for mothers with preexisting diabetes, chronic hypertension, or alcohol use during pregnancy.

An abstract reported that 28 people living in a Michigan town were exposed for 5–10 years to 8–14 ppm TCE in well water (Bernad et al., 1987, abstract). One child was born with multiple birth defects, with no further details.

4.8.3.1.1.4. Other adverse birth outcomes

TCE was previously used as a general anesthetic during pregnancy. One study measured the levels of TCE in maternal and newborn blood after use during 34 vaginal childbirths (Beppu, 1968). TCE was administered through a vaporizer from two to 98 minutes (mean 34.7 minutes) at volumes of 2 to 8 mL (mean 4.3 mL). Mean blood TCE concentrations were 2.80 ± 1.14 mg/dL in maternal femoral arteries; 2.36 ± 1.17 mg/dL in maternal cubital veins; 1.83 ± 1.08 mg/dL in umbilical vein; and 1.91 ± 0.95 mg/dL in the umbilical arteries. A significant correlation was seen for maternal arterial blood and infants‘ venous blood, and the concentration of the fetal blood was lower than that of the mother. Of these newborns, one had asphyxia and three "sleepy babies" had Apgar scores of 5–9; however, these results could not be correlated to length of inhalation and there was no difference in the TCE levels in the mother or newborn blood compared to those without adverse effects. Discussion included delayed newborn reflexes (raising the head and buttocks, bending the spine, and sound reflex), blood pressure, jaundice, and body weight gain; however, the results were compared to newborns exposed to other compounds, not to an unexposed population. This study also examined the concentration of TCE in one mother at 22-weeks gestation exposed for four minutes, after which the fetus was "artificially delivered." Maternal blood concentration was 3.0 mg/dL, and 0.9 mg/dL of TCE was found in the fetal heart, but not in other organs.

Another study of TCE administered during childbirth to the mother as an analgesic examined perinatal measures, including fetal pH, fetal partial pressure carbon dioxide (PCO_2) fetal base deficit, fetal partial pressure oxygen (PO_2), Apgar scores, and neonatal capillary blood (Phillips and Macdonald, 1971). The study consisted of 152 women whose fetus was considered to be at risk for hypoxia during labor. Out of this group, 51 received TCE (amount and route of exposure not reported). TCE caused fetal pH to fall more, base deficit increased more, and PO_2 fell more than the control group by fourfold or more compared to other analgesics used.

4.8.3.1.2. Postnatal developmental outcomes

4.8.3.1.2.1. Developmental neurotoxicity

The studies examining neurotoxic effects from TCE exposure are discussed in Section 4.3, and the human developmental neurotoxic effects are reiterated here.

4.8.3.1.2.1.1. Occupational studies

An occupational study examined the neurodevelopment of the offspring of 32 women exposed to various organic solvents during pregnancy (Laslo-Baker et al., 2004; Till et al., 2001a). Three of these women were exposed to TCE; however, no levels were measured and the results for examined outcomes are for total organic solvent exposure, and are not specific to TCE.

4.8.3.1.2.1.2. Geographic-based studies

A study of three residential cohorts (Woburn, Massachusetts; Alpha, Ohio; and Twin Cities, Minnesota) examined the neurological effects of TCE exposure in drinking water (White et al., 1997). For Woburn, Massachusetts, 28 individuals ranging from 9 to 55 years old were assessed, with exposure from a tanning factor and chemical plant at levels of 63–400 ppb for <1–12 years; the time between exposure and neurological examination was about 5 years. In this cohort, 6/13 children (46%) had impairments in the verbal naming/language domain. For Alpha, Ohio, 12 individuals ranging from 12 to 68 years old were assessed, with exposure from degreasing used at a manufacturing operation at levels of 3.3–330 ppb for 5–17 years; the time between exposure and neurological examination was 5–17 years. In this cohort, one of two children (50%) had impairments in the verbal naming/language domain. For Twin Cities, Minnesota, 14 individuals ranging from 8 to 62 years old were assessed, with exposure from an army ammunition plant at levels of 261–2,440 ppb for 0.25–25 years; the time between exposure and neurological examination was 4–22 years. In this cohort, four of four children (100%) had impairments in the verbal naming/language, memory, and academic domains and were diagnosed with moderate encephalopathy; and three of four children (75%) performed poorly on the WRAT-R Reading and Spelling and WAIS-R Information tests.

A case-control study was conducted to examine the relationship between multiple environmental agents and ASD (Windham et al., 2006). Cases (n = 284) and controls (n = 657) were born in 1994 in the San Francisco Bay Area. Cases were diagnosed before age 9 years. Exposure was determined by geocoding births to census tracts, and linking to hazardous air pollutants data. An elevated risk was seen for TCE in the upper 3rd quartile (OR: 1.37, 95% CI: 0.96–1.95), and a statistically significant elevated risk was seen for the upper 4th quartile (OR: 1.47, 95% CI: 1.03–2.08).

The TCE Subregistry (Burg and Gist, 1999; Burg et al., 1995), including 948 children <18 years old from 13 sites located in 3 states, was examined for any association of ingestion of drinking water contaminated with TCE and various health effects (ATSDR, 2003b; Burg and Gist, 1999; Burg et al., 1995). Exposure groups included: (1) maximum TCE exposure; (2) cumulative TCE exposure; (3) cumulative chemical exposure; and (4) duration of exposure. Exposed children 0–9 years old had statistically increased hearing impairment compared to controls (RR: 2.13, 99% CI: 1.12–4.07), with children <5 years old having a 5.2-fold increase over controls. Exposed children 0–9 years old also had statistically increased speech impairment (RR: 2.45, 99% CI: 1.31–4.58). In addition, anemia and other blood disorders were statistically higher for males 0–9 years old. The authors noted that exposure could have occurred prenatally or postnatally. There was further analysis on the 116 exposed children and 182 controls who were under 10 years old at the time that the baseline study was conducted by ATSDR. This analysis did not find a continued association with speech and hearing impairment in these children; however, the absence of acoustic reflexes (contraction of the middle ear muscles in response to sound) remained significant (ATSDR, 2003b). No differences were seen when stratified by prenatal and postnatal exposure.

Twenty-eight people living in a Michigan town were exposed for 5–10 years to 8–14 ppm TCE in well water (Bernad et al., 1987, abstract). Ten adults and 12 children completed a questionnaire on neurotoxic endpoints. Nine of the 12 children had poor learning ability, aggressive behavior, and low attention span.

4.8.3.1.2.2. Developmental immunotoxicity

The studies examining human immunotoxic effects from TCE exposure are discussed in Section 4.6.1. The studies reporting developmental effects are reiterated briefly here.

Two studies focused on immunological development in children after maternal exposure to VOCs (Lehmann et al., 2002; Lehmann et al., 2001). The first examined premature neonates (1,500–2,500 g) and neonates at risk of atopy (cord blood IgE >0.9 kU/L; double positive family atopy history) at 36 months of age (Lehmann et al., 2001). The median air level in children's bedrooms measured 0.42 μg/m^3. There was no association with allergic sensitization to egg white and milk, or to cytokine producing peripheral T-cells. The second examined healthy, full-term neonates (≥2,500 g; ≥37 weeks gestation) born in Lepzig, Germany (Lehmann et al., 2002).

The median air level in the children's bedrooms 3–4 weeks after birth measured 0.6 μg/m^3. A significant reduction of Th1 IL-2 producing T-cells was observed.

Byers et al. (1988) observed altered immune response in family members of children diagnosed with leukemia in Woburn, Massachusetts (Lagakos et al., 1986, see below for results of this study). The family members included 13 siblings under 19 years old at the time of exposure; however, an analysis looking at only these children was not done. This study is discussed in further detail in Section 4.6.1.

4.8.3.1.2.3. Other developmental outcomes

A study demonstrated the adverse effects of TCE used as an anesthetic in children during operations during 1964 in Poland to repair developmental defects of the jaw and face (Jasińska, 1965, translation). Fifty-five children ranging from 6 months to 10 years old were anesthetized with at least 10 mL TCE placed into an evaporator. Bradycardia occurred in two children, an accelerated heart rate of 20–25 beats per minute occurred in seven children, no arrhythmia was observed, and arterial blood pressure remained steady or dropped by 10 mmHg only. Respiratory acceleration was observed in 25 of the children, and was seen more in infants and younger children.

4.8.3.1.2.4. Childhood cancer

Several studies of parental occupational exposure were conducted in North America and the United Kingdom to determine an association with childhood cancer. A number of geographic-based studies were conducted in California; New Jersey; Woburn, Massachusetts; Endicott, New York; Phoenix, Arizona; and Tucson, Arizona. Specific categories of childhood cancers examined include leukemia, NHL, and CNS tumors.

4.8.3.1.2.4.1. Occupational studies

Brain tumors were observed in 98 children >10 years old at diagnosis from 1972–1977 in Los Angeles County (Peters et al., 1985; Peters et al., 1981). Exposure was determined by questionnaire. Two cases whose father had TCE exposure were reported: one case of oligodendroglioma in an 8-year-old whose father was a machinist, and astrocytoma in a 7-year-old whose father was an inspector for production scheduling and parts also exposed to methyl ethyl ketone (Peters et al., 1981). Peters et al. (1985) also briefly mentioned five cases of brain tumors in the offspring and no controls of paternal exposure to TCE (resulting in an inability to calculate an OR), but without providing any additional data.

A case-control study was conducted to assess an association between parental occupational exposure and neuroblastoma diagnosed in offspring <19 years old in the United States and Canada from May 1992 to April 1994 (De Roos et al., 2001). Paternal self-reported exposure to TCE was reported in 22 cases and 12 controls, resulting in an elevated risk of

neuroblastoma in the offspring (OR: 1.4, 95% CI: 0.7–2.9). Maternal exposure to TCE was not reported.

A case-control study of parental occupational exposure and childhood leukemia was conducted in Los Angeles County (Lowengart et al., 1987). Children (61 boys and 62 girls) diagnosed at <10 years old (mean age 4 years) from 1980 to 1984 were included in the analysis. Paternal occupation exposure to TCE was elevated for 1 year preconception (OR: 2.0, $p = 0.16$), prenatal (OR: 2.0, $p = 0.16$), and postnatal (OR: 2.7, $p = 0.7$) exposure periods. Maternal exposure to TCE was not reported.

A case-control study children diagnosed with acute lymphoblastic leukemia (ALL) examined parental occupational exposure to hydrocarbons in the United States and Canada (Shu et al., 1999). Children were under the age of 15 years at diagnosis during the years 1989–1993. Cases were confirmed with a bone marrow sample. Questionnaires on maternal and paternal exposures were given to 1,842 case-control pairs, resulting in 15 cases and 9 controls maternally exposed and 136 cases and 104 controls paternally exposed to TCE. There was an increased but nonsignificant risk for maternal exposure to TCE during preconception (OR: 1.8, 95% CI: 0.6–5.2), pregnancy (OR: 1.8, 95% CI: 0.5–6.4), postnatally (OR: 1.4, 95% CI: 0.5–4.1), or any of these periods (OR: 1.8, 95% CI: 0.8–4.1). However, there was no increased risk for paternal exposure to TCE.

Occupational exposure in communities in the United Kingdom was examined to determine an association with leukemia and NHL diagnosed in the offspring (McKinney et al., 1991). Paternal occupational exposure was elevated for exposure occurring during preconception (OR: 2.27, 95% CI: 0.84–6.16), prenatal (OR: 4.40, 95% CI: 1.15–21.01), and postnatal (OR: 2.66, 95% CI: 0.82–9.19) exposure periods. Risk from maternal preconception exposure was not elevated (OR: 1.16, 95% CI: 0.13–7.91). However, the number of cases examined in this study was low, particularly for maternal exposure.

4.8.3.1.2.4.2. Geographic-based studies

A California community exposed to TCE (0.09–97 ppb) in drinking water from contaminated wells was examined for cancer (Morgan and Cassady, 2002). A specific emphasis was placed on the examination of 22 cases of childhood cancer diagnosed before 15 years old. However, the incidence did not exceed those expected for the community for total cancer (SIR: 0.83, 99% CI: 0.44–1.40), CNS cancer (SIR: 1.05, 99% CI: 0.24–2.70), or leukemia (SIR: 1.09, 99% CI: 0.38–2.31).

An examination of drinking water was conducted in four New Jersey counties to determine an association with leukemia and NHL (Cohn et al., 1994b). A number of contaminants were reported, including VOCs and trihalomethanes. TCE was found as high as 67 ppb, and exposure categories were assigned to be >0.1, 0.1–5, and >5 ppb. A significantly elevated dose-response risk for ALL was observed for girls diagnosed before 20 years old (RR:

3.36, 95% CI: 1.29–8.28), which was increased among girls diagnosed before 5 years old (RR:4.54, 95% CI: 1.47–10.6). A significantly elevated dose-response risk for girls was also observed for total leukemia (RR: 1.43, 95% CI: 1.07–1.98).

The Woburn, Massachusetts community with contaminated well water experienced an increase in the incidence of childhood leukemia (Costas et al., 2002; MDPH, 1997b; Cutler et al., 1986; Lagakos et al., 1986). An initial study examined 12 cases of childhood leukemia diagnosed in children <15 years old between 1969 and 1979, when 5.2 cases were expected, and a higher risk was observed in boys compared to girls; however, no factors were observed to account for this increase (Cutler et al., 1986). Another study observed statistically significant positive association between access to contaminated water; 20 cases of childhood cancer were observed for both cumulative exposure metric (OR: 1.39, $p = 0.03$), and none vs. some exposure metric (OR: 3.03, $p = 0.02$) (Lagakos et al., 1986). Massachusetts Department of Public Health (MDPH, 1997b) conducted a case-control study of children <20 years old living in Woburn and diagnosed with leukemia between 1969 and 1989 (n = 21) and observed that consumption of drinking water increased the risk of leukemia (OR: 3.03, 95% CI: 0.82–11.28), with the highest risk from exposure during fetal development (OR: 8.33, 95% CI: 0.73–94.67). This study found that paternal occupational exposure to TCE was not related to leukemia in the offspring (MDPH, 1997b). In the most recent update, Costas et al. (2002) reported that between the years 1969 and 1997, 24 cases of childhood leukemia were observed when 11 were expected. Risk was calculated for cumulative exposure to contaminated drinking water 2 years prior to conception (OR_{adj}: 2.61, 95% CI: 0.47–14.97), during pregnancy (OR_{adj}: 8.33, 95% CI: 0.73–94.67), postnatal (OR_{adj}: 1.18, 95% CI: 0.28–5.05), and any of these time periods (OR_{adj}: 2.39, 95% CI: 0.54–10.59). A dose-response was observed during pregnancy only. Cases were more likely to be male (76%), <9 years old at diagnosis (62%), breast-fed (OR: 10.17, 95% CI: 1.22–84.50), and exposed during pregnancy (adjusted OR [OR_{adj}]: 8.33, 95% CI: 0.73–94.67). A dose-response was seen during the pregnancy exposure period, with the most exposed having an OR_{adj} of 14.30 (95% CI: 0.92–224.52). Other elevated risks observed included maternal alcohol intake during pregnancy (OR: 1.50, 95% CI: 0.54–4.20), having a paternal grandfather diagnosed with cancer (OR: 2.01, 95% CI: 0.73–5.58), father employed in a high risk industry (OR: 2.55, 95% CI: 0.78–8.30), and public water being the subject‘s primary beverage (OR: 3.03, 95% CI: 0.82–11.28). (Also see sections on spontaneous abortion, perinatal death, decreased birth weight, and congenital malformations for additional results from this cohort.)

The study of VOC exposure in Endicott, New York discussed above observed fewer than six cases of cancer that were diagnosed between 1980 and 2001 in children <20 years old, and did not exceed expected cases or types (ATSDR, 2006a). (See section on spontaneous abortion for study details, and sections on spontaneous abortion, decreased birth weight, and congenital malformations for additional results from this cohort.)

The AZ DHS conducted a number of studies of contaminated drinking water and 189 cases of childhood cancer (<20 years old) (Flood, 1997a; ADHS, 1990; Kioski et al., 1990a; Kioski et al., 1990b; Flood, 1988). In Maricopa County, which encompasses Phoenix and the surrounding area, TCE contamination (8.9 and 29 ppb in two wells) was associated with elevated levels of childhood leukemia (n = 67) in west central Phoenix during 1965–1986 (SIR: 1.67, 95% CI: 1.20–2.27) and 1982–1986 (SIR: 1.91, 95% CI: 1.11–3.12), but did not observe a significant increase in total childhood cancers, lymphoma, brain/CNS, or other cancers during these time periods (ADHS, 1990). (See above for results from this study on congenital anomalies.) A follow-up study retrospectively asked parents about exposures and found that residence within 2 miles of wells contaminated with TCE was not a risk factor for childhood leukemia, but identified a number of other risk factors (Flood, 1997a). A further study of East Phoenix, reported on TCE contamination found along with 1,1,1-trichloroethane and 25 other contaminants in well water (levels not reported) and found no increase in incidence of childhood leukemia (SIR: 0.85, 95% CI: 0.50–1.35) based on 16 cases (Kioski et al., 1990b). There were also 16 cases of other types of childhood cancer, but were too few to be analyzed separately. In Pima County, which encompasses Tucson and the surrounding area, TCE was found in drinking wells (1.1–239 ppb), along with 1,1-DCE, chloroform, and chromium and found a nonstatistically elevated risk of leukemia was observed (SIR: 1.50, 95% CI: 0.76–2.70), but no risk was observed for testicular cancer, lymphoma, or CNS/brain cancer (Kioski et al., 1990a).

4.8.3.1.3. Summary of human developmental toxicity

Epidemiological developmental studies examined the association between TCE exposure and a number of prenatal and postnatal developmental outcomes. Prenatal developmental outcomes examined include spontaneous abortion and perinatal death; decreased birth weight, SGA, and postnatal growth; congenital malformations; and other adverse birth outcomes. Postnatal developmental outcomes examined include developmental neurotoxicity, developmental immunotoxicity, other developmental outcomes, and childhood cancer related to TCE exposure.

More information on developmental outcomes is expected. A follow-up study of the Camp Lejeune cohort (ATSDR, 1998a) for birth defects and childhood cancers was initiated in 1999 (ATSDR, 2003a) and expected to be completed soon (ATSDR, 2009; U.S. GAO, 2007a, b). Out of a total of 106 potential cases of either birth defects or childhood cancer, 57 have been confirmed and will constitute the cases. These will be compared 548 control offspring of mothers who also lived at Camp Lejeune during their pregnancy from 1968 to 1985. As part of this study, a drinking water model was developed to determine a more accurate level and duration of exposure to these pregnant women (ATSDR, 2007a). Additional health studies have been suggested, including adverse neurological or behavioral effects or pregnancy loss.

4.8.3.2. Animal Developmental Toxicology Studies

A number of animal studies have been conducted to assess the potential for developmental toxicity of TCE. These include studies conducted in rodents by prenatal inhalation or oral exposures (summarized in Tables 4-96 and 4-97), as well as assessments in nonmammalian species (e.g., avian, amphibian, and invertebrate species) exposed to TCE during development. Studies have been conducted that provide information on the potential for effects on specific organ systems, including the developing nervous, immune, and pulmonary systems. Additionally, a number of research efforts have focused on further characterization of the mode of action for cardiac malformations that have been reported to be associated with TCE exposure.

Table 4-96. Summary of mammalian in vivo developmental toxicity studies—inhalation exposures

Reference[a]	Species/strain/ sex/number	Exposure level/ duration	NOAEL; LOAEL[b]	Effects
Carney et al. (2006)	**Rat, Sprague-Dawley, females, 27 dams/group**	**0, 50, 150, or 600 ppm (600 ppm = 3.2 mg/L)[c] 6 hrs/d; GDs 6–20**	**Maternal NOAEL: 150 ppm Maternal LOAEL: 600 ppm**	**↓ Body weight gain (22% less than control) on GDs 6–9 at 600 ppm.**
			Developmental NOAEL: 600 ppm	No evidence of developmental toxicity, including heart defects.
Dorfmueller et al. (1979)	Rat, Long-Evans, females, 30 dams/group	0 or 1,800 ± 200 ppm (9,674 ± 1,075 mg/m^3)[c] 2 wks, 6 hrs/d, 5 d/wk; prior to mating and/or on GDs 0–20	Maternal NOAEL: 1,800 ± 200 ppm	No maternal abnormalities.
			Developmental LOAEL: 1,800 ± 200 ppm	Statistically significant ↑ skeletal and soft tissue anomalies in fetuses from dams exposed during pregnancy only. No statistically significant treatment effects on behavior of offspring 10, 20, or 100 d postpartum. Body weight gains statistically significant ↓ in pups from dams with pregestational exposure.
Hardin et al. (1981)	Rat, Sprague-Dawley, female, nominal 30/group	0 or 500 ppm 6–7 hrs/d; GDs 1–19	Maternal NOAEL: 500 ppm	No maternal toxicity.
			Developmental NOAEL: 500 ppm	No embryonic or fetal toxicity.
	Rabbit, New Zealand white, female, nominal 20/group	0 or 500 ppm 6–7 hrs/d; GDs 1–24	Maternal NOAEL: 500 ppm	No maternal toxicity.
			Developmental LOAEL: 500 ppm	Hydrocephaly observed in two fetuses of two litters, considered equivocal evidence of teratogenic potential.
Healy et al. (1982)	**Rat, Wistar, females, 31–32 dams/group**	**0 or 100 ppm 4 hrs/d; GDs 8–21**	Maternal NOAEL: 100 ppm	No maternal abnormalities.
			Developmental LOAEL: 100 ppm	**Litters with total resorptions statistically significant ↑. Statistically significant ↓ fetal weight, and ↑ bipartite or absent skeletal ossification centers.**

Table 4-96. Summary of mammalian in vivo developmental toxicity studies—inhalation exposures

Reference[a]	Species/strain/ sex/number	Exposure level/ duration	NOAEL; LOAEL[a]	Effects
Schwetz et al. (1975)	**Rat, Sprague-Dawley, female, 20–35/group Mouse, Swiss-Webster, females, 30–40 dams/group**	**0 or 300 ppm** **7 hrs/d; GDs 6–15**	**Maternal LOAEL: 300 ppm**	**4–5% ↓ maternal body weight**
			Developmental NOAEL: 300 ppm	No embryonic or fetal toxicity; not teratogenic.
Westergren et al. (1984)	Mouse, NMRI, male and female, 6–12 offspring/group	0 or 150 ppm 24 hrs/d; 30 d (during 7 d of mating and until GD 22)	Developmental LOAEL[d]: 150 ppm[c]	Specific gravity of brains statistically significant ↓ at PNDs 0, 10, and 20–22. Similar effects at PNDs 20–22 in occipital cortex and cerebellum. No effects at 1 mo of age.

[a]**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5).
[b]NOAEL and LOAEL are based upon reported study findings.
[c]Dose conversions provided by study author(s).
[d]Parental observations not reported.

Table 4-97. Ocular defects observed (Narotsky et al., 1995)

Dose TCE (mg/kg-d)	Incidence (number affected pups/total number pups)[a]	Percentage of pups with eye defects
0	1/197	0.51
10.1	0/71	0.00
32	0/85	0.00
101	3/68	4.41
320	3/82	3.66
475	6/100	6.00
633	6/100	6.00
844	7/58	12.07
1,125	12/44	27.27

[a]Reported in Barton and Das (1996).

4.8.3.2.1. Mammalian studies

Studies that have examined the effects of TCE on mammalian development following either inhalation or oral exposures are described below and summarized in Tables 4-96 and 4-98, respectively.

Table 4-98. Summary of mammalian in vivo developmental toxicity studies—oral exposures

Reference[a]	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL[b]	Effects
Blossom and Doss (2007)	Mouse, MRL +/+, dams and both sexes offspring, 3 litters/group, 8–12 offspring/group	0, 0.5, or 2.5 mg/mL Parental mice and/or offspring exposed from GD 0 to 7–8 mo of age	Drinking water	Developmental LOAEL = 0.5 mg/mL[c]	At 0.5 mg/mL: statistically significant ↓ postweaning weight; statistically significant↑ IFNγ produced by splenic CD4+ cells at 5–6 wks; statistically significant ↓ splenic CD8+and B220+ lymphocytes; statistically significant ↑ IgG2a and histone; statistically significant altered CD4–/CD8– and CD4+/CD8+ thymocyte profile. At 2.5 mg/mL: statistically significant ↓ postweaning weight; statistically significant ↑ IFNγ produced by splenic CD4+ and CD8+ cells at 4–5 and 5–6 wks; statistically significant ↓ splenic CD4+, CD8+, and B220+ lymphocytes; statistically significant altered CD4+/CD8+ thymocyte profile.
Blossom et al. (2008)	Mouse, MRL +/+, dams and both sexes offspring, 8 litters/group, 3–8 offspring/group	0 or 0.1 mg/mL (maternal dose = 25.7 mg/kg-d; offspring PNDs 24–42 dose— 31.0 mg/kg-d Parental mice and/or offspring exposed from GD 0 to PND 42	Drinking water	Developmental LOAEL = 1,400 ppb[c]	At 0.1 mg/mL: at PND 20, statistically significant ↑ thymocyte cellularity and distribution, associated with statistically significant ↑ in thymocyte subset distribution; statistically significant ↑ reactive oxygen species generation in total thymocytes; statistically significant ↑ in splenic CD4+ T-cell production of IFN-γ and IL-2 in females and TNF-α in males at PND 42. Significantly impaired nest-building behaviors at PND 35. Increased aggressive activities, and increased oxidative stress and impaired thiol status in the cerebellar tissue of male offspring at PND 40.

Table 4-98. Summary of mammalian in vivo developmental toxicity studies—oral exposures (continued)

Reference[a]	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL[a]	Effects
Collier et al. (2003)	Rat, Sprague-Dawley, female, number dams/group not reported	0, 0.11, or 1.1 mg/mL (0, 830, or 8,300 µgM)[d] GDs 0–11	Drinking water	Developmental LOEL: 0.11 mg/mL	Embryos collected between GDs 10.5 and 11. Gene expression at 1.1 mg/mL TCE: 8 housekeeping genes ↑, and one gene ↓; 3 stress response genes ↑, IL-10 ↓; 2 cyto-skeletal/cell adhesion/blood related genes ↑, 3 genes ↓; 2 heart-specific genes ↑. Effects at 0.11 mg/mL reduced considerably. Two possible markers for fetal TCE exposure identified as Serca-2 Ca^{+2} ATPase and GPI-p137.
Cosby and Dukelow (1992)	Mouse, B6D2F1, female, 28–62 dams/group	0, 24, or 240 mg/kg-d GDs 1–5, 6–10, or 11–15	Gavage in corn oil	Maternal NOAEL: 240 mg/kg-d	No maternal toxicity.
				Developmental NOAEL: 240 mg/kg-d	No effects on embryonic or fetal development.
Dawson et al. (1993)	Rat, Sprague-Dawley, 116 females allocated to 11 groups	0, 1.5, or 1,100 ppm 2 mo before mating and/or during gestation	Drinking water	Maternal NOAEL: 1,100 ppm	No maternal toxicity.
				Developmental LOAEL: 1.5 ppm	Statistically significant ↑ in heart defects, primarily atrial septal defects, found at both dose levels in groups exposed prior to pregnancy and during pregnancy, as well as in group exposed to 1,100 ppm dose during pregnancy only. No statistically significant ↑ in congenital heart defects in groups exposed prior to pregnancy only.
Fisher et al. (2001); Warren et al. (2006)	Rat, Sprague-Dawley, female, 20–25 dams/group	0 or 500 mg/kg-d GDs 6–15	Gavage in soybean oil	Maternal NOAEL: 500 mg/kg-d	No maternal toxicity.
				Developmental NOAEL: 500 mg/kg-d	No developmental toxicity. The incidence of heart malformations for fetuses from TCE-treated dams (3–5%) did not differ from negative controls. No eye defects observed.

Table 4-98. Summary of mammalian in vivo developmental toxicity studies—oral exposures (continued)

Reference[a]	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL[a]	Effects
Fredriksson et al. (1993)	Mouse, NMRI, male pups, 12 pups from 3−4 different litters/group	0, 50, or 290 mg/kg-d PNDs 10–16	Gavage in a 20% fat emulsion prepared from egg lecithin and peanut oil	Developmental LOAEL: 50 mg/kg-d	Rearing activity statistically significant ↓ at both dose levels on PND 60.
George et al. (1986)	Rat, F334, male and female, 20 pairs/treatment group, 40 controls/sex	0, 0.15, 0.30, or 0.60% micro-encapsulated TCE Breeders exposed 1 wk premating, then for 13 wks; pregnant ♀s throughout pregnancy (i.e., 18 wks total)	Dietary	LOAEL: 0.15%	Open field testing in pups: a statistically significant dose-related trend toward ↑ time required for male and female pups to cross the first grid in the test devise.
Isaacson and Taylor (1989)	Rat, Sprague-Dawley, females, 6 dams/group	0, 312, or 625 mg/L (0, 4.0, or 8.1 mg/d)[d] Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Developmental LOAEL: 312 mg/L[b]	Statistically significant ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the hippocampus.
Johnson et al. (2003)	Rat, Sprague-Dawley, female, 9–13/group, 55 in control group	0, 2.5, 250, 1.5, or 1,100 ppm (0, 0.00045, 0.048, 0.218, or 129 mg/kg-d)[d] GDs 0–22	Drinking water	Developmental NOAEL: 2.5 ppb Developmental LOAEL: 250 ppb[b]	Statistically significant ↑ in percentage of abnormal hearts and the percentage of litters with abnormal hearts at ≥250 ppb.
Narotsky et al. (1995)	Rat, F344, females, 8–12 dams/group	0, 10.1, 32, 101, 320, 475, 633, 844, or 1,125 mg/kg-d GDs 6–15	Gavage in corn oil	Maternal LOAEL: 475 mg/kg-d	Statistically significant dose-related ↓ dam body weight gain at all dose levels on GDs 6–8 and 6–20. Delayed parturition at ≥475 mg/kg-d; ataxia at ≥633 mg/kg-d; mortality at 1,125 mg/kg-d.

Table 4-98. Summary of mammalian in vivo developmental toxicity studies—oral exposures (continued)

Reference[a]	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL[a]	Effects
Narotsky et al. (1995) (continued)				**Developmental NOAEL: 32 mg/kg-d Developmental LOAEL: 101 mg/kg-d**	**↑ full litter resorption and postnatal mortality at ≥425 mg/kg-d. Statistically significant prenatal loss at 1,125 mg/kg-d. Pup body weight ↓ (not statistically significant) on PNDs 1 and 6. Statistically significant ↑ in pups with eye defects at 1,125 mg/kg-d. Dose-related (not statistically significant) ↑ in pups with eye defects at ≥101 mg/kg-d.**
Narotsky and Kavlock (1995)	Rat, F344, females, 16–21 dams/group	0, 1,125, or 1,500 mg/kg-d GDs 6–19	Gavage in corn oil	Maternal LOAEL: 1,125 mg/kg-d	Ataxia, ↓ activity, piloerection; dose-related ↓ body weight gain.
				Developmental LOAEL: 1,125 mg/kg-d	Statistically significant ↑ full litter resorptions, ↓ live pups/litter; statistically significant ↓ pup body weight on PND 1; statistically significant ↑ incidences of microophthalmia and anophthalmia.
Noland-Gerbec et al. (1986)	Rat, Sprague-Dawley, females, 9–11 dams/group	0 or 312 mg/L (Average total intake of dams: 825 mg TCE over 61 d)[d] Dams (and pups) exposed from 14 d prior to mating until end of lactation	Drinking water	Developmental LOEL: 312 mg/L[b]	Statistically significant ↓ uptake of [^{3}H]-2-DG in whole brains and cerebella (no effect in hippocampus) of exposed pups at 7, 11, and 16 d, but returned to control levels by 21 d.

Table 4-98. Summary of mammalian in vivo developmental toxicity studies—oral exposures (continued)

Reference[a]	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL[a]	Effects
Peden-Adams et al. (2006)	**Mouse, B6C3F$_1$, dams and both sexes offspring, 5 dams/group; 5–7 pups/group at 3 wks; 4–5 pups/sex/group at 8 wks**	**0, 1,400, or 14,000 ppb Parental mice and/or offspring exposed during mating, and from GD 0 thru 3 or 8 wks of age**	**Drinking water**	**Developmental LOAEL: 1,400 ppb[c]**	**At 1,400 ppb: Suppressed PFC responses in males at 3 and 8 wks of age and in females at 8 wks of age. Delayed hypersensitivity response increased at 8 wks of age in females. At 14,000 ppb: Suppressed PFC responses in males and females at 3 and 8 wks of age. Splenic cell population decreased in 3-wk-old pups. Increased thymic T-cells at 8 wks of age. Delayed hypersensitivity response increased at 8 wks of age in males and females.**
Peden-Adams et al. (2008)	Mouse, MRL +/+, dams and both sexes offspring, unknown number litters/group, 6–10 offspring/sex/ group	0, 1,400, or 14,000 ppb (vehicle = 1% Emulphor) Parental mice and/or offspring exposed from GD 0 to 12 mo of age	Drinking water	Developmental LOAEL = 1,400 ppb[c]	At 1,400 ppb: splenic CD4–/CD8– cells statistically significant ↑ in females; thymic CD4+/CD8+ cells statistically significant ↓ in males; 18% ↑ in male kidney weight. At 14,000 ppb: thymic T-cell subpopulations (CD8+, CD4/CD8–, CD4+) statistically significant ↓ in males.
Taylor et al. (1985)	**Rat, Sprague-Dawley, females, number dams/group not reported**	**0, 312, 625, or 1,250 mg/L Dams (and pups) exposed from 14 d prior to mating until end of lactation**	**Drinking water**	**Developmental LOAEL: 312 mg/L[c]**	**Exploratory behavior statistically significant ↑ in 60- and 90-d old male rats at all treatment levels. Locomotor activity was higher in rats from dams exposed to 1,250 ppm TCE.**

[a]**Bolded studies** carried forward for consideration in dose-response assessment (see Chapter 5).
[b]NOAEL, LOAEL, and LOEL are based upon reported study findings.
[c]Dose conversions provided by study author(s).
[d]Maternal observations not reported.

4.8.3.2.1.1. Inhalation exposures

Dorfmueller et al. (1979) conducted a study in which TCE was administered by inhalation exposure to groups of approximately 30 female Long-Evans hooded rats at a concentration of 1,800 ± 200 ppm before mating only, during gestation only, or throughout the premating and gestation periods. Half of the dams were killed at the end of gestation and half

were allowed to deliver. There were no effects on body weight change or relative liver weight in the dams. The number of corpora lutea, implantation sites, live fetuses, fetal body weight, resorptions, and sex ratio were not affected by treatment. In the group exposed only during gestation, a significant increase in four specific sternebral, vertebral, and rib findings, and a significant increase in displaced right ovary were observed upon fetal skeletal and soft tissue evaluation. Mixed function oxidase enzymes (ethoxycoumarin and ethoxyresorbin), which are indicative of CYP and P448 activities, respectively, were measured in the livers of dams and fetuses, but no treatment-related findings were identified. Postnatal growth was significantly ($p < 0.05$) decreased in the group with gestation-only exposures. Postnatal behavioral studies, consisting of an automated assessment of ambulatory response in a novel environment on GDs 10, 20, and 100, did not identify any effect on general motor activity of offspring following in utero exposure to TCE.

In a study by Schwetz et al. (1975), pregnant Sprague-Dawley rats and Swiss Webster mice (30–40 dams/group) were exposed to TCE via inhalation at a concentration of 300 ppm for 7 hours/day on GDs 6–15. The only adverse finding reported was a statistically significant 4–5% decrease in maternal rat body weight. There were no treatment-related effects on pre- and postimplantation loss, litter size, fetal body weight, crown-rump length, or external, soft tissue, or skeletal findings.

Hardin et al. (1981) summarized the results of inhalation developmental toxicology studies conducted in pregnant Sprague-Dawley rats and New Zealand white rabbits for a number of industrial chemicals, including TCE. Exposure concentrations of 0 or 500 ppm TCE were administered for 6–7 hours/day, on GDs 1–19 (rats) or 1–24 (rabbits), and cesarean sections were conducted on GDs 21 or 30, respectively. There were no adverse findings in maternal animals. No statistically significant increase in the incidence of malformations was reported for either species; however, the presence of hydrocephaly in two fetuses of two TCE-treated rabbit litters was interpreted as a possible indicator of teratogenic potential.

Healy et al. (1982) did not identify any treatment-related fetal malformations following inhalation exposure of pregnant inbred Wistar rats to 0 or 100 ppm (535 mg/m^3) on GDs 8–21. In this study, significant differences between control and treated litters were observed as an increased incidence of total litter loss ($p < 0.05$), decreased mean fetal weight ($p < 0.05$), and increased incidence of minor ossification variations ($p = 0.003$) (absent or bipartite centers of ossification).

Carney et al. (2006) investigated the effects of whole-body inhalation exposures to pregnant Sprague-Dawley rats at nominal (and actual) chamber concentrations of 0, 50, 150, or 600 ppm TCE for 6 hours/day, 7 days/week, on GDs 6–20. This study was conducted under Good Laboratory Practice regulations according to current EPA and Organisation for Economic Co-operation and Development (OECD) regulatory testing guidelines (i.e., OPPTS 870.3700 and OECD GD 414). Maternal toxicity consisted of a statistically significant decrease (22%) in body

weight gain during the first 3 days of exposure to 600-ppm TCE, establishing a no-observed-effect concentration (NOEC) of 150 ppm for dams. No significant difference between control and TCE-treated groups was noted for pregnancy rates, number of corpora lutea, implantations, viable fetuses per litter, percentage pre- and postimplantation loss, resorption rates, fetal sex ratios, or gravid uterine weights. External, soft tissue, and skeletal evaluation of fetal specimens did not identify any treatment-related effects. No cardiac malformations were identified in treated fetuses. The fetal NOEC for this study was established at 600 ppm.

Westergren et al. (1984) examined brain specific gravity of NMRI mice pups following developmental exposures to TCE. Male and female mice were separately exposed 24 hours/day (except for limited periods of animal husbandry activities) to 0 or 150 ppm TCE for 30 days and mated during exposure for 7 days. Exposure of the females was continued throughout gestation, until the first litter was born. Offspring (6–12/group; litter origin not provided in report) were terminated on PNDs 1, 10, 21–22, or 30. The specific gravity of the brain frontal cortex, cortex, occipital cortex, and cerebellum were measured. The cortex specific gravity was significantly decreased at PND 1 ($p < 0.001$) and 10 ($p < 0.01$) in pups from exposed mice. There were also significant differences ($p < 0.05$) in the occipital cortex and cerebellum at PNDs 20–22. This was considered suggestive of delayed maturation. No significant differences between control and treated pups were observed at 1 month of age.

4.8.3.2.1.2. Oral exposures

A screening study conducted by Narotsky and Kavlock (1995) assessed the developmental toxicity potential of a number of pesticides and solvents, including TCE. In this study, F344 rats were administered TCE by gavage at 0, 1,125, and 1,500 mg/kg-day on GDs 6–19, and litters were examined on GDs 1, 3, and 6. TCE-related increased incidences of full-litter resorptions, decreased litter sizes, and decreased mean pup birth weights were observed at both treatment levels. Additionally, TCE treatment was reported to be associated with increased incidences of eye abnormalities (microphthalmia or anophthalmia). Increased incidences of fetal loss and percentage of pups with eye abnormalities were confirmed by Narotsky et al. (1995) in a preliminary dose-setting study that treated F344 rats with TCE by gavage doses of 475, 633, 844, or 1,125 mg/kg-day on GDs 6–15, and then in a 5 × 5 × 5 mixtures study that used TCE doses of 0, 10.1, 32, 101, and 320 mg/kg-day on GDs 6–15. In both studies, dams were allowed to deliver, and pups were examined postnatally. The incidence of ocular defects observed across all TCE treatment levels tested is presented in Table 4-97.

Other developmental findings in this study included increased full litter resorption at 475, 844, and 1,125 mg/kg-day; increased postnatal mortality at 425 mg/kg-day. Pup body weights were decreased (not significantly) on PNDs 1 and 6 at 1,125 mg/kg-day. In both the Narotsky and Kavlock (1995) and Narotsky et al. (1995) studies, significantly decreased maternal body weight gain was observed at the same treatment levels at which full litter resorption was noted.

Additionally, in Narotsky et al. (1995), maternal observations included delayed parturition at 475, 844, and 1,125 mg/kg-day, ataxia at 633 mg/kg-day, and mortality at 1,125 mg/kg-day.

Cosby and Dukelow (1992) administered TCE in corn oil by gavage to female B6D2F1 mice (28–62/group) on GDs 1–5, 6–10, or 11–15 (where mating = GD 1). Dose levels were 0, 1/100, and 1/10 of the oral LD_{50} (i.e., 0, 24.02, and 240.2 mg/kg body weight). Dams were allowed to deliver; litters were evaluated for pup count sex, weight, and crown-rump length until weaning (PND 21). Some litters were retained until 6 weeks of age, at which time gonads (from a minimum of 2 litters/group) were removed, weighed, and examined. No treatment-related reproductive or developmental abnormalities were observed.

A single dose of TCE was administered by gavage to pregnant CD-1 mice (9–19/group) at doses of 0, 0.1, or 1.0 µg/kg in distilled water, or 0, 48.3, or 483 mg/kg in olive oil, 24 hours after premating human chorionic gonadotropin (hCG) injection (Coberly et al., 1992). At 53 hours after the hCG-injection, the dams were terminated, and the embryos were flushed from excised oviducts. Chimera embryos were constructed, cultured, and examined. Calculated proliferation ratios did not identify any differences between control and treated blastomeres. A lack of treatment-related adverse outcome was also noted when the TCE was administered by i.p. injection to pregnant mice (16–39/group) at 24 and 48 hours post-hCG at doses of 0, 0.01, 0.02, or 10 µg/kg body weight.

In a study intended to confirm or refute the cardiac teratogenicity of TCE that had been previously observed in chick embryos, Dawson et al. (1990) continuously infused the gravid uterine horns of Sprague-Dawley rats with solutions of 0, 15, or 1,500 ppm TCE (or 1.5 or 150-ppm DCE) on GDs 7–22. At terminal cesarean section on GD 22, the uterine contents were examined, and fetal hearts were removed and prepared for further dissection and examination under a light microscope. Cardiac malformations were observed in 3% of control fetuses, 9% of the 15-ppm TCE fetuses ($p = 0.18$), and 14% of the 1,500-ppm TCE fetuses. ($p = 0.03$). There was a >60% increase in the percentage of defects with a 100-fold increase in dose. No individual malformation or combination of abnormalities was found to be selectively induced by treatment.

To further examine these TCE-induced cardiac malformations in rats, Dawson et al. (1993) administered 0, 1.5 or 1,100-ppm TCE in drinking water to female Sprague-Dawley rats. Experimental treatment regimens were: (1) a period of approximately 2 months prior to pregnancy plus the full duration of pregnancy; (2) the full duration of pregnancy only; or (3) an average of 3 months before pregnancy only. The average total daily doses of TCE consumed for each exposure group at both dose levels were

	1.5 ppm	1,100 ppm
Group 1	23.5 µL	1,206 µL
Group 2	0.78 µL	261 µL
Group 3	3.97 µL	1,185 µL

The study also evaluated 0, 0.15, or 110 ppm DCE in drinking water, with treatment administered: (1) 2 months prior to pregnancy plus the full duration of pregnancy, or (2) an average of 2 months before pregnancy only. At terminal cesarean section, uterine contents were examined, fetuses were evaluated for external defects, and the heart of each fetus was removed for gross histologic examination under a dissecting microscope, conducted without knowledge of treatment group. There were no differences between TCE-treated and control group relative to percentage of live births, implants, and resorptions. The percentage of cardiac defects in TCE-treated groups ranged from 8.2 to 13.0%, and was statistically significant as compared to the control incidence of 3%. The dose-response was relatively flat, even in spite of the extensive difference between the treatment levels. There was a broad representation of various types of cardiac abnormalities identified, notably including multiple transposition, great artery, septal, and valve defects (see Table 4-99). No particular combination of defects or syndrome predominated. Exposure before pregnancy did not appear to be a significant factor in the incidence of cardiac defects.

Table 4-99. Types of congenital cardiac defects observed in TCE-exposed fetuses

Cardiac abnormalities	Control	TCE concentrations					
		Premating		Premating/gestation		Gestation only	
		1,100 ppm	1.5 ppm	1,100 ppm	1.5 ppm	1,100 ppm	1.5 ppm
d-Transposition (right chest)	2						
l-Transposition (left chest)					2		1
Great artery defects				1	2		1
Atrial septal defects	1	7	3	19	5	7	4
Mitral valve defects				5	8		
Tricuspid valve defects		1		1	2		
Ventricular septal defects							
Subaortic	1			4	1	1	2
Membranous				2			
Muscular	2	1	1	4		4	1
Endocardial cushion defect	1					1	
Pulmonary valve defects			3	2	1		1
Aortic valve defects			1	2	2	2	
Situs inversus				1			
Total abnormalities	7	9	8	41	23	15	10
Total abnormal hearts	7	9	8	40	23	11	9

Source: (Dawson et al., 1993, Table 3).

In an attempt to determine a threshold for cardiac anomalies following TCE exposures, Johnson et al. (Johnson et al., 2005, 2003) compiled and reanalyzed data from five studies

conducted from 1989 to 1995. In these studies, TCE was administered in drinking water to Sprague-Dawley rats throughout gestation (i.e., a total of 22 days) at levels of 2.5 ppb (0.0025 ppm), 250 ppb (0.25 ppm), 1.5, or 1,100 ppm. The dams were terminated on the last day of pregnancy and fetuses were evaluated for abnormalities of the heart and great vessels. The control data from the five studies were combined prior to statistical comparison to the individual treated groups, which were conducted separately. The study author reported that significant increases in the percentage of abnormal hearts and the percentage of litters with abnormal hearts were observed in a generally dose-responsive manner at ≥250 ppb (see Table 4-100).

Table 4-100. Types of heart malformations per 100 fetuses

Type of defect/100 fetuses	Control	TCE dose group			
		1,100 ppm	1.5 ppm	250 ppb	2.5 ppb
Abnormal looping	0.33		1		
Coronary artery/sinus				1.82	
Aortic hypoplasia			0.55		
Pulmonary artery hypoplasia			0.55		
Atrial septal defect	1.16	6.67	2.21	0.91	
Mitral valve defect	0.17			0.91	
Tricuspid valve defect				0.91	
Ventricular septal defect					
Perimembranous (subaortic)	0.33	2.86	1.66		
Muscular	0.33	0.95	0.55		
Atriventricular septal defect	0.17	0.95			
Pulmonary valve defect					
Aortic valve defects		1.9		0.91	
Fetuses with abnormal hearts (n)	13	11	9	5	0
Total fetuses (n)	606	105	181	110	144
Litters with fetuses with abnormal hearts/litter (n)	9/55	6/9	5/13	4/9	0/12
Litter with fetuses with abnormal hearts/number litters (%)	16.4	66.7	38.5	44.4	0.0

Source: (Johnson et al., 2003, Table 2, p. 290).

In a study by Fisher et al. (2001), pregnant Sprague-Dawley rats were administered daily gavage doses on GDs 6–15 of TCE (500 mg/kg-day), TCA (300 mg/kg-day), or DCA (300 mg/kg-day). Cesarean delivery of fetuses was conducted on GD 21. Water and soybean oil negative control groups, and a retinoic acid positive control group were also conducted simultaneously. Maternal body weight gain was not significantly different from control for any of the treated groups. No significant differences were observed for number of implantations, resorptions, or litter size. Mean fetal body weight was reduced by treatment with TCA and DCA. The incidence of heart malformations was not significantly increased in treated groups as

compared to controls. The fetal rate of cardiac malformations ranged from 3 to 5% across the TCE, TCA, and DCA dose groups and from 6.5 to 2.9% for the soybean and water control dose groups, respectively. It was suggested that the apparent differences between the results of this study and the Dawson et al. (1993) study may be related to factors such as differences in purity of test substances or in the rat strains, or differences in experimental design (e.g., gavage vs. drinking water, exposure only during the period of organogenesis versus during the entire gestation period, or the use of a staining procedure). The rats from this study were also examined for eye malformations to follow-up on the findings of Narotsky (1995). As reported in Warren et al. (2006), gross evaluation of the fetuses as well as computerized morphometry conducted on preserved and sectioned heads revealed no ocular anomalies in the groups treated with TCE. This technique allowed for quantification of the lens area, global area, medial canthus, distance, and interlocular distance. DCA treatment was associated with statistically significant reductions in the lens area, globe area, and interlocular distance. All four measures were reduced in the TCA-treated group, but not significantly. The sensitivity of the assay was demonstrated successfully with the use of a positive control group that was dosed on GDs 6–15 with a known ocular teratogen, retinoic acid (15 mg/kg-day).

Johnson et al. (1998b; 1998a) conducted a series of studies to determine whether specific metabolites of TCE or DCE were responsible for the cardiac malformations observed in rats following administration during the period of organogenesis. Several metabolites of the two chemicals were administered in drinking water to Sprague-Dawley rats from GDs 1 to 22. These included carboxy methylcystine, dichloroacetaldehyde, dichlorovinyl cystine, monochloroacetic acid, TCA, trichloroacetaldehyde, and TCOH. DCA, a primary common metabolite of TCE and DCE, was not included in these studies. The level of each metabolite administered in the water was based upon the dosage equivalent expected if 1,100 ppm (the limit of solubility) TCE broke down completely into that metabolite. Cesarean sections were performed on GD 22, uterine contents were examined, and fetuses were processed and evaluated for heart defects according to the procedures used by Dawson et al. (1993). No treatment-related maternal toxicity was observed for any metabolite group. Adverse fetal outcomes were limited to significantly increased incidences of fetuses with abnormal hearts (see Table 4-101). Significant increases in fetuses with cardiac defects (on a per-fetus and per-litter basis) were observed for only one of the metabolites evaluated (i.e., TCA [2,730 ppm, equivalent to a dose of 291 mg/kg-day]). Notably, significant increases in fetuses with cardiac malformations were also observed with 1.5 or 1,100 ppm TCE (0.218 or 129 mg/kg-day), or with 0.15 or 110 ppm DCE (0.015 or 10.64 mg/kg-day), but in each case, only with prepregnancy-plus-pregnancy treatment regimens. The cardiac abnormalities observed were diverse and did not segregate to any particular anomaly or grouping. Dose related increases in response were observed for the overall number of fetuses with any cardiac malformation for both TCE and DCE; however, no dose-related increase occurred for any specific cardiac anomaly (Johnson et al., 1998a).

Table 4-101. Congenital cardiac malformations

Heart abnormalities	Treatment group												
	Normal water	TCE p+p 1,100 ppm	TCE p+p 1.5 ppm	TCE p 1,100 ppm	DCE p+p 110 ppm	DCE p+p 0.15 ppm	TCA p 2,730 ppm	MCA p 1,570 ppm	TCEth p 1,249 ppm	TCAld p 1,232 ppm	DCAld p 174 ppm	CMC p 473 ppm	DCVC p 50 ppm
Abnormal looping	2	–	2	–	–	–	–	–	–	–	–	–	–
Aortic hypoplasia	–	1	1	–	1	–	1	–	1	–	1	–	1
Pulmonary artery hypoplasia	–	–	1	–	–	–	2	1	–	–	2	–	–
Atrial septal defects	7	19	5	7	11	7	3	3	–	2	–	–	1
Mitral valve defects, hypoplasia or ectasia	1	5	8	–	4	3	1	–	1	2	–	–	1
Tricuspid valve defects, hypoplasia or ectasia	–	1	1	–	1	–	–	–	1	–	–	–	–
Ventricular septal defects													
Perimembranous[a]	2	6	2	1	4	1	4	–	–	3	–	1	–
Muscular	2	4	–	4	2	1	1	–	1	–	–	2	2
Atrioventricual septal defects	1	–	–	1	1	–	–	–	–	–	–	–	–
Pulmonary valve defects	–	2	1	–	1	–	1	3	1	1	–	–	–
Aortic valve defects	–	2	2	2	2	3	–	–	1	–	–	1	–
Situs inversus	–	1	–	–	–	–	–	–	–	–	–	–	–
Total													
Abnormal hearts	15	41	23	15	25	15	13	7	6	8	3	4	5
Fetuses with abnormal hearts	13	40[b]	22[b]	11[b]	24[b]	14[b]	12[b]	6	5	8	3	4	5
Fetuses	605	434	255	105	184	121	114	132	121	248	101	85	140

[a]Subaortic.
[b]Per-fetus statistical significance (Fisher‘s exact test).

p = pregnancy; p+p = pregnancy and prepregnancy

Source: (Johnson et al., 1998b, Table 2, p. 997).

The TCE metabolites TCA and DCA were also studied by Smith et al. (1992; 1989). Doses of 0, 330, 800, 1,200, or 1,800 mg/kg TCA were administered daily by gavage to Long-Evan hooded rats on GDs 6–15. Similarly, DCA was administered daily by gavage to Long-Evans rats on GDs 6–15 in two separate studies, at 0, 900, 1,400, 1,900, or 2,400 mg/kg-day and 0, 14, 140, or 400 mg/kg-day. Embryo lethality and statistically or biologically significant incidences of orbital anomalies (combined soft tissue and skeletal findings) were observed for TCA at ≥800 mg/kg-day, and for DCA at ≥900 mg/kg-day. Fetal growth (body weight and crown-rump length) was affected at ≥330 mg/kg-day for TCE and at ≥400 mg/kg-day for DCA. For TCA, the most common cardiac malformations observed were levocardia at ≥330 mg/kg-day and interventricular septal defect at ≥800 mg/kg-day. For DCA, levocardia was observed at ≥900 mg/kg-day, interventricular septal defect was observed at ≥1,400 mg/kg-day, and a defect between the ascending aorta and right ventricle was observed in all treated groups (i.e., ≥14 mg/kg-day, although the authors appeared to discount the single fetal finding at the lowest dose tested). Thus, NOAELs were not definitively established for either metabolite, although it appears that TCA was generally more potent than DCA in inducing cardiac abnormalities.

These findings were followed up by a series of studies on DCA reported by Epstein et al. (1992), which were designed to determine the most sensitive period of development and further characterize the heart defects. In these studies, Long-Evans hooded rats were dosed by gavage with a single dose of 2,400 mg/kg-day on selected GDs (6–8, 9–11, or 12–15); with a single dose of 2,400 mg/kg on days 10, 11, 12, or 13; or with a single dose of 3,500 mg/kg on days 9, 10, 11, 12, or 13. The heart defects observed in these studies were diagnosed as high interventricular septal defects rather than membranous type interventricular septal defects. The authors hypothesized that high intraventricular septal defects are a specific type of defect produced by a failure of proliferating interventricular septal tissue to fuse with the right tubercle of the atrioventricular cushion tissue. This study identified GDs 9 through 12 as a particularly sensitive period for eliciting high interventricular septal defects. It was postulated that DCA interferes with the closure of the tertiary interventricular foramen, allowing the aorta to retain its embryonic connection with the right ventricle. Further, it was suggested that the selectivity of DCA in inducing cardiac malformations may be due to the disruption of a discrete cell population.

TCE, DCE, and TCA were administered in drinking water to pregnant Sprague-Dawley rats on GDs 0–11 (Collier et al., 2003). Treatment levels were 0, 110, or 1,100 ppm (i.e., 0, 830, or 8,300 µgM) TCE; 0, 11, or 110 ppm (i.e., 0, 110, or 1,100 µgM) DCE; 0, 2.75, or 27.3 mg/mL (i.e., 0, 10, or 100 mM) TCA. Embryos (including hearts) were harvested between embryonic days 10.5–11, since this is the stage at which the developmental processes of myoblast differentiation, cardiac looping, atrioventricular valve formation, and trabeculation would typically be occurring. A PCR-based subtraction scheme was used to identify genes that were differentially regulated with TCE or metabolite exposure. Numerous differentially regulated

gene sequences were identified. Upregulated transcripts included genes associated with stress response (Hsp 70) and homeostasis (several ribosomal proteins). Downregulated transcripts included extracellular matrix components (GPI-p137 and vimentin) and Ca^{2+} responsive proteins (Serca-2 Ca^{2+}-ATPase and β–catenin). Serca-2 Ca^{2+} and GPI-p137 were identified as two possible markers for fetal TCE exposure. Differential regulation of expression of these markers by TCE was confirmed by dot blot analysis and semiquantitative real time PCR with decreased expression seen at levels of TCE exposure between 100 and 250 ppb (0.76 and 1.9 μM).

4.8.3.2.1.2.1. Developmental neurotoxicity and developmental immunotoxicity

Several studies were conducted that included assessments of the effects of TCE oral exposure on the developing nervous system (Blossom et al., 2008; Fredriksson et al., 1993; Isaacson and Taylor, 1989; George et al., 1986; Noland-Gerbec et al., 1986; Dorfmueller et al., 1979) or immune system (Blossom et al., 2008; Peden-Adams et al., 2008; Blossom and Doss, 2007; Peden-Adams et al., 2006). These studies, summarized below, are addressed in additional detail in Sections 4.3 (nervous system) and 4.6.2.1.2 (immune system).

4.8.3.2.1.2.2. Developmental neurotoxicity

Fredriksson et al. (1993) conducted a study in male NMRI weanling mice (12/group, selected from 3−4 litters), which were exposed to TCE by gavage at doses of 0 (vehicle), 50, or 290 mg/kg-day TCE in a fat emulsion vehicle, on PNDs 10–16. Locomotor behavior (horizontal movement, rearing, and total activity) were assessed over three 20-minute time periods at GDs 17 and 60. There were no effects of treatment in locomotor activity at PND 17. At PND 60, the mice treated with 50 and 290 mg/kg-day TCE showed a significant ($p < 0.01$) decrease in rearing behavior at the 0–20- and 20–40-minute time points, but not at the 40–60 minute time point. Mean rearing counts were decreased by over 50% in treated groups as compared to control. Horizontal activity and total activity were not affected by treatment.

Open field testing was conducted in control and high-dose F1 weanling F344 rat pups in an NTP reproduction and fertility study with continuous breeding (George et al., 1986). In this study, TCE was administered at dietary levels of 0, 0.15, 0.30, or 0.60%. The open field testing revealed a significant ($p < 0.05$) dose-related trend toward an increase in the time required for male and female pups to cross the first grid in the testing device, suggesting an effect on the ability to react to a novel environment.

Taylor et al. (1985) administered TCE in drinking water (0, 312, 625, or 1,250 ppm) to female Sprague-Dawley rats for 14 days prior to breeding, and from GD 0 through PND 21. The number of litters/group was not reported, nor did the study state how many pups per litter were evaluated for behavioral parameters. Exploratory behavior was measured in the pups in an automated apparatus during a 15-minute sampling period on PNDs 28, 60, and 90. Additionally, wheel-running, feeding, and drinking behavior was monitored 24 hours/day on PNDs 55–60.

The number of exploratory events was significantly increased by approximately 25–50% in 60- and 90-day old male TCE-treated rats at all dose levels, with the largest effect observed at the highest dose level tested, although there were no effects of treatment on the number of infrared beam-breaks. No difference between control and treated rats was noted for pups tested on PND 28. Wheel-running activity was increased approximately 40% in 60-day-old males exposed to 1,250-ppm TCE as compared to controls. It is notable that adverse outcomes reported in the developmentally-exposed offspring on this study were observed long after treatment ceased.

Using a similar treatment protocol, the effects of TCE on development of myelinated axons in the hippocampus was evaluated by Isaacson and Taylor (1989) in Sprague-Dawley rats. Female rats (6/group) were exposed in the drinking water from 14 days prior to breeding and through the mating period; the dams and their pups were then exposed throughout the prenatal period and until PND 21, when they were sacrificed. The dams received 0, 312, or 625 ppm (0, 4, or 8.1 mg/day) TCE in the drinking water. Myelinated fibers were counted in the hippocampus of 2–3 pups per treatment group at PND 21, revealing a decrease of approximately 40% in myelinated fibers in the CA1 area of the hippocampus of pups from dams at both treatment levels, with no dose-response relationship. There was no effect of TCE treatment on myelination in several other brain regions including the internal capsule, optic tract, or fornix.

A study by Noland-Gerbec et al. (1986) examined the effect of pre- and perinatal exposure to TCE on 2-deoxyglucose (2-DG) uptake in the cerebellum, hippocampus, and whole brain of neonatal rats. Sprague-Dawley female rats (9–11/group) were exposed via drinking water to 0 or 312 mg TCE/L distilled water from 14 days prior to mating until their pups were euthanized at GD 21. The total TCE dose received by the dams was 825 mg over the 61-day exposure period. Pairs of male neonates were euthanized on PNDs 7, 11, 16, and 21. There was no significant impairment in neonatal weight or brain weight attributable to treatment, nor were other overt effects observed. 2-DG uptake was significantly reduced from control values in neonatal whole brain (9–11%) and cerebellum (8–16%) from treated rats at all ages studied, and hippocampal 2-DG uptake was significantly reduced (7–21% from control) in treated rats at all ages except at PND 21.

In a study by Blossom et al. (2008), MRL +/+ mice were treated in the drinking water with 0 or 0.1 mg/mL TCE from maternal GD 0 through offspring PND 42. Based on drinking water consumption data, average maternal doses of TCE were 25.7 mg/kg-day, and average offspring (PNDs 24–42) doses of TCE were 31.0 mg/kg-day. In this study, a subset of offspring (three randomly selected neonates from each litter) was evaluated for righting reflex on PNDs 6, 8, and 10; bar-holding ability on PNDs 15 and 17; and negative geotaxis on PNDs 15 and 17; none of these were impaired by treatment. In an assessment of offspring nest building on PND 35, there was a significant association between impaired nest quality and TCE exposure; however, TCE exposure did not have an effect on the ability of the mice to detect social and

nonsocial odors on PND 29 using olfactory habituation and dishabituation methods. Resident intruder testing conducted on PND 40 to evaluate social behaviors identified significantly more aggressive activities (i.e., wrestling and biting) in TCE-exposed juvenile male mice as compared to controls. Cerebellar tissue homogenates from the male TCE-treated mice had significantly lower GSH levels and GSH:GSSG ratios, indicating increased oxidative stress and impaired thiol status; these have been previously reported to be associated with aggressive behaviors (Franco et al., 2006). Qualitative histopathological examination of the brain did not identify alterations indicative of neuronal damage or inflammation. Although the study author attempted to link the treatment-related alterations in social behaviors to the potential for developmental exposures to TCE to result in autism in humans, this association is not supported by data and is considered speculative at this time.

As previously noted, postnatal behavioral studies conducted by Dorfmueller et al. (1979) did not identify any changes in general motor activity measurements of rat offspring on PNDs 10, 20, and 100 following maternal gestational inhalation exposure to TCE at 1,800 ± 200 ppm.

4.8.3.2.1.2.3. Developmental immunotoxicity

Peden-Adams et al. (2006) assessed the potential for developmental immunotoxicity following TCE exposures. In this study, $B6C3F_1$ mice (5/sex/group) were administered TCE via drinking water at dose levels of 0, 1,400 or 14,000 ppb from maternal GD 0 to either PND 3 or 8, when offspring lymphocyte proliferation, NK cell activity, SRBC-specific IgM production (PFC response), splenic B220+ cells, and thymus and spleen T-cell immunophenotypes were assessed. (A total of 5–7 pups per group were evaluated at week 3, and the remainder were evaluated at week 8.) Observed positive responses consisted of suppressed PFC responses in males at both ages and both TCE treatment levels, and in females at both ages at 14,000 ppb and at 8 weeks of age at 1,400 ppb. Spleen numbers of B220+ cells were decreased in 3-week-old pups at 14,000 ppb. Pronounced increases in all thymus T-cell subpopulations (CD4+, CD8+, CD4+/CD8+, and CD4–/CD8–) were observed at 8 weeks of age. Delayed hypersensitivity response, assessed in offspring at 8 weeks of age, was increased in females at both treatment levels and in males at 14,000 ppb only. No treatment-related increase in serum anti-dsDNA antibody levels was found in the offspring at 8 weeks of age.

In a study by Blossom and Doss (2007), TCE was administered to groups of pregnant MRL +/+ mice in drinking water at levels of 0, 0.5, or 2.5 mg/mL. TCE was continuously administered to the offspring until young adulthood (i.e., 7–8 weeks of age). Offspring postweaning body weights were significantly decreased in both treated groups. Decreased spleen cellularity and reduced numbers of CD4+, CD8+, and B220+ lymphocyte subpopulations were observed in the postweaning offspring. Thymocyte development was altered by TCE exposures (significant alterations in the proportions of double-negative subpopulations and

inhibition of in vitro apoptosis in immature thymocytes). A dose-dependent increase in CD4+ and CD8+ T-lymphocyte IFNγ was observed in peripheral blood by 4–5 weeks of age, although these effects were no longer observed at 7–8 weeks of age. Serum antihistone autoantibodies and total IgG_{2a} were significantly increased in treated offspring; however, no histopathological signs of autoimmunity were observed in the liver and kidneys at sacrifice.

Blossom et al. (2008) administered TCE to MRL +/+ mice (8 dams/group) in the drinking water at levels of 0 or 0.1 mg/mL from GD 0 through PND 42. Average maternal doses of TCE were 25.7 mg/kg-day, and average offspring (PNDs 24–42) doses of TCE were 31.0 mg/kg-day. Subsets of offspring were sacrificed at PNDs 10 and 20, and thymus endpoints (i.e., total cellularity, CD4+/CD8+ ratios, CD24 differentiation markers, and double-negative subpopulation counts) were evaluated. Evaluation of the thymus identified a significant treatment-related increase in cellularity, accompanied by alterations in thymocyte subset distribution, at PND 20 (sexes combined). TCE treatment also appeared to promote T-cell differentiation and maturation at PND 42. Indicators of oxidative stress were measured in the thymus at PNDs 10 and 20, and in the brain at PND 42, and ex vivo evaluation of cultured thymocytes indicated increased reactive oxygen species generation. Mitogen-induced intracellular cytokine production by splenic CD4+ and CD8+ T-cells was evaluated in juvenile mice and brain tissue was examined at PND 42 for evidence of inflammation. Evaluation of peripheral blood indicated that splenic CD4+ T-cells from TCE-exposed PND 42 mice produced significantly greater levels of IFN-γ and IL-2 in males and TNF-α in both sexes. There was no effect on cytokine production on PND 10 or 20.

Peden-Adams et al. (2008) administered TCE to MRL+/+ mice (unspecified number of dams/group) in drinking water at levels of 0, 1,400, or 14,000 ppb from GD 0 and continuing until the offspring were 12 months of age. At 12 months of age, final body weight; spleen, thymus, and kidney weights; spleen and thymus lymphocyte immunophenotyping (CD4 or CD8); splenic B-cell counts; mitogen-induced splenic lymphocyte proliferation; serum levels of autoantibodies to dsDNA and GA, periodically measured from 4 to 12 months of age; and urinary protein measures were recorded. Reported sample sizes for the offspring measurements varied from 6 to 10 per sex per group; the number of source litters represented within each sample was not specified. The only organ weight alteration was an 18% increase in kidney weight in the 1,400 ppb males. Splenic CD4–/CD8– cells were altered in female mice (but not males) at 1,400 ppm only. Splenic T-cell populations, numbers of B220+ cells, and lymphocyte proliferation were not affected by treatment. Populations of thymic T-cell subpopulations (CD8+, CD4–/CD8–, and CD4+) were significantly decreased in male but not female mice following exposure to 14,000 ppb TCE, and CD4+/CD8+ cells were significantly reduced in males by treatment with both TCE concentrations. Autoantibody levels (anti-dsDNA and anti-GA) were not increased in the offspring over the course of the study.

Although all of the developmental immunotoxicity studies with TCE (Peden-Adams et al., (2006), (2008); Blossom and Doss, (2007); Blossom et al., (2008)) exposed the offspring during critical periods of pre- and postnatal immune system development, they were not designed to assess issues such as posttreatment recovery, latent outcomes, or differences in severity of response that might be attributed to the early life exposures.

4.8.3.2.1.3. i.p. exposures

The effect of TCE on pulmonary development was evaluated in a study by Das and Scott (1994). Pregnant Swiss-Webster mice (5/group) were administered a single i.p. injection of TCE in peanut oil at doses of 0 or 3,000 mg/kg on GD 17 (where mating = day 1). Lungs from GDs 18 and 19 fetuses and from neonates on PNDs 1, 5, and 10 were evaluated for phospholipid content, DNA, and microscopic pathology. Fetal and neonatal (PND 1) mortality was significantly increased ($p < 0.01$) in the treated group. Pup body weight and absolute lung weight were significantly decreased ($p < 0.05$) on PND 1, and mean absolute and relative (to body weight) lung weights were significantly decreased on GDs 18 and 19. Total DNA content (µg/mg lung) was similar between control and treated mice, but lung phospholipid was significantly ($p < 0.05$) reduced on GD 19 and significantly increased ($p < 0.05$) on PND 10 in the TCE-treated group. Microscopic examination revealed delays in progressive lung morphological development in treated offspring, first observed at GD 19 and continuing at least through PND 5.

4.8.3.2.2. Studies in nonmammalian species

4.8.3.2.2.1. Avian

Injection of White Leghorn chick embryos with 1, 5, 10, or 25 µmol TCE per egg on days 1 and 2 of embryogenesis demonstrated mortality, growth defects, and morphological anomalies at evaluation on day 14 (Bross et al., 1983). These findings were consistent with a previous study that had been conducted by Elovaara et al. (1979). Up to 67% mortality was observed in the treated groups, and most of the surviving embryos were malformed (as compared to a complete absence of malformed chicks in the untreated and mineral-oil-treated control groups). Reported anomalies included subcutaneous edema, evisceration (gastroschisis), light dermal pigmentation, beak malformations, club foot, and patchy feathering. Retarded growth was observed as significantly ($p < 0.05$) reduced crown-rump, leg, wing, toe, and beak lengths as compared to untreated controls. This study did not identify any liver damage or cardiac anomalies.

In a study by Loeber et al. (1988), 5, 10, 15, 20, or 25 µmol TCE was injected into the air space of White Longhorn eggs at embryonic stages 6, 12, 18, or 23. Embryo cardiac development was examined in surviving chicks in a double-blinded manner at stages 29, 34, or 44. Cardiac malformations were found in 7.3% of TCE-treated hearts, compared to 2.3% of

saline controls and 1.5% of mineral oil controls. The observed defects included septal defects, cor biloculare, conotruncal abnormalities, atrioventricular canal (AVC) defects, and abnormal cardiac muscle.

Drake et al. (2006b) injected embryonated White Leghorn chicken eggs (Babcock or Bovan strains) with 0, 0.4, 8, or 400 ppb TCE per egg during the period of cardiac valvuloseptal morphogenesis (i.e., 2–3.3 days incubation). The injections were administered in four aliquots at Hamberger and Hamilton (HH) stages 13, 15, 17, and 20, which spanned the major events of cardiac cushion formation, from induction through mesenchyme transformation and migration. Embryos were harvested 22 hours after the last injection (i.e., HH 24 or HH 30) and evaluated for embryonic survival, apoptosis, cellularity and proliferation, or cardiac function. Survival was significantly reduced for embryos at 8 and 400 ppb TCE at HH 30. Cellular morphology of cushion mesenchyme, cardiomyocytes, and endocardiocytes was not affected by TCE treatment; however, the proliferative index was significantly increased in the AVC cushions at both treatment levels and in the outflow tract (OFT) cushions at 8 ppb. This resulted in significant cushion hypercellularity for both the OFT and AVC of TCE-treated embryos. Similar outcomes were observed in embryos when TCA or TCOH was administered, and the effects of TCA were more severe than for TCE. Doppler ultrasound assessment of cardiac hemodynamics revealed no effects of TCE exposure on cardiac cycle length or heart rate; however, there was a reduction in dorsal aortic blood flow, which was attributed to a 30.5% reduction in the active component of atrioventricular blood flow. Additionally the passive-to-active atrioventricular blood flow was significantly increased in treated embryos, and there was a trend toward lower stroke volume. The overall conclusion was that exposure to 8 ppb TCE during cushion morphogenesis reduced the cardiac output of the embryos in this study. The findings of cardiac malformations and/or mortality following in ovo exposure to chick embryos with 8 ppb TCE during the period of valvuloseptal morphogenesis has also been confirmed by Rufer et al. (2010; 2008).

In a follow-up study, Drake et al. (2006a) injected embryonated White Leghorn chicken eggs with TCE or TCA during the critical window of avian heart development, beginning at HH stage 3+ when the primary heart field is specified in the primitive streak and ending approximately 50 hours later at HH stage 17, at the onset of chambering. Total dosages of 0, 0.2, 2, 4, 20, or 200 nmol (equivalent to 0, 0.4, 4, 8, 40, or 400 ppb) were injected in four aliquots into each egg yolk during this window (i.e., at stages 3+, 6, 13, and 17: hours 16, 24, 46, and 68). Embryos were harvested at 72 hours, 3.5 days, 4 days or 4.25 days (HH stages 18, 21, 23, or 24, respectively) and evaluated for embryonic survival, cardiac function, or cellular parameters. Doppler ultrasound technology was utilized to assess cardiovascular effects at HH 18, 21, and 23. In contrast to the results of Drake et al. (2006b), all of the functional parameters assessed (i.e., cardiac cycle length, heart rate, stroke volume, and dorsal aortic and atrioventricular blood flow) were similar between control and TCE- or TCA-treated embryos. The authors attributed this difference in response between studies to dependence upon developmental stage at the time

of exposure. In this case, the chick embryo was relatively resistant to TCE when exposure occurred during early cardiogenic stages, but was extremely vulnerable when TCE exposure occurred during valvuloseptal morphogenesis. It was opined that this could explain why some researchers have observed no developmental cardiac effects after TCE exposure to mammalian models, while others have reported positive associations.

4.8.3.2.2.2. Amphibian

The developmental toxicity of TCE was evaluated in the *Frog Embryo Teratogenesis Assay: Xenopus* by Fort et al. (1993; 1991). Late *Xenopus laevis* blastulae were exposed to TCE, with and without exogenous metabolic activation systems, or to TCE metabolites (DCA, TCA, TCOH, or oxalic acid), and developmental toxicity ensued. Findings included alterations in embryo growth, and increased types and severity of induced malformations. Findings included cardiac malformations that were reportedly similar to those that had been observed in avian studies. It was suggested that a mixed function oxidase-mediated reactive epoxide intermediate (i.e., TCE-oxide) may play a significant role in observed developmental toxicity in in vitro tests.

Likewise, McDaniel et al. (2004) observed dose-dependent increases in developmental abnormalities in embryos of four North American amphibian species (wood frogs, green frogs, American toads, and spotted salamanders) following 96-hour exposures to TCE. The median effective concentration (EC_{50}) for malformations was 40 mg/L for TCE in green frogs, while American toads were less sensitive (with no EC_{50} at the highest concentration tested—85 mg/L). Although significant mortality was not observed, the types of malformations noted would be expected to compromise survival in an environmental context.

4.8.3.2.2.3. Invertebrate

The response of the daphnid *Ceriodaphnia dubia* to six industrial chemicals, including TCE, was evaluated by Niederlehner et al. (1998). Exposures were conducted for 6–7 days, according to standard EPA testing guidelines. Lethality, impairment of reproduction, and behavioral changes, such as narcosis and abnormal movement, were observed with TCE exposures. The reproductive sublethal effect concentration value for TCE was found to be 82 μM.

4.8.3.2.3. In vitro studies

Rat whole embryo cultures were used by Saillenfait et al. (1995) to evaluate the embryotoxicity of TCE, tetrachloroethylene, and four metabolites (TCA, DCA, CH, and trichloroacetyl chloride). In this study, explanted embryos of Sprague-Dawley rats were cultured in the presence of the test chemicals for 46 hours and subsequently evaluated. Concentration-dependant decreases in growth and differentiation, and increases in the incidence of morphologically abnormal embryos were observed for TCE at ≥5 mM.

Whole embryo cultures were also utilized by Hunter et al. (1996) in evaluating the embryotoxic potential of a number of disinfection byproducts, including the TCE metabolites DCA and TCA. CD-1 mouse conceptuses (GD 9; 3–6 somites) were cultured for 24–26 hours in treated medium. DCA levels assessed were 0, 734, 1,468, 4,403, 5,871, 7,339, 11,010, or 14,680 μM; TCA levels assessed were 0, 500, 1,000, 2,000, 3,000, 4,000, and 5,000 μM. For DCA, neural tube defects were observed at levels ≥5,871 μM, heart defects were observed at ≥7,339 μM, and eye defects were observed at levels ≥11,010 μM. For TCA, neural tube defects were observed at levels ≥2,000 μM, heart and eye defects were observed at ≥3,000 μM. The heart defects for TCA were reported to include incomplete looping, a reduction in the length of the heart beyond the bulboventricular fold, and a marked reduction in the caliber of the heart tube lumen. Overall benchmark concentrations (i.e., the lower limit of the 95% CI required to produce a 5% increase in the number of embryos with neural tube defects) were 2,451.9 μM for DCA and 1,335.8 μM for TCA (Richard and Hunter, 1996).

Boyer et al. (2000) used an in vitro chick-AVC culture to test the hypothesis that TCE might cause cardiac valve and septal defects by specifically perturbing epithelial-mesenchymal cell transformation of endothelial cells in the AVC and outflow tract areas of the heart. AV explants from Stage 16 White Leghorn chick embryos were placed in hydrated collagen gels, with medium and TCE concentrations of 0, 50, 100, 150, 200, or 250 ppm. TCE was found to block the endothelial cell-cell separation process that is associated with endothelial activation as well as to inhibit mesenchymal cell formation across all TCE concentrations tested. TCE did not, however, have an effect on the cell migration rate of fully formed mesenchymal cells. TCE-treatment was also found to inhibit the expression of transformation factor Mox-1 and extracellular matrix protein fibrillin 2, two protein markers of epithelial-mesenchyme cell transformation.

4.8.3.3. Discussion/Synthesis of Developmental Data

In summary, an overall review of the weight of evidence in humans and experimental animals is suggestive of the potential for developmental toxicity with TCE exposure. A number of developmental outcomes have been observed in the animal toxicity and the epidemiological data, as discussed below. These include adverse fetal/birth outcomes including death (spontaneous abortion, perinatal death, pre- or postimplantation loss, resorptions), decreased growth (low birth weight, SGA, IUGR, decreased postnatal growth), and congenital malformations, in particular cardiac defects. Postnatal developmental outcomes include developmental neurotoxicity, developmental immunotoxicity, and childhood cancer.

4.8.3.3.1. Adverse fetal and early neonatal outcomes

Studies that demonstrate adverse fetal or early neonatal outcomes are summarized in Table 4-102. In human studies of prenatal TCE exposure, increased risk of spontaneous abortion

was observed in some studies (ATSDR, 2001; Taskinen et al., 1994; Windham et al., 1991), but not in others (ATSDR, 2008b, 2001; Goldberg et al., 1990; Lindbohm et al., 1990; Taskinen et al., 1989; Lagakos et al., 1986). In addition, perinatal deaths were observed after 1970, but not before 1970 (Lagakos et al., 1986). In rodent studies that examined offspring viability and survival, there was an indication that TCE exposure may have resulted in increased pre-and/or postimplantation loss (Kumar et al., 2000b; Narotsky and Kavlock, 1995; Healy et al., 1982), and in reductions in live pups born as well as in postnatal and postweaning survival (George et al., 1986; George et al., 1985).

Table 4-102. Summary of adverse fetal and early neonatal outcomes associated with TCE exposures

Positive finding	Species	Reference
Spontaneous abortion, miscarriage, pre-and/or postimplantation loss	Human	ATSDR (2001)[a]; Taskinen et al. (1994)[a]; Windham et al. (1991)
	Rat	Kumar et al. (2000b); Healy et al. (1982); Narotsky and Kavlock (1995); Narotsky et al. (1995)
Perinatal death, reduction in live births	Human	Lagakos et al. (1986)[b]
	Mouse	George et al. (1985)
	Rat	George et al. (1986)
Postnatal and postweaning survival	Mouse	George et al. (1985)
	Rat	George et al. (1986)
Decreased birth weight, SGA, postnatal growth	Human	ATSDR (1998a); ATSDR (2006a); Rodenbeck et al. (2000)[c]; Windham et al. (1991)
	Mouse	George et al. (1985)
	Rat	George et al. (1986); Healy et al. (1982); Narotsky and Kavlock (1995); Narotsky et al. (1995)

[a]Not significant.
[b]Observed for exposures after 1970, but not before.
[c]Increased risk for very low birth weight but not low birth weight or full-term low birth weight.

Decreased birth weight and SGA was observed (ATSDR, 2006a; Rodenbeck et al., 2000; ATSDR, 1998a; Windham et al., 1991); however, no association was observed in other studies (Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). While comprising both occupational and environmental exposures, these human studies are, overall, not highly informative due to their small numbers of cases and limited exposure characterization or to the fact that exposures to mixed solvents were involved. However, decreased fetal weight, live birth weights and postnatal growth were also observed in rodents, (Narotsky and Kavlock, 1995; George et al., 1986; George et al., 1985; Healy et al., 1982), adding to the weight of evidence for this endpoint. It is noted that the rat studies reporting effects on fetal or neonatal viability and growth used F344 or Wistar

rats, while several other studies, which used Sprague-Dawley rats, reported no increased risk in these developmental measures (Carney et al., 2006; Hardin et al., 1981; Schwetz et al., 1975).

Overall, based on weakly suggestive epidemiologic data and fairly consistent laboratory animal data, it can be concluded that TCE exposure poses a potential hazard for prenatal losses and decreased growth or birth weight of offspring.

4.8.3.3.2. Cardiac malformations

A discrete number of epidemiological studies and studies in laboratory animal models have identified an association between TCE exposures and cardiac defects in developing embryos and/or fetuses. These are listed in Table 4-103. Additionally, a number of avian and rodent in vivo studies and in vitro assays have examined various aspects of the induction of cardiac malformations.

Table 4-103. Summary of studies that identified cardiac malformations associated with TCE exposures

Finding	Species	References
Cardiac defects	Human	ATSDR (2008b, 2006a); Yauck et al. (2004)
	Rat	Dawson et al. (1993, 1990); Johnson et al. (2003); Johnson et al. (2005); Johnson et al. (1998b; 1998a)[a]; Smith et al. (1989), (1992)[a]; Epstein et al. (1992)[a]
	Chicken	Bross et al. (1983); Boyer et al. (2000); Loeber et al. (1988); Drake et al. (2006a; 2006b); Mishima et al. (2006); Rufer et al. (2010; 2008)
Altered heart rate	Human	Jasinka (1965, translation)

[a]Metabolites of TCE.

In humans, an increased risk of cardiac defects has been observed after exposure to TCE in studies reported by ATSDR (2008b, 2006a) and Yauck et al. (2004), although others saw no significant effect (Bove, 1996; Bove et al., 1995; Goldberg et al., 1990; Lagakos et al., 1986), possibly due to a small number of cases. In addition, altered heart rate was seen in one study (Jasińska, 1965, translation). A cohort of water contamination in Santa Clara County, California is often cited as a study of TCE exposure and cardiac defects; however, the chemical of exposure is in fact trichloroethane, not TCE (Deane et al., 1989; Swan et al., 1989).

In laboratory animal models, avian studies were the first to identify adverse effects of TCE exposure on cardiac development. As described in Section 4.8.3.2.2.1, cardiac malformations have been reported in chick embryos exposed to TCE (Rufer et al., 2008; Drake et al., 2006a; Drake et al., 2006b; Mishima et al., 2006; Boyer et al., 2000; Loeber et al., 1988; Bross et al., 1983). Additionally, a number of studies were conducted in rodents in which

cardiac malformations were observed in fetuses following the oral administration of TCE to maternal animals during gestation (Johnson et al., 2005, 2003; Dawson et al., 1993, 1990) (see Section 4.8.3.2.1.2). Cardiac defects were also observed in rats following oral gestational treatment with metabolites of TCE (Johnson et al., 1998b; Johnson et al., 1998a; Epstein et al., 1992; Smith et al., 1992; Smith et al., 1989).

However, cardiac malformations were not observed in a number of other studies in laboratory animals in which TCE was administered during the period of cardiac organogenesis and fetal visceral findings were assessed. These included inhalation studies in rats (Carney et al., 2006; Healy et al., 1982; Hardin et al., 1981; Dorfmueller et al., 1979; Schwetz et al., 1975) and rabbits (Hardin et al., 1981), and gavage studies in rats (Fisher et al., 2001; Narotsky and Kavlock, 1995; Narotsky et al., 1995) and mice (Cosby and Dukelow, 1992).

It is generally recognized that response variability among developmental bioassays conducted with the same chemical agent may be related to factors such as the study design (e.g., the species and strain of laboratory animal model used, the day(s) or time of day of dose administration in relation to critical developmental windows, the route of exposure, the vehicle used, the day of study termination), or the study methodologies (e.g., how fetuses were processed, fixed, and examined; what standard procedures were used in the evaluation of morphological landmarks or anomalies; and whether there was consistency in the fetal evaluations that were conducted). In the case of studies that addressed cardiac malformations, there is additional concern as to whether detailed visceral observations were conducted and whether or not cardiac evaluation was conducted using standardized dissection procedures (e.g., with the use of a dissection microscope or including confirmation by histopathological evaluation, and whether the examinations were conducted by technicians who were trained and familiar with fetal cardiac anatomy). Furthermore, interpretation of the findings can be influenced by the analytical approaches applied to the data as well as by biological considerations such as the historical incidence data for the species and strain of interest. These issues have been critically examined in the case of the TCE developmental toxicity studies (Watson et al., 2006; Hardin et al., 2005).

In the available animal developmental studies with TCE, differences were noted in the procedures used to evaluate fetal cardiac morphology following TCE gestational exposures across studies, and some of these differences may have resulted in inconsistent fetal outcomes and/or the inability to detect cardiac malformations. Most of the studies that did not identify cardiac anomalies used a traditional free-hand sectioning technique (as described in Wilson, 1965) on fixed fetal specimens (Healy et al., 1982; Hardin et al., 1981; Dorfmueller et al., 1979; Schwetz et al., 1975). Detection of cardiac anomalies can be enhanced through the use of a fresh dissection technique as described by Staples (1974) and Stuckhardt and Poppe (1984); a significant increase in treatment-related cardiac heart defects was observed by Dawson et al. (1990) when this technique was used. Further refinement of this fresh dissection technique was

employed by Dawson and colleagues at the University of Arizona (UA), resulting in several additional studies that reported cardiac malformations (Johnson et al., 2005, 2003; Dawson et al., 1993). However, two studies conducted in an attempt to verify the teratogenic outcomes of the UA laboratory studies used the same or similar enhanced fresh dissection techniques and were unable to detect cardiac anomalies (Carney et al., 2006; Fisher et al., 2001). Although the Carney et al. (2006) study was administered via inhalation (a route that has not previously been shown to produce positive outcomes), the Fisher et al. (2001) study was administered orally and included collaboration between industry and UA scientists. It was suggested that the apparent differences between the results of the Fisher et al. (2001) study and the Dawson et al. (1993) and Johnson et al. (2003) studies may be related to factors such as differences in purity of test substances or in the rat strains, or differences in experimental design (e.g., gavage vs. drinking water, exposure only during the period of organogenesis versus during the entire gestation period, or the use of a staining procedure).

It is notable that all studies that identified cardiac anomalies following gestational exposure to TCE or its metabolites were: (1) conducted in rats and (2) dosed by an oral route of exposure (gavage or drinking water). Cross-species and route-specific differences in fetal response may be due in part to toxicokinetic factors. Although a strong accumulation and retention of TCA was found in the amniotic fluid of pregnant mice following inhalation exposures to TCE (Ghantous et al., 1986), other toxicokinetic factors may be critical. The consideration of toxicokinetics in determining the relevance of murine developmental data for human risk assessment is briefly discussed by Watson et al. (2006). There are differences in the metabolism of TCE between rodent and humans in that TCE is metabolized more efficiently in rats and mice than humans, and a greater proportion of TCE is metabolized to DCA in rodents versus to TCA in humans. Studies that examined the induction of cardiac malformations with gestational exposures of rodents to various metabolites of TCE identified TCA and DCA as putative cardiac teratogens. Johnson et al. (1998b; 1998a) and Smith et al. (1989) reported increased incidences of cardiac defects with gestational TCA exposures, while Smith et al. (1992) and Epstein et al. (1992) reported increased incidences following DCA exposures.

In all studies that observed increased cardiac defects, either TCE or its metabolites were administered during critical windows of in utero cardiac development, primarily during the entire duration of gestation, or during the period of major organogenesis (e.g., GDs 6–15 in the rat). The study by Epstein et al. (1992) used dosing with DCA on discrete days of gestation and had identified GDs 9 through 12 as a particularly sensitive period for eliciting high interventricular septal defects associated with exposures to TCE or its metabolites.

In the oral studies that identified increased incidences of cardiac malformations following gestational exposure to TCE, there was a broad range of administered doses at which effects were observed. In drinking water studies, Dawson et al. (1993) observed cardiac anomalies at 1.5 and 1,100 ppm (with no NOAEL) and Johnson et al. (2005, 2003) reported effects at 250 ppb

(with a NOAEL of 2.5 ppb). One concern is the lack of a clear dose-response for the incidence of any specific cardiac anomaly or combination of anomalies was not identified, a disparity for which no reasonable explanation for this disparity has been put forth.

The analysis of the incidence data for cardiac defects observed in the Dawson et al. (1993, 1990) and Johnson et al. (2005, 2003) studies has been critiqued (Watson et al., 2006). Issues of concern that have been raised include the statistical analyses of findings on a per-fetus (rather than the more appropriate per-litter) basis (Benson, 2004). Johnson et al. was further criticized for the use of nonconcurrent control data in the analysis (Hardin et al., 2004). In response, the study author has further explained procedures used (Johnson et al., 2004) and has provided individual litter incidence data to the EPA for independent statistical analysis (P. Johnson, personal communication, 2008) (see Section 5.1.2.8). In sum, while the studies by Dawson et al. (1993, 1990) and Johnson et al. (2005, 2003), have significant limitations, there is insufficient reason to dismiss their findings.

4.8.3.3.2.1. Mode of action for cardiac malformations

A number of in vitro studies have been conducted to further characterize the potential for alterations in cardiac development that have been attributed to exposures with TCE and/or its metabolites. It was noted that many of the cardiac defects observed in humans and laboratory species (primarily rats and chickens) involved septal and valvular structures.

During early cardiac morphogenesis, outflow tract and AV endothelial cells differentiate into mesenchymal cells. These mesenchymal cells have characteristics of smooth muscle-like myofibroblasts and form endocardial cushion tissue, which is the primordia of septa and valves in the adult heart. Events that take place in cardiac valve formation in mammals and birds are summarized by NRC (2006) and reproduced in Table 4-104.

Table 4-104. Events in cardiac valve formation in mammals and birds[a]

Stage and event	Structural description[b]
Early cardiac development	The heart is a hollow, linear, tube-like structure with two cell layers. The outer surface is a myocardial cell layer, and the inner luminal surface is an endothial layer. Extracellular matrix is between the two cell layers.
Epithelial-mesenchymal cell transformation	A subpopulation of endothelial cells lining the AVC detaches from adjacent cells and invades the underlying extracellular matrix. Three events occur: ➢ Endothelial cell activation (avian stage 14) ➢ Mesenchymal cell formation (avian stage 16) ➢ Mesenchymal cell migration into the extracellular matrix (avian stages 17 and 18).
Mesenchymal cell migration and proliferation	Endothelial-derived mesenchymal cells migrate toward the surrounding myocardium and proliferate to populate the AVC extracellular matrix.
Development of septa and valvular structures	Cardiac mesenchyme provides cellular constituents for: ➢ Septum intermedium ➢ Valvular leaflets of the mitral and tricuspid AV valves. The septum intermedium subsequently contributes to: ➢ Lower portion of the interatrial septum ➢ Membranous portion of the interventricular septum.

[a]As summarized in NRC (2006).
[b]Markwald et al. (1996; 1984); Boyer et al. (2000).

Methods have been developed to extract the chick stage 16 AVC from the embryo and culture it on a hydrated collagen gel for 24–48 hours, allowing evaluation of the described stages of cardiac development and their response to chemical treatment. Factors that have been shown to influence the induction of endocardial cushion tissue include molecular components such as fibronectin, laminin, and galactosyltransferase (Loeber and Runyan, 1990; Mjaatvedt et al., 1987), components of the extracellular matrix (Mjaatvedt et al., 1991), and smooth muscle α-actin and transforming growth factor β3 (Nakajima et al., 1997; Ramsdell and Markwald, 1997).

Boyer et al. (2000) utilized the in vitro chick AVC culture system to examine the molecular mechanism of TCE effects on cardiac morphogenesis. AVC explants from stage 16 chick embryos (15/treatment level) were placed onto collagen gels and treated with 0, 50, 100, 150, 200, or 250 ppm TCE and incubated for a total of 54 hours. Epithelial-mesenchymal transformation, endothelial cell density, cell migration, and immunohistochemistry were evaluated. TCE treatment was found to inhibit endothelial cell activation and normal mesenchymal cell transformation, endothelial cell-cell separation, and protein marker expression (i.e., transcription factor Mox-1 and extracellular matrix protein fibrillin 2). Mesenchymal cell migration was not affected, nor was the expression of smooth muscle α-actin. The study authors proposed that TCE may cause cardiac valvular and septal malformations by inhibiting endothelial separation and early events of mesenchymal cell formation. Hoffman et al. (2004) proposed alternatively that TCE may be affecting the adhesive properties of the endocardial cells. No experimental data are currently available that address the levels of TCE in cardiac

tissue in vivo, resulting in some questions (Dugard, 2000) regarding the relevance of these mechanistic findings to human health risk assessment.

In a study by Mishima et al. (2006), White Leghorn chick whole embryo cultures (stage 13 and 14) were used to assess the susceptibility of endocardial epithelial-mesenchymal transformation in the early chick heart to TCE at analytically determined concentrations of 0, 10, 20, 40, or 80 ppm. This methodology maintained the anatomical relationships of developing tissues and organs, while exposing precisely staged embryos to quantifiable levels of TCE and facilitating direct monitoring of developmental morphology. Following 24 hours of incubation, the numbers of mesenchymal cells in the inferior and superior AV cushions were counted. TCE treatment significantly reduced the number of mesenchymal cells in both the superior and inferior AV cushions at 80 ppm.

Ou et al. (2003) examined the possible role of endothelial nitric oxide synthase (which generates nitric oxide that has an important role in normal endothelial cell proliferation and hence normal blood vessel growth and development) in TCE-mediated toxicity. Cultured proliferating bovine coronary endothelial cells were treated with TCE at 0–100 μM and stimulated with a calcium ionophore to determined changes in endothelial cells and the generation of endothelial nitric oxide synthase, nitric oxide, and superoxide anion. TCE was shown to alter heat shock protein interactions with endothelial nitric oxide synthase and induce endothelial nitric oxide synthase to shift nitric oxide to superoxide-anion generation. These findings provide insight into how TCE impairs endothelial proliferation.

Several studies have also identified a TCE-related perturbation of several proteins involved in regulation of intracellular Ca^{2+}. After 12 days of maternal exposure to TCE in drinking water, *Serca2a* (sarcoendoplasmic reticulum Ca^{2+} ATPase) mRNA expression was reduced in rat embryo cardiac tissues (Collier et al., 2003). Selmin et al. (2008) conducted a microarray analysis of a P19 mouse stem cell line exposed to 1-ppm TCE in vitro, identifying altered expression of *Ryr2* (ryanodine receptor isoform 2), a Ca^{2+} release channel that is important in normal rhythmic heart activity (Gyorke and Terentyev, 2008). Alterations in Ca^{2+} cycling and resulting contractile dysfunction is a recognized pathogenic mechanism of cardiac arrhythmias and sudden cardiac death (Lehnart et al., 2008; Yano et al., 2008; Leandri et al., 1995). Caldwell et al. (2008c) used real-time PCR and digital imaging microscopy to characterize the effects of various doses of TCE on gene expression and Ca^{2+} response to vasopressin in rat cardiac myocytes (H9c2) *Serca2a* and *Ryr2* expression were reduced at 12 and 48 hours following exposure to TCE. Additionally, Ca^{2+} response to vasopressin was altered following TCE treatment. Makwana et al. (2010) dosed chick embryos in ovo with 8 or 800 ppb TCE; real time-PCR analysis of RNA isolated during specific windows of cardiac development demonstrated effects on the expression of genes associated with reduced blood flow. Although it has been hypothesized that TCE might interfere with the folic acid/methylation pathway in liver and kidney and alter gene regulation by epigenetic

mechanisms, Caldwell et al. (2010) found that the effects of TCE exposure on normal gene expression in rat embryonic hearts was not altered by the administration of exogenous folate. Overall, these data suggest that TCE may disrupt the ability to regulate cellular Ca^{2+} fluxes, altering blood flow and leading to morphogenic consequences in the developing heart. This remains an open area of research.

Thus, in summary, a number of studies have been conducted in an attempt to characterize the mode of action for TCE-induced cardiac defects. A major research focus has been on disruptions in cardiac valve formation, using avian in ovo and in vitro studies. These studies demonstrated treatment-related alterations in endothelial cushion development that could plausibly be associated with defects involving septal and valvular morphogenesis in rodents and chickens. However, a broad array of cardiac malformations has been observed in animal models following TCE exposures (Johnson et al., 2005, 2003; Dawson et al., 1993), and other evidence of molecular disruption of Ca^{2+} during cardiac development has been examined (Caldwell et al., 2008c; Selmin et al., 2008; Collier et al., 2003), suggesting the possible existence of multiple modes of action. The observation of defective myocardial development in a mouse model deficient for gp130, a signal transducer receptor for IL-6 (Yoshida et al., 1996), suggests the potential involvement of immune-mediated effects.

4.8.3.3.2.2. Association of PPARα with developmental outcomes

The PPARs are ligand activated receptors that belong to the nuclear hormone receptor family. Three isotypes have been identified (PPARα, PPARδ [also known as PPARβ], and PPARγ). These receptors, upon binding to an activator, stimulate the expression of target genes implicated in important metabolic pathways. In rodents, all three isotypes show specific time- and tissue-dependent patterns of expression during fetal development and in adult animals. In development, they have been especially implicated in several aspects of tissue differentiation (e.g., of the adipose tissue, brain, placenta, and skin). Epidermal differentiation has been linked strongly with PPARα and PPARδ (Michalik et al., 2002). PPARα starts late in development, with increasing levels in organs such as liver, kidney, intestine, and pancreas; it is also transiently expressed in fetal epidermis and CNS (Braissant and Wahli, 1998) and has been linked to phthalate-induced developmental and testicular toxicity (Corton and Lapinskas, 2005). Liver, kidney, and heart are the sites of highest PPARα expression (Toth et al., 2007). PPARδ and PPARγ have been linked to placental development and function, with PPARγ found to be crucial for vascularization of the chorioallantoic placenta in rodents (Wendling et al., 1999), and placental anomalies mediated by PPARγ have been linked to rodent cardiac defects (Barak et al., 2008). While it might be hypothesized that there is some correlation between PPAR signaling, fetal deaths, and/or cardiac defects observed following TCE exposures in rodents, no definitive data have been generated that elucidate a possible PPAR-mediated mode of action for these outcomes.

4.8.3.3.2.3. Summary of the weight of evidence on cardiac malformations

The evidence for an association between TCE exposures in the human population and the occurrence of congenital cardiac defects is not particularly strong. Many of the epidemiological study designs were not sufficiently robust to detect exposure-related birth defects with a high degree of confidence. However, two well-conducted studies by ATSDR (2008b, 2006a) clearly demonstrated an elevation in cardiac defects. It could be surmised that the identified cardiac defects were detected because they were severe, and that additional cases with less severe cardiac anomalies may have gone undetected.

The animal data provide strong, but not unequivocal, evidence of the potential for TCE-induced cardiac malformations following oral exposures during gestation. Strengths of the evidence are the duplication of the adverse response in several studies from the same laboratory group, detection of treatment-related cardiac defects in both mammalian and avian species (i.e., rat and chicken), general cross-study consistency in the positive association of increased cardiac malformations with test species (i.e., rat), route of administration (i.e., oral), and the methodologies used in cardiac morphological evaluation (i.e., fresh dissection of fetal hearts). Furthermore, when differences in response are observed across studies, they can generally be attributed to obvious methodological differences, and a number of in ovo and in vitro studies demonstrate a consistent and biologically plausible mode of action for one type of malformation observed. Weaknesses in the evidence include lack of a clear dose-related response in the incidence of cardiac defects, and the broad variety of cardiac defects observed, such that they cannot all be grouped easily by type or etiology.

Taken together, the epidemiological and animal study evidence raise sufficient concern regarding the potential for developmental toxicity (increased incidence of cardiac defects) with in utero TCE exposures.

4.8.3.3.3. Other structural developmental outcomes

A summary of other structural developmental outcomes that have been associated with TCE exposures is presented in Table 4-105.

Table 4-105. Summary of other structural developmental outcomes associated with TCE exposures

Finding	Species	References
Eye/ear birth anomalies	Human	Lagakos et al. (1986)
	Rat	Narotsky (1995); Narotsky and Kavlock (1995)
Oral cleft defects	Human	Bove (1996); Bove et al. (1995); Lagakos et al. (1986); Lorente et al. (2000)
Kidney/urinary tract disorders	Human	Lagakos et al. (1986)
Musculoskeletal birth anomalies	Human	Lagakos et al. (1986)
Anemia/blood disorders	Human	Burg and Gist (1999)
Lung/respiratory tract disorders	Human	Lagakos et al. (1986)
	Mouse	Das and Scott (1994)
Skeletal	Rat	Healy et al. (1982)
Other[a]	Human	ATSDR (2001)

[a]As reported by the authors.

In humans, a variety of birth defects other than cardiac have been observed. These include total birth defects (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Flood, 1988) CNS birth defects (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986), eye/ear birth anomalies (Lagakos et al., 1986); oral cleft defects (Lorente et al., 2000; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986); kidney/urinary tract disorders (Lagakos et al., 1986); musculoskeletal birth anomalies (Lagakos et al., 1986); anemia/blood disorders (Burg and Gist, 1999); and lung/respiratory tract disorders (Lagakos et al., 1986). While some of these results were statistically significant, they have not been reported elsewhere. Occupational cohort studies, while not reporting positive results, are generally limited by the small number of observed or expected cases of birth defects (Lorente et al., 2000; Taskinen et al., 1989; Tola et al., 1980).

In experimental animals, a statistically significant increase in the incidence of fetal eye defects, primarily micropththalmia and anopththalmia, manifested as reduced or absent eye bulge, was observed in rats following gavage administration of 1,125 mg/kg-day TCE during the period of organogenesis (Narotsky and Kavlock, 1995; Narotsky et al., 1995). Dose-related nonsignificant increases in the incidence of F344 rat pups with eye defects were also observed at lower dose levels (101, 320, 475, 633, and 844 mg/kg-day) in the Narotsky et al. (1995) study (also reported in Barton and Das, 1996). However, no other developmental or reproductive toxicity studies identified abnormalities of eye development following TCE exposures. For example, in a study reported by Warren et al. (2006), extensive computerized morphometric ocular evaluation was conducted in Sprague-Dawley rat fetuses that had been examined for cardiac defects by Fisher et al. (2001); the dams had been administered TCE (500 mg/kg-day),

DCA (300 mg/kg-day), or TCA (300 mg/kg-day) during GDs 6–15. No ocular defects were found with TCE exposures; however, significant reductions in the lens area, globe area, and interocular distance were observed with DCA exposures, and nonsignificant decreases in these measures as well as the medial canthus distance were noted with TCA exposures. Developmental toxicity studies conducted by Smith et al. (1992; 1989) also identified orbital defects (combined soft tissue and skeletal abnormalities) in Long-Evans rat fetuses following GD 6–15 exposures with TCA and DCA (statistically or biologically significant at ≥800 and ≥900 mg/kg-day, respectively). Overall, the study evidence indicates that TCE and its oxidative metabolites can disrupt ocular development in rats. In addition to the evidence of alteration to the normal development of ocular structure, these findings may also be an indicator of disruptions to nervous system development. It has been suggested by Warren et al. (2006) and Williams and DeSesso (2008) that the effects of concern (defined as statistically significant outcomes) are observed only at high dose levels and are not relevant to risk assessment for environmental exposures. On the other hand, Barton and Das (1996) point out that BMD modeling of the quantal eye defect incidence data provides a reasonable approach to the development of oral toxicity values for TCE human health risk assessment. It is also noted that concerns may exist not only for risks related to low level environmental exposures, but also for risks resulting from acute or short-term occupational or accidental exposures, which may be associated with much higher inadvertent doses.

It was also notable that a study using a single i.p. dose of 3,000 mg/kg TCE to mice during late gestation (GD 17) identified apparent delays in lung development and increased neonatal mortality (Das and Scott, 1994). No further evaluation of this outcome has been identified in the literature.

Healy et al. (1982) did not identify any treatment-related fetal malformations following inhalation exposure of pregnant inbred Wistar rats to 0 or 100 ppm (535 mg/m^3) on GDs 8–21. In this study, significant differences between control and treated litters were observed as an increased incidence of minor ossification variations (p = 0.003) (absent or bipartite centers of ossification).

4.8.3.3.4. Developmental neurotoxicity

Studies that address effects of TCE on the developing nervous system are discussed in detail in Section 4.3, addressed above in the sections on human developmental toxicity (see Section 4.8.3) and on mammalian studies (see Section 4.8.3.2.1) by route of exposure, and summarized in Table 4-106. The available data collectively suggest that the developing brain is susceptible to TCE exposures.

Table 4-106. Summary of developmental neurotoxicity associated with TCE exposures

Positive findings	Species	References
CNS defects, neural tube defects	Human	ATSDR (2001)
		Bove (1996); Bove et al. (1995)
		Lagakos et al. (1986)
Eye defects	Rat	Narotsky (1995); Narotsky and Kavlock (1995)
Delayed newborn reflexes	Human	Beppu (1968)
Impaired learning or memory	Human	Bernad et al. (1987), abstract
		White et al. (1997)
Aggressive behavior	Human	Bernad et al. (1987), abstract
	Rat	Blossom et al. (2008)
Hearing impairment	Human	ATSDR (2003b); Burg et al. (1995); Burg and Gist (1999)
		Beppu (1968)
Speech impairment	Human	ATSDR (2003b); Burg et al. (1995); Burg and Gist (1999)
		White et al. (1997)
Encephalopathy	Human	White et al. (1997)
Impaired executive function	Human	White et al. (1997)
Impaired motor function	Human	White et al. (1997)
Attention deficit	Human	Bernad et al. (1987), abstract
ASD	Human	Windham et al. (2006)
Delayed or altered biomarkers of CNS development	Rat	Isaacson and Taylor (1989); Noland-Gerbec et al. (1986); Westergren et al. (1984)
Behavioral alterations	Mice	Blossom et al. (2008); Fredriksson et al. (1993)
	Rat	George et al. (1986); Taylor et al. (1985)

In humans, CNS birth defects were observed in a few studies (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). Postnatally, observed adverse effects in humans include delayed newborn reflexes following use of TCE during childbirth (Beppu, 1968), impaired learning or memory (White et al., 1997; Bernad et al., 1987, abstract); aggressive behavior (Bernad et al., 1987, abstract); hearing impairment (ATSDR, 2003b; Burg and Gist, 1999; Burg et al., 1995; Beppu, 1968); speech impairment (Burg and Gist, 1999; White et al., 1997; Burg et al., 1995); encephalopathy (White et al., 1997); impaired executive and motor function (White et al., 1997); attention deficit (White et al., 1997; Bernad et al., 1987, abstract), and ASD (Windham et al., 2006). While there are broad developmental neurotoxic effects that have been associated with TCE exposure, there are many limitations in the studies.

More compelling evidence for the adverse effect of TCE exposure on the developing nervous system is found in the animal study data, although a rigorous evaluation of potential

outcomes has not been conducted. For example, there has not been an assessment of cognitive function (i.e., learning and memory) following developmental exposures to TCE, nor have most of the available studies characterized the pre- or postnatal exposure of the offspring to TCE or its metabolites. Nevertheless, there is evidence of treatment-related alterations in brain development and in behavioral parameters (e.g., spontaneous motor activity and social behaviors) associated with exposures during neurological development. The animal study database includes the following information: following inhalation exposures of 150 ppm to mice during mating and gestation, the specific gravity of offspring brains were significantly decreased at postnatal time points through the age of weaning; however, this effect did not persist to 1 month of age (Westergren et al., 1984). In studies reported by Taylor et al. (1985), Isaacson and Taylor (1989), and Noland-Gerbec et al. (1986), 312 mg/L exposures in drinking water that were initiated 2 weeks prior to mating and continued to the end of lactation resulted, respectively, in: (1) significant increases in exploratory behavior at GDs 60 and 90; (2) reductions in myelination in the brains of offspring at weaning; and (3) significantly decreased uptake of 2-deoxyglucose in the neonatal rat brain (suggesting decreased neuronal activity). Ocular malformations in rats observed by Narotsky (1995) and Narotsky and Kavlock (1995) following maternal gavage doses of 1,125 mg/kg-day during gestation may also be indicative of alterations of nervous system development. Gestational exposures to mice (Fredriksson et al., 1993) resulted in significantly decreased rearing activity on GD 60, and dietary exposures during the course of a continuous breeding study in rats (George et al., 1986) found a significant trend toward increased time to cross the first grid in open field testing. In a study by Blossom et al. (2008), alterations in social behaviors (deficits in nest-building quality and increased aggression in males) were observed in pubertal-age MRL +/+ mice that had been exposed to 0.1 mg/mL TCE via drinking water during prenatal and postnatal development (until PND 42). Dorfmueller et al. (1979) was the only study that assessed neurobehavioral endpoints following in utero exposure (maternal inhalation exposures of 1,800 ± 200 ppm during gestation) and found no adverse effects that could be attributed to TCE exposure. Specifically, an automated assessment of ambulatory response in a novel environment on GDs 10, 20 and 100, did not identify any effect on general motor activity of offspring.

4.8.3.3.5. Developmental immunotoxicity

Studies that address the developmental immunotoxic effects of TCE are discussed in detail in Section 4.6, addressed above in the sections on human developmental toxicity (see Section 4.8.3) and on mammalian studies (see Section 4.8.3.2.1) by route of exposure, and summarized in Table 4-107.

Table 4-107. Summary of developmental immunotoxicity associated with TCE exposures

Finding	Species (strain)	References
Significant reduction in Th1 IL-2 producing cells	Human	Lehmann et al. (2002)
Altered immune response	Human	Byers et al. (1988)
Suppression of PFC responses, increased T-cell subpopulations, decreased spleen cellularity, and increased hypersensitivity response	Mouse ($B6C3F_1$)	Peden-Adams et al. (2006)
Altered splenic and thymic T-cell subpopulations	Mouse (MRL +/+)	Peden-Adams et al. (2008)
Altered thymic T-cell subpopulations; transient increased proinflammatory cytokine production by T-cells; increased autoantibody levels and IgG	Mouse (MRL +/+)	Blossom and Doss (2007)
Increased proinflammatory cytokine production by T-cells	Mouse (MRL +/+)	Blossom et al. (2008)

Two epidemiological studies that addressed potential immunological perturbations in children that were exposed to TCE were reported by Lehmann et al. (2002; 2001). In the 2001 study, no association was observed between TCE and allergic sensitization to egg white and milk, or to cytokine producing peripheral T-cells, in premature neonates and 36-month-old neonates that were at risk of atopy. In the 2002 study, there was a significant reduction in Th1 IL-2 producing cells. Another study observed altered immune response in family members of those diagnosed with childhood leukemia, including 13 siblings under 19 years old at the time of exposure, but an analysis looking at only these children was not done (Byers et al., 1988).

Several studies were identified (Blossom et al., 2008; Peden-Adams et al., 2008; Blossom and Doss, 2007; Peden-Adams et al., 2006) that assessed the potential for developmental immunotoxicity in mice following oral (drinking water) TCE exposures during critical pre- and postnatal stages of immune system development. Peden-Adams et al. (2006) noted evidence of immune system perturbation (suppression of PFC responses, increased T-cell subpopulations, decreased spleen cellularity, and increased hypersensitivity response) in $B6C3F_1$ offspring following in utero and 8 weeks of postnatal exposures to TCE. Evidence of autoimmune response was not observed in the offspring of this nonautoimmune-prone strain of mice. However, in a study by Peden-Adams et al. (2008) MRL +/+ mice, which are autoimmune-prone, were exposed from conception until 12 months of age. Consistent with the Peden-Adams et al. (2006) study, no evidence of increased autoantibody levels was observed in the offspring. In two other studies focused on autoimmune responses following drinking water exposures of MRL +/+ mice to TCE during in utero development and continuing until the time of sexual maturation, Blossom and Doss (2007) and Blossom et al. (2008) reported some peripheral blood changes that were indicative of treatment-related autoimmune responses in offspring. Positive response levels were 0.5 and 2.5 mg/mL for Blossom and Doss (2007) and 0.1 mg/mL for Blossom et al. (2008). None of these studies were designed to extensively evaluate recovery, latent outcomes, or differences in severity of response that might be attributed to the early life

exposures. Consistency in response in these animal studies was difficult to ascertain due to the variations in study design (e.g., animal strain used, duration of exposure, treatment levels evaluated, timing of assessments, and endpoints evaluated). Likewise, the endpoints assessed in the few epidemiological studies that evaluated immunological outcomes following developmental exposures to TCE were dissimilar from those evaluated in the animal models, and so provided no clear cross-species correlation. The most sensitive immune system response noted in the studies that exposed developing animals were the decreased PFC and increased hypersensitivity observed by Peden-Adams et al. (2006); treatment-related outcomes were noted in mice exposed in the drinking water at a concentration of 1,400 ppb. None of the other studies that treated mice during immune system development assessed these same endpoints; therefore, direct confirmation of these findings across studies was not possible. It is noted, however, that similar responses were not observed in studies in which adult animals were administered TCE (e.g., Woolhiser et al., 2006), suggesting increased susceptibility in the young. Differential lifestage-related responses have been observed with other diverse chemicals (e.g., diethylstilbestrol; diazepam; lead; 2,3,7,8-tetrachlorobenzo-*p* dioxin; and tributyltin oxide) in which immune system perturbations were observed at lower doses and/or with greater persistence when tested in developing animals as compared to adults (Luebke et al., 2006). Thus, such an adverse response with TCE exposure is considered biologically plausible and an issue of concern for human health risk assessment.

4.8.3.3.6. Childhood cancers

A summary of childhood cancers that have been associated with TCE exposures discussed above is presented in Table 4-108. A summary of studies that observed childhood leukemia is also discussed in detail in Sections 4.6.1.2 and 4.8.3.1.2.4 contains details of epidemiologic studies on childhood brain cancer.

Table 4-108. Summary of childhood cancers associated with TCE exposures

Finding	Species	References
Leukemia	Human	AZ DHS (ADHS, 1990; Flood, 1988)
		AZ DHS (Kioski et al., 1990a)
		Cohn et al. (1994b)
		Cutler et al. (1986); Costas et al. (2002); Lagakos et al. (1986); MDPH (1997c)
		Lowengart et al. (1987)
		McKinney et al. (1991)
		Shu et al. (1999)
Neuroblastoma	Human	De Roos et al. (2001)
		Peters et al. (1985; 1981)

A nonsignificant increased risk of leukemia diagnosed during childhood has been observed in a number of studies examining TCE exposure (Costas et al., 2002; Shu et al., 1999; MDPH, 1997b; Cohn et al., 1994b; McKinney et al., 1991; ADHS, 1990; Kioski et al., 1990a; Flood, 1988; Lowengart et al., 1987; Lagakos et al., 1986). However, other studies did not observed an increased risk for childhood leukemia after TCE exposure (Morgan and Cassady, 2002; Flood, 1997b; Kioski et al., 1990b), possibly due to the limited number of cases or the analysis based on multiple solvents.

CNS cancers during childhood have been reported on in a few studies. Neuroblastomas were not statistically elevated in one study observing parental exposure to multiple chemicals, including TCE (De Roos et al., 2001). Brain tumors were observed in another study, but the OR could not be determined (Peters et al., 1985; Peters et al., 1981). CNS cancers were not elevated in other studies (Morgan and Cassady, 2002; Kioski et al., 1990a). Other studies did not see an excess risk of total childhood cancers (ATSDR, 2006a; Morgan and Cassady, 2002).

A follow-up study of the Camp Lejeune cohort that will examine childhood cancers (along with birth defects) was initiated in 1999 (ATSDR, 2003a), is expected to be completed soon (ATSDR, 2009; U.S. GAO, 2007b, a), and may provide additional insight.

No studies of cancers in experimental animals in early lifestages have been identified.

4.9. OTHER SITE-SPECIFIC CANCERS

4.9.1. Esophageal Cancer

Increasing esophageal cancer incidence has been observed in males, but not females in the United States between 1975 and 2002, a result of increasing incidence of esophageal adenocarcinoma (Ward et al., 2006). Males also have higher age-adjusted incidence and mortality rates (incidence, 7.8 per 100,000; mortality, 7.8 per 100,000) than females (incidence, 2.0 per 100,000; mortality, 1.7 per 100,000) (Ries et al., 2008). Survival for esophageal cancer remains poor, and age-adjusted mortality rates are just slightly lower than incidence rates. Major risk factors associated with esophageal cancer are smoking and alcohol for squamous cell carcinoma, typically found in the upper third of the esophagus, and obesity, gastroesophageal reflux, and Barrett‘s esophagus for adenocarcinoma that generally occurs in the lower esophagus (Ward et al., 2006).

Seventeen epidemiologic studies on TCE exposure reported RRs for esophageal cancer (Clapp and Hoffman, 2008; Radican et al., 2008; Sung et al., 2007; ATSDR, 2006a; Boice et al., 2006b; Zhao et al., 2005; ATSDR, 2004a; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Ritz, 1999a; Blair et al., 1998; Greenland et al., 1994; Siemiatycki, 1991; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988). Ten studies had high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis (Radican et al., 2008; Boice et al., 2006b; Zhao et al., 2005; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Ritz,

1999a; Blair et al., 1998; Greenland et al., 1994; Siemiatycki, 1991). Four studies with TCE exposure potential assigned to individual subjects (Blair et al., 1998 [Incidence]; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994) did not present RR estimates for esophageal cancer and TCE exposure nor did two other studies, which carry less weight in the analysis because of design limitations (Henschler et al., 1995; Sinks et al., 1992). Only Raaschou-Nielsen et al. (2003) examined esophageal cancer histologic type, an important consideration given differences between suspected risk factors for adenocarcinoma and those for squamous cell carcinoma. Appendix B identifies these studies' design and exposure assessment characteristics.

Several population case-control studies (Ramanakumar et al., 2008; Santibanez et al., 2008; Weiderpass et al., 2003; Engel et al., 2002; Parent et al., 2000b; Gustavsson et al., 1998; Yu et al., 1988) examined esophageal cancer and organic solvents or occupational job titles with past TCE use documented (Bakke et al., 2007). RR estimates in case-control studies that examine metal occupations or job titles, or solvent exposures are found in Table 4-109. The lack of exposure assessment to TCE, low prevalence of exposure to chlorinated hydrocarbon solvents, or few exposed cases and controls in those studies lowers their sensitivity for informing evaluations of TCE and esophageal cancer.

Table 4-109. Selected observations from case-control studies of TCE exposure and esophageal cancer

Study population	Exposure group	All esophageal cancers		Squamous cell cancer		Adenocarcinoma		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Population of regions in Eastern Spain								Santibañez et al. (2008)
	Metal molders, welders, etc.	0.94 (0.14, 6.16)	3	0.40 (0.05, 3.18)	2	3.55 (0.28, 44.70)	1	
	Metal-processing plant operators	1.14 (0.29, 4.44)	5	1.23 (0.23, 6.51)	4	0.86 (0.08, 8.63)	1	
	Chlorinated hydrocarbon solvents							
	Low exposure	1.05 (0.15, 7.17)	2		0	4.92 (0.69, 34.66)	2	
	High exposure	1.76 (0.40, 7.74)	6	2.18 (0.41, 11.57)	5	3.03 (0.28, 32.15)	1	
Population of Montreal, Canada								Ramanakumar et al. (2008); Parent et al. (2000b)
	Painter, Metal coatings							
	Any exposure	1.3 (0.4, 4.2)	6					
	Substantial exposure	4.2 (1.1, 17.0)	4					
	Solvents							
	Any exposure	1.1 (0.7, 1.7)	39	1.4 (0.8, 2.5)	30			
	Nonsubstantial exposure	1.0 (0.5, 1.9)	16	1.3 (0.6, 2.6)	12			
	Substantial exposure	1.1 (0.6, 1.9)	39	1.4 (0.8, 2.5)	30			
Population of Sweden								Jansson et al., (2006; 2005)
	Organic solvents							
	No exposure			1.0	145	1.0	128	
	Moderate exposure			0.7 (0.4, 1.5)	15	1.2 (0.6, 2.3)	14	
	High exposure			1.3 (0.7, 2.3)	21	1.4 (0.7, 2.5)	18	
	Test for trend			$p = 0.47$		$p = 0.59$		
	No exposure			1.0		1.0		
	Moderate exposure			0.5 (0.1, 3.9)[a]	1	0.4 (0.1, 1.5)[a]	2	
	High exposure			0.4 (0.1, 1.8)[a]	2	0.9 (0.5, 1.6)[a]	12	
	Test for trend			$p = 0.44$		$p = 0.36$		

Table 4-109. Selected observations from case-control studies of TCE exposure and esophageal cancer (continued)

Study population	Exposure group	All esophageal cancers		Squamous cell cancer		Adenocarcinoma		Reference
		RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	RR (95% CI)	Number of observable events	
Population of Finland (Females)								Weiderpass et al. (2003)
	Chlorinated hydrocarbon solvents							
	Low level exposure	0.95 (0.54, 1.66)	Not reported					
	High level exposure	0.62 (0.34, 1.13)	Not reported					
Population of New Jersey, Connecticut, Washington State								Engel et al. (2002)
	Precision metal workers	Not reported		0.7 (0.3, 1.5)	12	1.4 (0.8, 2.3)	25	
	Metal product manufacturing	Not reported		0.8 (0.3, 1.8)	15	1.3 (0.8, 2.3)	26	

[a]Jansson et al. (2006) is a registry-based study of the Swedish Construction Worker Cohort. RRs are incidence rate ratios from Cox regression analysis using calendar time and adjustment for attained age, calendar period at entry into the cohort, tobacco smoking status at entry into the cohort and BMI at entry into the cohort.

Table 4-110 presents risk estimates for TCE exposure and esophageal cancer observed in cohort, PMR, case-control, and geographic-based studies. Ten studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) reported risk estimates for esophageal cancer (Radican et al., 2008; Boice et al., 2006b; Zhao et al., 2005; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Ritz, 1999a; Blair et al., 1998; Greenland et al., 1994; Siemiatycki, 1991). Some evidence for association with esophageal cancer and overall TCE exposure comes from studies with high likelihood of TCE exposure (5.6, 95% CI: 0.7, 44.5 (Blair et al., 1998) and 1.88, 95% CI: 0.61, 5.79 [Radican et al. (2008), which was an update of Blair et al. (1998) with an additional 10 years of follow-up]; 4.2, 95% CI: 1.5, 9.2, (Hansen et al., 2001); 1.2, 95% CI: 0.84, 1.57 (Raaschou-Nielsen et al., 2003)]. Two studies support an association with adenocarcinoma histologic type of esophageal cancer and TCE exposure [five of the six observed esophageal cancers were adenocarcinomas [<1 expected; Hansen et al. (2001)]; 1.8, 95% CI: 1.2, 2.7 (Raaschou-Nielsen et al., 2003). Risk estimates in other studies are based on few deaths, low statistical power to detect a doubling of esophageal cancer risk, and CIs that include a risk estimate of 1.0 (no increased risk).

Table 4-110. Summary of human studies on TCE exposure and esophageal cancer

Exposure group	RR (95% CI)	Number of observable events	Reference
Cohort studies—incidence			
Aerospace workers (Rocketdyne)			Zhao et al. (2005)
Any exposure to TCE	Not reported		
Low cumulative TCE score	1.00[a]	9	
Med cumulative TCE score	1.66 (0.62, 4.41)[b]	8	
High TCE score	0.82 (0.17, 3.95)[b]	2	
p for trend	$p = 0.974$		
All employees at electronics factory (Taiwan)			Sung et al. (2007)
Males	Not reported		
Females	1.16 (0.0.14, 4.20)[c]	2	
Danish blue-collar worker with TCE exposure			Raaschou-Nielsen et al. (2003)
Any exposure, all subjects	1.2 (0.84, 1.57)	44	
Any exposure, males	1.1 (0.81, 1.53)	40	
Any exposure, females	2.0 (0.54, 5.16)	4	
Any exposure, males	1.8 (1.15, 2.73)[d]	23	
Any exposure, females		0 (0.4 exp)[d]	
Exposure lag time			
20 yrs	1.7 (0.8, 3.0)[d]	10	
Employment duration			
<1 yr	1.7 (0.6, 3.6)[d]	6	
1–4.9 yrs	1.9 (0.9, 3.6)[d]	9	
≥5 yrs	1.9 (0.8, 3.7)[d]	8	
Subcohort with higher exposure			
Any TCE exposure	1.7 (0.9, 2.9)[d]	13	
Employment duration			
1–4.9 yrs	1.6 (0.6, 3.4)[d]	6	
≥5 yrs	1.9 (0.8, 3.8)[d]	7	

Table 4-110. Summary of human studies on TCE exposure and esophageal cancer (continued)

Exposure group	RR (95% CI)	Number of observable events	Reference
Biologically-monitored Danish workers	4.0 1.5, 8.72)	6	Hansen et al. (2001)
Any TCE exposure, males	4.2 (1.5, 9.2)	6	
Adenocarcinoma histologic type	3.6 (1.2, 8.3)[e]	5	
Any TCE exposure, females		0 (0.1 exp)	
Cumulative exposure (Ikeda)			
<17 ppm-yr	6.5 (1.3, 19)	3	
≥17 ppm-yr	4.2 (1.5, 9.2)	3	
Mean concentration (Ikeda)			
<4 ppm	8.0 (2.6, 19)	5	
4+ ppm	1.3 (0.02, 7.0)	1	
Employment duration			
<6.25 yr	4.4 (0.5, 16)	2	
≥6.25 yr	6.6 (1.8, 17)	4	
Aircraft maintenance workers from Hill Air Force Base			Blair et al. (1998)
TCE subcohort	Not reported		
Males, cumulative exposure			
0	1.0[a]		
<5 ppm-yr	Not reported		
5–25 ppm-yr	Not reported		
>25 ppm-yr	Not reported		
Females, cumulative exposure			
0	1.0[a]		
<5 ppm-yr	Not reported		
5–25 ppm-yr	Not reported		
>25 ppm-yr	Not reported		
Biologically-monitored Finnish workers			Anttila et al. (1995)
All subjects	Not reported		
Mean air-TCE (Ikeda extrapolation)			
<6 ppm	Not reported		
6+ ppm	Not reported		

Table 4-110. Summary of human studies on TCE exposure and esophageal cancer (continued)

Exposure group	RR (95% CI)	Number of observable events	Reference
Cardboard manufacturing workers in Arnsburg, Germany			Henschler et al. (1995)
Exposed workers	Not reported		
Biologically-monitored Swedish workers			Axelson et al. (1994)
Any TCE exposure, males	Not reported		
Any TCE exposure, females	Not reported		
Cardboard manufacturing workers, Atlanta area, Georgia			Sinks et al. (1992)
All subjects	Not reported		
Cohort and PMR studies-mortality			
Computer manufacturing workers (IBM), New York			Clapp and Hoffman (2008)
Males	1.12 (0.30, 2.86)[f]		
	5.24 (0.13, 29.2)[f]		
Aerospace workers (Rocketdyne)			
Any TCE (utility/eng flush)	0.88 (0.18, 2.58)	3	Boice et al. (2006b)
Any exposure to TCE	Not reported		Zhao et al. (2005)
Low cumulative TCE score	1.00[a]	18	
Medium cumulative TCE score	1.40 (0.70, 2.82)[b]	15	
High TCE score	1.27 (0.52, 3.13)[b]	7	
p for trend	$p = 0.535$		
View-Master employees			ATSDR (2004a)
Males	0.62 (0.02, 3.45)[f]	1	
Females		0 (1.45 exp)[f]	
All employees at electronics factory (Taiwan)			Chang et al. (2003)
Males		0 (3.34 exp)	
Females		0 (0.83 exp)	
United States uranium-processing workers (Fernald)			Ritz (1999a)
Any TCE exposure	Not reported		
Light TCE exposure, >2-yr duration	2.61 (0.99, 6.88)[g]	12	
Moderate TCE exposure, >2-yr duration		0	

Table 4-110. Summary of human studies on TCE exposure and esophageal cancer (continued)

Exposure group	RR (95% CI)	Number of observable events	Reference
Aerospace workers (Lockheed)			Boice et al. (1999)
Routine exposure	0.83 (0.34, 1.72)	7	
Routine-intermittent[a]	Not presented	11	
Duration of exposure			
0 yr	1.0[a]	28	
<1 yr	0.23 (0.05, 0.99)	2	
1–4 yrs	0.57 (0.20, 1.67)	4	
≥5 yrs	0.91 (0.38, 2.22)	7	
p for trend	$p > 0.20$		
Aerospace workers (Hughes)			Morgan et al. (1998)
TCE subcohort	Not reported		
Low intensity (<50 ppm)			
High intensity (>50 ppm)			
TCE subcohort (Cox Analysis)	Not reported		
Never exposed			
Ever exposed			
Peak	Not reported		
No/Low			
Medium/high			
Cumulative	Not reported		
Referent			
Low			
High			
Aircraft maintenance workers (Hill Air Force Base, Utah)			Blair et al. (1998)
TCE subcohort	5.6 (0.7, 44.5)[a]	10	
Males, cumulative exposure			
0	1.0[a]		
<5 ppm-yr	Not reported[h]	3	
5–25 ppm-yr	Not reported[h]	2	
>25 ppm-yr	Not reported[h]	4	

Table 4-110. Summary of human studies on TCE exposure and esophageal cancer (continued)

Exposure group	RR (95% CI)	Number of observable events	Reference
Females, cumulative exposure			Blair et al. (1998) (continued)
0	1.0[a]		
<5 ppm-yr	3.6 (0.2, 58)	1	
5–25 ppm-yr		0	
>25 ppm-yr		0	
TCE subcohort	1.88 (0.61, 5.79)	17	Radican et al. (2008)
Males, cumulative exposure	1.66 (0.48, 5.74)	15	
0	1.0[a]		
<5 ppm-yr	1.84 (0.48, 7.14)	7	
5–25 ppm-yr	1.33 (0.27, 6.59)	3	
>25 ppm-yr	1.67 (0.40, 7.00)	5	
Females, cumulative exposure	2.81 (0.25, 31.10)	2	
0	1.0[a]		
<5 ppm-yr	3.99 (0.25, 63.94)	1	
5–25 ppm-yr	9,.59 (0.60, 154.14)	1	
>25 ppm-yr		0	
Cardboard manufacturing workers in Arnsburg, Germany			Henschler et al. (1995)
TCE exposed workers	Not reported		
Unexposed workers	Not reported		
Deaths reported to among GE pension fund (Pittsfield, Massachusetts)	0.95 (0.1, 3.17)[i]	13	Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia	Not reported		Sinks et al. (1992)
U.S. Coast Guard employees			Blair et al. (1989)
Marine inspectors	0.72 (0.09, 2.62)	2	
Noninspectors	0.74 (0.09, 2.68)	2	
Aircraft manufacturing plant employees (Italy)			Costa et al. (1989)
All subjects	0.21 (0.01, 1.17)	1	
Rubber Workers	Not reported[i]		Wilcosky et al. (1984)
Aircraft manufacturing plant employees (San Diego, California)			Garabrant et al. (1988)
All subjects	1.14 (0.62, 1.92)	14	

Table 4-110. Summary of human studies on TCE exposure and esophageal cancer (continued)

Exposure group	RR (95% CI)	Number of observable events	Reference
Case-control studies			
Population of Montreal, Canada			Siemiatycki et al. (1991); Parent et al. (2000b)
Any TCE exposure	0.5 (0.1, 2.5)[j]	1	
Substantial TCE exposure	0.8 (0.1, 4.6)[j]	1	
Geographic-based studies			
Residents in two study areas in Endicott, New York	0.78 (0.29, 1.70)	6	ATSDR (2006a)
Residents of 13 census tracts in Redlands, California	Not reported		Morgan and Cassady (2002)
Finnish residents			Vartiainen et al. (1993)
Residents of Hausjarvi	Not reported		
Residents of Huttula	Not reported		

[a]Internal referents, workers not exposed to TCE.
[b]Ritz (1999a) and Zhao et al. (2005) reported RRs for the combined site of esophagus and stomach.
[c]Sung et al. (2007) and Chang et al. (2005)—SIR for females and reflects a 10-yr lag period.
[d]SIR for adenocarcinoma of the esophagus.
[e]The SIR for adenocarcinoma histologic type cannot be calculated because Hansen et al. (2001) do not present expected numbers for adenocarcinoma histologic type of esophageal cancer. An approximation of the SIR for adenocarcinoma histologic type is presented using the expected number of total number of expected esophageal cancers for males (n = 1.4). The expected numbers of esophageal adenocarcinomas in males will be lower; Hansen et al. (2001) noted the proportion of adenocarcinomas among the comparable Danish male population during the later period of the study (1990–1996) as 38%. A rough approximation of the expected number of esophageal carcinomas would be 0.5 expected cases and an approximated SIR of 9.4 (3.1, 22).
[f]PMR.
[g]Adjusted RRs for >2-year exposure duration and 15-year lag from 1st exposure.
[h]No esophageal cancer deaths occurred in the referent population in Blair et al. (1998) and RR could not be calculated for this reason.
[i]OR from nested case-control analysis.
[j]90% CI.

Seven other studies (Clapp and Hoffman, 2008; Sung et al., 2007; ATSDR, 2006a, 2004a; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988) with lower likelihood for TCE exposure, in addition to limited statistical power and other design limitations, observed RR estimates between 0.21 (95% CI: 0.0.01, 1.17) (Costa et al., 1989) and 1.14 (95% CI: 0.62, 1.92) (Garabrant et al., 1988). For these reasons, esophageal cancer observations in these studies are not inconsistent with Blair et al. (1998) and its update Radican et al. (2008), Hansen et al. (2001), or Raaschou-Nielsen et al. (2003). No study reported a statistically significant deficit in the esophageal cancer risk estimate and overall of TCE exposure. Of those studies with exposure-response analyses, a pattern of increasing esophageal cancer RR with increasing exposure metric is not generally noted (Radican et al., 2008; Zhao et al., 2005; Boice et al., 1999; Blair et al., 1998; Siemiatycki, 1991) except for Hansen et al. (2001) and Raaschou-Nielsen et al. (2003). In

these last two studies, esophageal cancer RR estimates associated with long employment duration were slightly higher [SIR: 6.6, 95% CI: 1.8, 17 (Hansen et al., 2001); SIR: 1.9, 95% CI: 0.8, 3.7 (Raaschou-Nielsen et al., 2003)] than those for short employment duration [SIR: 4.4, 95% CI: 0.5, 16 (Hansen et al., 2001); SIR: 1.7, 95% CI: 0.6, 3.6 (Raaschou-Nielsen et al., 2003)]. Hansen et al. (2001) also reported risk for two other TCE exposure surrogates, average intensity and cumulative exposure, and in both cases, observed lower risk estimates with the higher exposure surrogate.

Meta-analysis is not adopted as a tool for examining the body of epidemiologic evidence on esophageal cancer and TCE exposure given the absence of reported RR estimates in several of the studies in which there is a high likelihood of TCE exposure in individual study subjects and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review (Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994).

Overall, three cohort studies in which there is a high likelihood of TCE exposure in individual study subjects and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review provide some evidence of association for esophageal cancer and TCE exposure. The finding in two of these studies of esophageal risk estimates among subjects with long employment duration were higher than those associated with low employment duration provides additional evidence (Raaschou-Nielsen et al., 2003; Hansen et al., 2001). The cohort studies are unable to directly examine possible confounding due to suspected risk factors for esophageal cancer such as smoking, obesity, and alcohol. The use of an internal referent group, similar in SES status as exposed subjects, is believed to minimize but may not completely control for possible confounding related to smoking and health status (Blair et al., (1998); Radican et al., (2008); Zhao et al., (2005); Boice et al., (2006b). Observation of a higher risk for adenocarcinoma histologic type than for a combined category of esophageal cancer in Raaschou-Nielsen et al. (2003) also suggests minimal confounding from smoking. Smoking is not identified as a possible risk factor for the adenocarcinoma histologic type of esophageal cancer, but is believed to be a risk factor for squamous cell histologic type. Furthermore, the magnitude of lung cancer risk in Raaschou-Nielsen et al. (2003) suggests that a high smoking rate is unlikely. The lack of association with overall TCE exposure and the absence of exposure-response patterns in the other studies of TCE exposure may reflect limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. These studies do not provide evidence against an association between TCE exposure and esophageal cancer.

4.9.2. Bladder Cancer

Twenty-five epidemiologic studies present risk estimates for bladder cancer (Radican et al., 2008; Sung et al., 2007; ATSDR, 2006a; Boice et al., 2006b; Chang et al., 2005; Zhao et al., 2005; ATSDR, 2004a, b; Chang et al., 2003; Raaschou-Nielsen et al., 2003; Morgan and

Cassady, 2002; Hansen et al., 2001; Pesch et al., 2000a; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Sinks et al., 1992; Siemiatycki, 1991; Mallin, 1990; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Shannon et al., 1988). Table 4-111 presents risk estimates for TCE exposure and bladder cancer observed in cohort, case-control, and geographic-based studies. Thirteen studies, all either cohort or case-control studies, which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) or which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review, reported RR estimates for bladder or urothelial cancer between 0.6 (Siemiatycki, 1991) and 1.7 (Boice et al., 2006b) and overall TCE exposure. RR estimates were generally based on small numbers of cases or deaths, except for one study (Raaschou-Nielsen et al., 2003), with the result of wide CIs on the estimates. Of these studies, two reported statistically significant elevated bladder or urothelial cancer risks with the highest cumulative TCE exposure category (2.71, 95% CI: 1.10, 6.65 (Morgan et al., 1998); 1.8, 95% CI: 1.2, 2.7 (Pesch et al., 2000a) and five presented risk estimates and categories of increasing cumulative TCE exposure (Radican et al., 2008; Zhao et al., 2005; Pesch et al., 2000a; Blair et al., 1998; Morgan et al., 1998). Risk estimates in Morgan et al. (1998), Pesch et al. (2000a), and Zhao et al. (2005) appeared to increase with increasing cumulative TCE exposure with the *p*-value for trend of 0.07 in Zhao et al. (2005), the only study to present a formal statistical test for linear trend. Risk estimates did not appear to either increase or decrease with increasing cumulative TCE exposure in Blair et al. (1998) or its update Radican et al. (2008), which added another 10 years of follow-up. Twelve additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease statistical power and study sensitivity (Sung et al., 2007; ATSDR, 2006a; Chang et al., 2005; ATSDR, 2004a; Chang et al., 2003; Morgan and Cassady, 2002; Sinks et al., 1992; Mallin, 1990; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Shannon et al., 1988).

Table 4-111. Summary of human studies on TCE exposure and bladder cancer

Exposure group		RR (95% CI)	Number of observable events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al. (2005)
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	1.00[a]	20	
	Medium cumulative TCE score	1.54 (0.81, 2.92)[b]	19	
	High TCE score	1.98 (0.93, 4.22)[b]	11	
	p for trend	$p = 0.069$		
	TCE, 20-yr exposure lag			
	Low cumulative TCE score	1.00[a]	20	
	Medium cumulative TCE score	1.76 (0.61, 5.10)[c]	20	
	High TCE score	3.68 (0.87, 15.5)[c]	10	
	p for trend	$p = 0.064$		
All employees at electronics factory (Taiwan)				
	Males	Not reported		Sung et al. (2007)
	Females	0.34 (0.07, 1.00)	10	
	Males	1.06 (0.45, 2.08)[d]	8	Chang et al. (2005)
	Females	1.09 (0.56, 1.91)[d]	12	
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al. (2003)
	Any exposure, all subjects	1.1 (0.92, 1.21)	220	
	Any exposure, males	1.0 (0.89, 1.18)	203	
	Any exposure, females	1.6 (0.93, 2.57)	17	
Biologically-monitored Danish workers		1.0 (0.48, 1.86)	10	Hansen et al. (2001)
	Any TCE exposure, males	1.1 (0.50, 2.0)	10	
	Any TCE exposure, females	0.5 expected	0	
Aircraft maintenance workers from Hill Air Force Base				Blair et al. (1998)
	TCE subcohort	Not reported		
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	1.7 (0.6, 4.4)	13	
	5–25 ppm-yr	1.7 (0.6, 4.9)	9	
	>25 ppm-yr	1.4 (0.5, 4.1)	9	

Table 4-111. Summary of human studies on TCE exposure and bladder cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
	Females, cumulative exposure			Blair et al. (1998) (continued)
	0	1.0[a]		
	<5 ppm-yr	1.1 (0.1, 10.8)	1	
	5–25 ppm-yr		0	
	>25 ppm-yr	1.0 (0.1, 9.1)	1	
Biologically-monitored Finnish workers				Anttila et al. (1995)
	All subjects	0.82 (0.27, 1.90)	5	
Biologically-monitored Swedish workers				Axelson et al. (1994)
	Any TCE exposure, males	1.02 (0.44, 2.00)	8	
	Any TCE exposure, females	Not reported		
Cohort and PMR studies-mortality				
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	1.66 (0.54, 3.87)	5	Boice et al. (2006b)
	Any exposure to TCE	Not reported		Zhao et al. (2005)
	Low cumulative TCE score	1.00[a]	8	
	Med cumulative TCE score	1.27 (0.43, 3.73)[b]	6	
	High TCE score	1.15 (0.29, 4.51)[b]	3	
	p for trend	$p = 0.809$		
	TCE, 20-yr exposure lag			
	Low cumulative TCE score	1.00[a]	8	
	Medium cumulative TCE score	0.95 (0.15, 6.02)[c]	7	
	High TCE score	1.85 (0.12, 27.7)[c]	2	
	p for trend	$p = 0.533$		
View-Master employees				ATSDR (2004a)
	Males	1.22 (0.15, 4.40)		
	Females	0.78 (0.09, 2.82)		
United States uranium-processing workers (Fernald)				Ritz (1999a)
	Any TCE exposure	Not reported		
	Light TCE exposure, >2-yr duration	Not reported		
	Moderate TCE exposure, >2-yr duration	Not reported		
Aerospace workers (Lockheed)				Boice et al. (1999)
	Routine exposure	0.55 (0.18, 1.28)	5	
	Routine-intermittent[a]	Not reported		

Table 4-111. Summary of human studies on TCE exposure and bladder cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Aerospace workers (Hughes)				Morgan et al. (1998)
	TCE subcohort	1.36 (0.59, 2.68)	8	
	Low intensity (<50 ppm)	0.51 (0.01, 2.83)	1	
	High intensity (>50 ppm)	1.79 (0.72, 3.69)	7	
	TCE subcohort (Cox Analysis)			
	Never exposed	1.0[a]		
	Ever exposed	2.05 (0.86, 4.85)[e]	8	
	Peak			
	No/low	1.0[a]		
	Medium/high	1.41 (0.52, 3.81)	5	
	Cumulative			
	Referent	1.0[a]		
	Low	0.69 (0.09, 5.36)	1	
	High	2.71 (1.10, 6.65)	7	
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
	TCE subcohort	1.2 (0.5, 2.9)[a]	17	
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	1.8 (0.5, 6.2)	7	
	5–25 ppm-yr	2.1 (0.6, 8.0)	5	
	>25 ppm-yr	1.0 (0.2, 5.1)	3	
	Females, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr		0	
	5–25 ppm-yr		0	
	>25 ppm-yr	0.8 (0.1, 7.5)	1	
	TCE subcohort	0.80 (0.41, 1.58)	25	Radican et al. (2008)
	Males, cumulative exposure	1.05 (0.47, 2.35)	24	
	0	1.0[a]		
	<5 ppm-yr	0.96 (0.37, 2.51)	9	
	5–25 ppm-yr	1.77 (0.70, 4.52)	10	
	>25 ppm-yr	0.67 (0.15, 2.95)	5	

Table 4-111. Summary of human studies on TCE exposure and bladder cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
	Females, cumulative exposure	0.22 (0.03, 1.83)	1	Radican et al. (2008) (continued)
	0	1.0[a]		
	<5 ppm-yr		0	
	5–25 ppm-yr	2.86 (0.27, 29.85)	1	
	>25 ppm-yr		0	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	TCE exposed workers	Not reported		
	Unexposed workers	Not reported		
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		0.85 (0.32, 2.23)[f]	20	Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia				Sinks et al. (1992)
		0.3 (0.0, 1.6)	1	
U.S. Coast Guard employees				Blair et al. (1989)
	Marine inspectors	0.50 (0.06, 1.79)	2	
	Noninspectors	0.90 (0.18, 2.62)	3	
Aircraft manufacturing plant employees (Italy)				Costa et al. (1989)
	All subjects	0.74 (0.30, 1.53)	7	
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)
	All subjects	1.26 (0.74, 2.03)	17	
Lamp manufacturing workers (GE)		0.93 (0.19, 2.72)	3	Shannon et al. (1988)
Case-control studies				
Population of five regions in Germany				Pesch et al. (2000a)
	Any TCE exposure	Not reported		
	Males	Not reported		
	Females	Not reported		
	Males			
	Medium	0.8 (0.6, 1.2)[g]	47	
	High	1.3 (0.8, 1.7)[g]	74	
	Substantial	1.8 (1.2, 2.7)[g]	36	
Population of Montreal, Canada				Siemiatycki (1991); Siemiatycki et al. (1994)
	Any TCE exposure	0.6 (0.3, 1.2)	8	
	Substantial TCE exposure	0.7 (0.3, 1.6)	5	
Geographic-based studies				
Residents in two study areas in Endicott, New York				ATSDR (2006a)
		0.71 (0.38, 1.21)	13	
Residents of 13 census tracts in Redlands, California				Morgan and Cassady (2002)
		0.98 (0.71, 1.29)[h]	82	
Finnish residents				Vartiainen et al. (1993)
	Residents of Hausjarvi	Not reported		

Table 4-111. Summary of human studies on TCE exposure and bladder cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
	Residents of Huttula	Not reported		
Residents of 9 county area in Northwestern Illinois				Mallin (1990)
	All zip codes in study area			
	Males	1.4 (1.1, 1.9)	47	
	Females	1.8 (1.2, 2.7)	21	
	Cluster community			
	Males	1.7 (1.1, 2.6)	21	
	Females	2.6 (1.2, 4.7)	10	
	Adjacent community			
	Males	1.2 (0.6, 2.0)	12	
	Females	1.6 (0.5, 3.8)	5	
	Remainder of zip code areas			
	Males	1.4 (0.8, 2.2)	14	
	Females	1.4 (0.5, 3.0)	6	

[a]Internal referents, workers not exposed to TCE.
[b]RR estimates for TCE exposure after adjustment for 1st employment, SES status, and age at event.
[c]RR estimates for TCE exposure after adjustment for 1st employment, SES status, age at event, and all other carcinogen exposures, including hydrazine.
[d]Chang et al. (2005) and Costa et al. (1989) report estimated risks for a combined site of all urinary organ cancers.
[e]Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex and decade (EHS, 1997).
[f]OR from nested case-control analysis.
[g]OR for urothelial cancer, a category of bladder, ureter, and renal pelvis cancers) and cumulative TCE exposure, as assigned using a JTEM approach (Pesch et al., 2000a).
[h]99% CI.

Meta-analysis is not adopted as a tool for examining the body of epidemiologic evidence on bladder cancer and TCE.

Overall, three cohort or case-control studies in which there is a high likelihood of TCE exposure in individual study subjects and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review provide some evidence of association for bladder or urothelial cancer and high cumulative TCE exposure (Zhao et al., 2005; Pesch et al., 2000a; Morgan et al., 1998). The case-control study of Pesch et al. (2000a) adjusted for age, study center, and cigarette smoking, with a finding of a statistically significant risk estimate between urothelial cancer and the highest TCE exposure category. Cancer cases in this study are of several sites (bladder, ureter, and renal pelvis), and grouping different site-specific cancers with possible etiologic heterogeneity may introduce misclassification bias. The cohort studies are unable to directly examine possible confounding due to suspected risk factors for esophageal cancer such as smoking, obesity, and alcohol. The use of an internal referent group, similar in

SES status as exposed subjects, by Morgan et al. (1998) and Zhao et al. (2005) is believed to minimize but may not completely control for possible confounding related to smoking and health status. The lack of association with overall TCE exposure in other studies and the absence of exposure-response patterns with TCE exposure in Blair et al. (1998) and Radican et al. (2008) may reflect limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. These studies do not provide evidence against an association between TCE exposure and bladder cancer.

4.9.3. CNS and Brain Cancers

Brain cancer is examined in most cohort studies and in one case-control study (Clapp and Hoffman, 2008; Radican et al., 2008; Sung et al., 2007; Boice et al., 2006b; Chang et al., 2005; Zhao et al., 2005; Chang et al., 2003; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Henschler et al., 1995; Greenland et al., 1994; Heineman et al., 1994; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988). Overall, these epidemiologic studies do not provide strong evidence for or against association between TCE and brain cancer in adults (see Table 4-112). RR estimates in well-designed and -conducted cohort studies, Axelson et al. (1994), Anttila et al. (1995), Blair et al. (1998), its follow-up reported in Radican et al. (2008), Morgan et al. (1998), Boice et al. (1999), Zhao et al. (2005), and Boice et al. (2006b), are near a risk of 1.0 and imprecise, CIs all include a risk estimate of 1.0. All studies except Raaschou-Nielsen et al. (2003), observations are based on few events and lowered statistical power. Bias resulting from exposure misclassification is likely in these studies, although of a lower magnitude compared to other cohort studies identified in Table 4-112, and may partly explain observations. Exposure misclassification is also likely in the case-control study of occupational exposure of Heineman et al. (1994) who do not report association with TCE exposure.

Table 4-112. Summary of human studies on TCE exposure and brain cancer

Exposure group	RR (95% CI)	Number of observable events	Reference
Cohort studies—incidence			
Aerospace workers (Rocketdyne)			Zhao et al. (2005)
Any exposure to TCE	Not reported		
Low cumulative TCE score	1.00[a]	7	
Medium cumulative TCE score	0.46 (0.09, 2.25)[b]	2	
High TCE score	0.47 (0.06, 3.95)[b]	1	
p for trend	*p* = 0.382		
All employees at electronics factory (Taiwan)			
Males	Not reported		Sung et al. (2007)
Females	1.07 (0.59, 1.80)[c]		
Males	0.40 (0.05, 1.46)	2	Chang et al. (2005)
Females	0.97 (0.54, 1.61)	15	
Danish blue-collar worker with TCE exposure			Raaschou-Nielsen et al. (2003)
Any exposure, all subjects	1.0 (0.84, 1.24)	104	
Any exposure, males	1.0 (0.76, 1.18)	85	
Any exposure, females	1.1 (0.67, 1.74)	19	
Biologically-monitored Danish workers	0.3 (0.01, 1.86)	1	Hansen et al. (2001)
Any TCE exposure, males	0.4 (0.01, 2.1)	1	
Any TCE exposure, females	0.5 expected	0	
Aircraft maintenance workers from Hill Air Force Base			Blair et al. (1998)
TCE subcohort	Not reported		
Males, cumulative exposure			
0	1.0[a]		
<5 ppm-yr	2.0 (0.2, 19.7)	3	
5–25 ppm-yr	3.9 (0.4, 34.9)	4	
>25 ppm-yr	0.8 (0.1, 13.2)	1	
Females, cumulative exposure			
0	1.0[a]		
<5 ppm-yr		0	
5–25 ppm-yr		0	
>25 ppm-yr		0	
Biologically-monitored Finnish workers			Anttila et al. (1995)
All subjects	1.09 (0.50, 2.07)	9	
Mean air-TCE (Ikeda extrapolation)			
<6 ppm	1.52 (0.61, 3.13)	7	
6+ ppm	0.76 (0.01, 2.74)	2	
Biologically-monitored Swedish workers			Axelson et al. (1994)
Any TCE exposure, males	Not reported		
Any TCE exposure, females	Not reported		

Table 4-112. Summary of human studies on TCE exposure and brain cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Cohort and PMR studies-mortality				
Computer manufacturing workers (IBM), New York				Clapp and Hoffman (2008)
	Males	1.90 (0.52, 4.85)	4	
	Females		0	
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	0.81 (0.17, 2.36)	3	Boice et al. (2006b)
	Any exposure to TCE	Not reported		Zhao et al. (2005)
	Low cumulative TCE score	1.00[a]	12	
	Medium cumulative TCE score	0.42 (0.12, 1.50)	3	
	High TCE score	0.83 (0.23, 3.08)	3	
	p for trend	$p = 0.613$		
View-Master employees				ATSDR (2004a)
	Males	Not reported		
	Females	Not reported		
All employees at electronics factory (Taiwan)				Chang et al. (2003)
	Males	0.96 (0.01, 5.36)	1	
	Females	0.96 (0.01, 5.33)	1	
United States uranium-processing workers (Fernald)				Ritz (1999a)
	Any TCE exposure	Not reported		
	Light TCE exposure, >2-yr duration, 0 lag	1.81 (0.49, 6.71)[d]	6	
	Moderate TCE exposure, >2-yr duration, 0 lag	3.26 (0.37, 28.9)[d]	1	
	Light TCE exposure, >5-yr duration, 15-yr lag	5.41 (0.87, 33.9)[d]	3	
	Moderate TCE exposure, >5-yr duration, 15-yr lag	14.4 (1.24, 167)[d]	1	
Aerospace workers (Lockheed)				Boice et al. (1999)
	Routine exposure	0.54 (0.15, 1.37)	4	
	Routine-intermittent[a]	Not presented		
Aerospace workers (Hughes)				Morgan et al. (1998)
	TCE subcohort	0.99 (0.64, 1.47)	4	
	Low intensity (<50 ppm)[d]	0.73 (0.09, 2.64)	2	
	High intensity (>50 ppm)[d]	0.44 (0.05, 1.58)	2	
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al. (1998)
	TCE subcohort	0.8 (0.2, 2.2)[a]	11	

Table 4-112. Summary of human studies on TCE exposure and brain cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
	Males, cumulative exposure			Blair et al. (1998) (continued)
	0	1.0[a]		
	<5 ppm-yr	0.7 (0.7, 3,3)	3	
	5–25 ppm-yr	2.0 (0.5, 8.4)	5	
	>25 ppm-yr	0.9 (0.2, 4.4)	2	
	Females, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr		0	
	5–25 ppm-yr		0	
	>25 ppm-yr		0	
	TCE subcohort	1.02 (0.39, 2.67)	17	Radican et al. (2008)
	Males, cumulative exposure	1.26 (0.43, 3.75)	17	
	0	1.0[a]		
	<5 ppm-yr	1.46 (0.44, 4.86)	8	
	5–25 ppm-yr	1.74 (0.49, 6.16)	6	
	>25 ppm-yr	0.66 (0.15, 2.95)	3	
	Females, cumulative exposure		0	
	0			
	<5 ppm-yr			
	5–25 ppm-yr			
	>25 ppm-yr			
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al. (1995)
	TCE exposed workers	3.70 (0.09, 20.64)	1	
	Unexposed workers	9.38 (1.93, 27.27)	3	
Deaths reported to GE pension fund (Pittsfield, Massachusetts)		0.93 (0.32, 2.69)[e]	16	Greenland et al. (1994)
Cardboard manufacturing workers, Atlanta area, Georgia				Sinks et al. (1992)
		Not reported		
U.S. Coast Guard employees				Blair et al. (1989)
	Marine inspectors	1.70 (0.55, 3.95)	5	
	Noninspectors	1.36 (0.44, 3.17)	5	
Aircraft manufacturing plant employees (Italy)				Costa et al. (1989)
	All subjects	0.79 (0.16, 2.31)	3	
Aircraft manufacturing plant employees (San Diego, California)				Garabrant et al. (1988)
	All subjects	0.78 (0.42, 1.34)	16	

Table 4-112. Summary of human studies on TCE exposure and brain cancer (continued)

Exposure group		RR (95% CI)	Number of observable events	Reference
Case-control studies				
Children's Cancer Group/Pediatric Oncology Group				De Roos et al. (2001)
	Any TCE exposure	1.64 (0.95, 2.84)	37	
	Neuroblastoma, ≤15 yrs of age			
	Paternal TCE exposure			
	Self-reported exposure	1.4 (0.7, 2.9)	22	
	IH assignment of probable exposure	0.9 (0.3, 2.5)	9	
Population of So. LA, NJ, Philadelphia, PA				Heineman et al. (1994)
	Any TCE exposure	1.1 (0.8, 1.6)	128	
	Low exposure	1.1 (0.7, 1.7)	27	
	Medium exposure	1.1 (0.6, 1.8)	42	
	High exposure	1.1 (0.5, 2.8)	12	
	p for trend	0.45		
Geographic-based studies				
Residents in two study areas in Endicott, New York				ATSDR (2006a)
	Brain/CNS, ≤19 yrs of age	Not reported	<6	
Residents of 13 census tracts in Redlands, California				Morgan and Cassady (2002)
	Brain/CNS, <15 yrs of age	1.05 (0.24, 2.70)[f]	6	
Resident of Tucson Airport Area, Arizona				AZ DHS (1995, 1990)
	Brain/CNS, ≤19 yrs of age			
	1970–1986	0.84 (0.23, 2.16)	3	
	1987–1991	0.78 (0.26, 2.39)	2	

[a]Internal referents, workers not exposed to TCE.
[b]RRs for TCE exposure after adjustment for 1st employment, SES status, and age at event.
[c]SIR from analyses lagging exposure 10 years prior to end of follow-up or date of incident cancer.
[d]RRs for TCE exposure after adjustment for time since 1st hired, external and internal radiation dose, and same chemical at a different level.
[e]OR from nested case-control analysis.
[f]99% CI.

Three geographic-based studies and one case-control study examined childhood brain cancer (ATSDR, 2006a; Morgan and Cassady, 2002; De Roos et al., 2001; ADHS, 1995, 1990). The strongest study, De Roos et al. (2001), a population case-control study that examined paternal exposure, used expert judgment to evaluate the probably of TCE exposure from self-reported information in an attempt to reduce exposure misclassification bias. The OR estimate in this study was 0.9 (95% CI: 0.3, 2.5). Like many population case-control studies, a low prevalence of TCE exposure was found, and only nine fathers were identified with probable TCE exposure by the industrial hygiene review, which greatly impacted statistical power. There is some concern for childhood brain cancer and organic solvent exposure based on Peters et al.

(1981) whose case-control study of childhood brain cancer reported to the Los Angeles County Cancer Surveillance Program observed a high OR estimate for paternal employment in the aircraft industry (OR: ∞, $p < 0.001$). This study does not present an OR for TCE exposure only although it did identify two of the 14 case and control fathers with previous employment in the aircraft industry reported exposure to TCE.

4.10. SUSCEPTIBLE LIFESTAGES AND POPULATIONS

Variation in response among segments of the population may be due to age, genetics, and ethnicity, as well as to differences in lifestyle, nutrition, and disease status. These could be potential risk factors that play an important role in determining an individual's susceptibility and sensitivity to chemical exposures. Available studies on TCE toxicity in relation to some of these risk factors including lifestage, gender, genetics, race/ethnicity, preexisting health status, and lifestyle are discussed below. However, there is a general lack of data demonstrating the modulation of health effects from TCE exposure based on these factors. Additional data examining these factors would provide further understanding of the populations that may be more susceptible to the health effects from TCE exposure. Others have also reviewed factors related to human variability and their potential for susceptibility to TCE (NRC, 2006; Clewell et al., 2000; Pastino et al., 2000; ATSDR, 1998b, 1997c; Barton et al., 1996; Davidson and Beliles, 1991).

4.10.1. Lifestages

Individuals of different lifestages are physiologically, anatomically, and biochemically different. Early (infants and children) and later (the elderly) lifestages differ greatly from adulthood in body composition, organ function, and many other physiological parameters that can influence the toxicokinetics of chemicals and their metabolites in the body (Guzelian et al., 1992). The limited data on TCE exposure among these segments of the population—particularly individuals in early lifestages—suggest they may have greater susceptibility than does the general population. This section presents and evaluates the pertinent published literature available to assess how individuals of differing lifestages may respond differently to TCE.

4.10.1.1. Early Lifestages

4.10.1.1.1. Early lifestage-specific exposures

Section 2.4 describes the various exposure pathways of concern for TCE. For all postnatal lifestages, the primary exposure routes of concern include inhalation and contaminated drinking water. In addition, there are exposure pathways to TCE are unique to early lifestages. Fetal and infant exposure to TCE can occur through placental transfer and breast milk consumption if the mother has been exposed, and could potentially increase overall TCE exposure. Placental transfer of TCE has been demonstrated in humans (Laham, 1970; Beppu,

1968), rats (Withey and Karpinski, 1985), mice (Ghantous et al., 1986), rabbits (Beppu, 1968), and sheep and goats (Helliwell and Hutton, 1950). Similarly, TCE has been found in breast milk in humans (Fisher et al., 1997; Pellizzari et al., 1982), goats (Hamada and Tanaka, 1995), and rats (Fisher et al., 1990). Pellizzari et al. (1982) conducted a survey of environmental contaminants in human milk, using samples from cities in the northeastern region of the United States and one in the southern region and detected TCE in 8 milk samples taken from 42 lactating women. No details of when the samples were taken postpartum, milk lipid content, or TCE concentration in milk or blood were reported. Fisher et al. (1997) predicted that a nursing infant would consume 0.496 mg TCE during a 24-hour period. In lactating rats exposed to 600 ppm (3,225 mg/m^3) TCE for 4 hours resulted in concentrations of TCE in milk of 110 µg/mL immediately following the cessation of exposure (Fisher et al., 1990).

Direct childhood exposures to TCE from oral exposures may also occur. A contamination of infant formula resulted in levels of 13 ppb (Fan, 1988). Children consume high levels of dairy products, and TCE has been found in butter and cheese (Wu and Schaum, 2000). In addition, TCE has been found in food and beverages containing fats such as margarine (Wallace et al., 1984), grains, and peanut butter (Wu and Schaum, 2000), all of which children consume in high amounts. A number of studies have examined the potential adverse effects of prenatal or postnatal exposure to drinking water contaminated with TCE (ATSDR, 2001; Sonnenfeld et al., 2001; Rodenbeck et al., 2000; Burg and Gist, 1999; ATSDR, 1998b; White et al., 1997; see Section 4.10.2.1; Bove, 1996; Bove et al., 1995; Goldberg et al., 1990; Bernad et al., 1987, abstract; Lagakos et al., 1986). TCE in residential water may also be a source of dermal or inhalation exposure during bathing and showering (Franco et al., 2007; Lee et al., 2002; Wu and Schaum, 2000; Giardino and Andelman, 1996; Weisel and Jo, 1996; Fan, 1988); it has been estimated that showering and bathing scenarios in water containing 3 ppm TCE, a child of 22 kg receives a higher dose (about 1.5 times) on a mg/kg basis than a 70 kg adult (Fan, 1988).

Direct childhood inhalation exposure to TCE have been documented in both urban and rural settings. A study of VOCs measured personal, indoor, and outdoor TCE in 284 homes, with 72 children providing personal measures and time-activity diaries (Adgate et al., 2004b). The intensive-phase of the study found a mean personal level of 0.8 µg/m^3 and mean indoor and outdoor levels of 0.6 µg/m^3, with urban homes have significantly higher indoor levels of TCE than nonurban homes ($t = 2.3$, $p = 0.024$) (Adgate et al., 2004b). A similar study of personal, indoor, and outdoor TCE was conducted in two inner-city elementary schools as well as in the homes of 113 children along with time-activity diaries, and found a median a median personal level of 0.3 µg/m^3, a median school indoor level of 0.2 µg/m^3, a median home indoor level of 0.3 µg/m^3, and a median outdoor level of 0.3 µg/m^3 in the winter, with slightly lower levels in the spring (Adgate et al., 2004a). Studies from Leipzig, Germany measured the median air level of TCE in children‘s bedrooms to be 0.42 µg/m^3 (Lehmann et al., 2001) and 0.6 µg/m^3

(Lehmann et al., 2002). A study of VOCs in Hong Kong measured air levels in schools, including an 8-hour average of 1.28 μg/m^3, which was associated with the lowest risk of cancer in the study (Guo et al., 2004). Another found air TCE levels to be highest in school/work settings, followed by outside, in home, in other, and in transit settings (Sexton et al., 2007). Measured indoor air levels ranged from 0.18 to 140 μg/m^3 for children exposed through vapor intrusion from soil vapor (ATSDR, 2006a). Contaminated soil may be a source of either dermal or ingestion exposure of TCE for children (Wu and Schaum, 2000).

Additional TCE exposure has also been documented to have occurred during medical procedures. TCE was used in the past as an anesthetic during childbirth (Phillips and Macdonald, 1971; Beppu, 1968) and surgery during childhood (Jasińska, 1965). These studies are discussed in more detail in Section 4.8.3.1.1. In addition, the TCE metabolite, CH, has been used as an anesthetic for children for CAT scans (Steinberg, 1993).

Dose received per body weight for 3 ppm TCE via oral, dermal, dermal plus inhalation, and bathing scenarios was estimated for a 10-kg infant, a 22-kg child, and a 70-kg adult (Fan, 1988) (see Table 4-113). For the oral route (drinking water), an infant would receive a higher daily dose than a child, and the child more than the adult. For the dermal and dermal plus inhalation route, the child would receive more than the adult. For the bathing scenario, the infant and child would receive comparable amounts, more than the adult.

Table 4-113. Estimated lifestage-specific daily doses for TCE in water[a]

	Body weight		
	Infant (10 kg)	**Child (22 kg)**	**Adult (70 kg)**
Drinking water	0.3 mg/kg	0.204 mg/kg	0.086 mg/kg
Showering—dermal	–	0.1 mg/kg	0.064 mg/kg
Showering—dermal and inhalation	–	0.129 mg/kg	0.083 mg/kg
Bathing—15 min	–	0.24 mg/kg	0.154 mg/kg
Bathing—5 min	0.08 mg/kg	0.08 mg/kg	0.051 mg/kg

[a]Adapted from Fan (1988).

4.10.1.1.2. Early lifestage-specific toxicokinetics

Chapter 3 describes the toxicokinetics of TCE. However, toxicokinetics in developmental lifestages are distinct from toxicokinetics in adults (Benedetti et al., 2007; Ginsberg et al., 2004a; Ginsberg et al., 2004b; Hattis et al., 2003; Ginsberg et al., 2002) due to, for example, altered ventilation rates, percentage of adipose tissue, and metabolic enzyme expression. Early lifestage-specific information is described below for absorption, distribution, metabolism, and excretion, followed by available early lifestage-specific PBPK models.

4.10.1.1.2.1. Absorption

As discussed in Section 3.1, exposure to TCE may occur via inhalation, ingestion, and dermal absorption. In addition, prenatal exposure may result in absorption via the transplacental route. Exposure via inhalation is proportional to the ventilation rate, duration of exposure, and concentration of expired air, and children have increased ventilation rates per kg body weight compared to adults, with an increased alveolar surface area per kg body weight for the first 2 years (U.S. EPA, 2008c). It is not clear to what extent dermal absorption may be different for children compared to adults; however, infants have a twofold increase in surface area compared to adults, although similar permeability (except for premature babies) compared to adults (U.S. EPA, 2008c).

4.10.1.1.2.2. Distribution

Both human and animal studies provide clear evidence that TCE distributes widely to all tissues of the body (see Section 3.2). For lipophilic compounds such as TCE, percentage adipose tissue, which varies with age, will affect absorption and retention of the absorbed dose. Infants have a lower percentage of adipose tissue per body weight than adults, resulting in a higher concentration of the lipophilic compound in the fat of the child (NRC, 1993).

During pregnancy of humans and experimental animals, TCE is distributed to the placenta (Ghantous et al., 1986; Withey and Karpinski, 1985; Laham, 1970; Beppu, 1968; Helliwell and Hutton, 1950). In humans, TCE has been found in newborn blood after exposure to TCE during childbirth with ratios of concentrations in fetal:maternal blood ranging from approximately 0.5 to approximately 2 (Laham, 1970). In childhood, blood level concentrations of TCE were found to range from 0.01 to 0.02 ng/mL (Sexton et al., 2005). Pregnant rats exposed to TCE vapors on GD 17 resulted in concentrations of TCE in fetal blood approximately one-third the concentration in corresponding maternal blood, and was altered based upon the position along the uterine horn (Withey and Karpinski, 1985). TCE has also been found in the organs of prenatal rabbits including the brain, liver, kidneys, and heart (Beppu, 1968). Rats prenatally exposed to TCE had increased levels measured in the brain at PND 10, compared to rats exposed as adults (Rodriguez et al., 2007). TCE can cross the blood:brain barrier during both prenatal and postnatal development, and may occur to a greater extent in younger children. It is also important to note that it has been observed in mice that TCE can cycle from the fetus into the amniotic fluid and back to the fetus (Ghantous et al., 1986).

Studies have examined the differential distribution by age to a mixture of six VOCs including TCE to children aged 3–10 years and adults aged 20–82 years (Mahle et al., 2007) and in rats at PND 10, 2 months (adult), and 2 years (aged) (Mahle et al., 2007; Rodriguez et al., 2007). In humans, the blood:air partition coefficient for male or female children was

significantly lower compared to adult males (Mahle et al., 2007). In rats, the difference in tissue:air partition coefficients increased with age (Mahle et al., 2007). Higher peak concentrations of TCE in the blood were observed in the PND 10 rat compared to the adult rat after inhalation exposure, likely due to the lower metabolic capacity of the young rats (Rodriguez et al., 2007).

4.10.1.1.2.3. Metabolism

Section 3.3 describes the enzymes involved in the metabolism of TCE, including CYP and GST. Expression of these enzymes changes during various stages of fetal development (Shao et al., 2007; Dorne et al., 2005; Hines and McCarver, 2002; Hakkola et al., 1998a; 1998b; van Lieshout et al., 1998; Hakkola et al., 1996a; Hakkola et al., 1996b) and during postnatal development (Blake et al., 2005; Dorne et al., 2005; Tateishi et al., 1997), and may result in altered susceptibility.

Expression of CYP enzymes have been shown to play a role in decreasing the metabolism of TCE during pregnancy in rats, although metabolism increased in young (3-week-old) rats compared to adult (18-week-old) rats (Nakajima et al., 1992b). For TCE, CYP2E1 is the main metabolic CYP enzyme, and expression of this enzyme has been observed in humans in prenatal brain tissue at low levels beginning at 8 weeks of gestation and increasing throughout gestation (Brzezinski et al., 1999). Very low levels of CYP2E1 have been detected in some samples of fetal liver during the second trimester (37% of samples) and third trimester (80% of samples) (Johnsrud et al., 2003; Carpenter et al., 1996), although hepatic expression surges immediately after birth in most cases (Johnsrud et al., 2003; Vieira et al., 1996) and in most infants, reaches adult values by 3 months of age (Johnsrud et al., 2003; Vieira et al., 1996).

Although there is some uncertainty as to which GST isoforms mediate TCE conjugation, it should be noted that their expression changes with fetal development (McCarver and Hines, 2002; Raijmakers et al., 2001; van Lieshout et al., 1998).

4.10.1.1.2.4. Excretion

The major processes of excretion of TCE and its metabolites are discussed in Section 3.4, yet little is known about whether there are age-related differences in excretion of TCE. The major pathway for elimination of TCE is via exhalation, and its metabolites via urine and feces, and it is known that renal processes are not mature until about 6 months of age (NRC, 1993). Only one case study was identified that measured TCE or its metabolites in exhaled breath and urine in a 17-year-old who ingested a large quantity of TCE (Brüning et al., 1998). TCE has also been measured in the breast milk in lactating women (Fisher et al., 1997; Pellizzari et al., 1982), goats (Hamada and Tanaka, 1995), and rats (Fisher et al., 1990).

4.10.1.1.2.5. PBPK models

Early lifestage-specific information regarding absorption, distribution, metabolism, and excretion needs to be considered for a child-specific and chemical-specific PBPK model. To adequately address the risk to infants and children, age-specific parameters for these values should be used in PBPK models that can approximate the internal dose an infant or child receives based on a specific exposure level (see Section 3.5).

Fisher and colleagues developed PBPK models to describe the toxicokinetics of TCE in the pregnant rat (Fisher et al., 1989), lactating rat and nursing pup (Fisher et al., 1990). The prenatal study demonstrates that approximately two-thirds of maternal exposure to both TCE and TCA reached the fetus after maternal inhalation, gavage, or drinking water exposure (Fisher et al., 1989). After birth, only 2% of maternal exposure to TCE reaches the pup; however, 15 and 30% of maternal TCA reaches the pup after maternal inhalation and drinking water exposure, respectively (Fisher et al., 1990). One analysis of PBPK models examined the variability in response to VOCs including TCE between adults and children, and concluded that the intraspecies uncertainty factor (UF) for pharmacokinetics is sufficient to capture variability between adults and children (Pelekis et al., 2001).

4.10.1.1.3. Early lifestage-specific effects

Although limited data exist on TCE toxicity as it relates to early lifestages, there is enough information to discuss the qualitative differences. In addition to the evidence described below, Section 4.8 contains information on reproductive and developmental toxicity. In addition, Sections 4.3 on neurotoxicity and Section 4.6 on immunotoxicity characterize a wide array of postnatal developmental effects.

4.10.1.1.3.1. Differential noncancer outcomes in early lifestages

Some adverse health outcomes, in particular birth defects, are observed only after early lifestage exposure to TCE. A summary of structural developmental outcomes that have been associated with TCE exposures is presented in Sections 4.8.3.3.

Cardiac birth defects have been observed after exposure to TCE in humans (ATSDR, 2006a; Yauck et al., 2004; Goldberg et al., 1990; Lagakos et al., 1986), rodents (Johnson et al., 2005, 2003; Johnson et al., 1998b; Johnson et al., 1998a; Dawson et al., 1993; Smith et al., 1992; Dawson et al., 1990; Smith et al., 1989), and chicks (Rufer et al., 2008; Drake et al., 2006a; Drake et al., 2006b; Mishima et al., 2006; Boyer et al., 2000; Loeber et al., 1988; Bross et al., 1983). However, it is notable that cardiac malformations were not observed in a number of other studies in humans (Taskinen et al., 1989; Lagakos et al., 1986; Tola et al., 1980), rodents (Carney et al., 2006; Fisher et al., 2001; Narotsky and Kavlock, 1995; Narotsky et al., 1995; Coberly et al., 1992; Cosby and Dukelow, 1992; Healy et al., 1982; Hardin et al., 1981;

Dorfmueller et al., 1979; Schwetz et al., 1975), and rabbits (Hardin et al., 1981). See Section 4.8.3.3.2.3 for further discussion on cardiac malformations.

Structural CNS birth defects were observed in humans (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). In addition, a number of postnatal nonstructural adverse effects on the CNS system have been observed in humans and experimental animals following prenatal exposure to TCE. See Sections 4.3.10 and 4.8.3.3.4 for further discussion on developmental neurotoxicity.

A variety of other birth defects have been observed—including eye/ear birth anomalies in humans and rats (Narotsky and Kavlock, 1995; Narotsky et al., 1995; Lagakos et al., 1986); lung/respiratory tract disorders in humans and mice (Das and Scott, 1994; Lagakos et al., 1986); and oral cleft defects (Bove, 1996; Bove et al., 1995; Lagakos et al., 1986), kidney/urinary tract disorders, musculoskeletal birth anomalies (Lagakos et al., 1986), and anemia/blood disorders (Burg and Gist, 1999) in humans. See Section 4.8.3.3.3 for further discussion on other structural developmental outcomes. A current follow-up study of the Camp Lejeune cohort will examine birth defects and may provide additional insight (ATSDR, 2009; U.S. GAO, 2007b, a; ATSDR, 2003a).

4.10.1.1.3.2. Susceptibility to noncancer outcomes in early lifestages

There are a number of adverse health outcomes observed after exposure to TCE that are observed in both children and adults. Below is a discussion of differential exposure, incidence, and/or severity in early lifestages compared to adulthood.

Occupational TCE poisonings via inhalation exposure resulted in an elevated percentage of cases in the adolescents aged 15–19 years old compared those ≥20 years old (McCarthy and Jones, 1983). In addition, there is concern for intentional exposure to TCE during adolescence, including a series of deaths involving inhaling typewriter correction fluid (King et al., 1985) a case of glue sniffing likely associated with cerebral infarction in a 12-year-old boy with a 2-year history of exposure (Parker et al., 1984), and a case of attempted suicide by ingestion of 70 mg TCE in a 17-year-old boy (Brüning et al., 1998).

4.10.1.1.3.2.1. Neurotoxicity

Adverse CNS effects observed after early lifestage exposure to TCE in humans include delayed newborn reflexes (Beppu, 1968); impaired learning or memory (White et al., 1997; Bernad et al., 1987); aggressive behavior (Blossom et al., 2008; Bernad et al., 1987); hearing impairment (Burg and Gist, 1999; Burg et al., 1995); speech impairment (Burg and Gist, 1999; White et al., 1997; Burg et al., 1995); encephalopathy (White et al., 1997); impaired executive and motor function (White et al., 1997); attention deficit (Bernad et al., 1987) (White et al., 1997); and ASD (Windham et al., 2006). One analysis observed a trend for increased adversity during development, with those exposed during childhood demonstrating more deficits than

those exposed during adulthood (White et al., 1997). In experimental animals, observations include decreased specific gravity of newborn brains until weaning (Westergren et al., 1984), reductions in myelination in the brains at weaning, significantly decreased uptake of 2-deoxyglucose in the neonatal rat brain, significant increase in exploratory behavior (Isaacson and Taylor, 1989; Noland-Gerbec et al., 1986; Taylor et al., 1985), decreased rearing activity (Fredriksson et al., 1993), and increased time to cross the first grid in open field testing (George et al., 1986).

Few studies addressed whether or not children are more susceptible to CNS effects compared to adults (Burg and Gist, 1999; White et al., 1997; Burg et al., 1995). An analysis of three residential exposures of TCE observed speech impairments in younger children and not at any other lifestage (White et al., 1997). A national TCE exposure registry also observed statistically significant speech impairment and hearing impairment in 0–9 year olds and no other age group (Burg and Gist, 1999; Burg et al., 1995). However, a follow-up study did not find a continued association with speech and hearing impairment in these children, although the absence of acoustic reflexes remained significant (ATSDR, 2002). See Section 4.3 for further information on CNS toxicity, and Section 4.8.3.3.4 for further information on developmental neurotoxicity.

4.10.1.1.3.2.2. Liver toxicity

No early lifestage-specific effects were observed after TCE exposure. See Section 4.5 for further information on liver toxicity.

4.10.1.1.3.2.3. Kidney toxicity

Residents of Woburn, Massachusetts including 4,978 children were surveyed on residential and medical history to examine an association between observed adverse health outcomes and wells contaminated with TCE and other chemicals; among these children, an association was observed for higher cumulative exposure measure and history of kidney and urinary tract disorders (primarily kidney or urinary tract infections) and with lung and respiratory disorders (asthma, chronic bronchitis, or pneumonia) (Lagakos et al., 1986). Comparisons were not made for the adults living in this community. See Section 4.4 for further information on kidney toxicity.

4.10.1.1.3.2.4. Immunotoxicity

Several studies in exposure to TCE in early lifestages of humans (Lehmann et al., 2002; Lehmann et al., 2001) and experimental animals (Blossom et al., 2008; Peden-Adams et al., 2008; Blossom and Doss, 2007; Peden-Adams et al., 2006; Adams et al., 2003) were identified that assessed the potential for developmental immunotoxicity. While some noted evidence of immune system perturbation (Blossom et al., 2008; Blossom and Doss, 2007; Peden-Adams et

al., 2006; Adams et al., 2003; Lehmann et al., 2002), others did not (Peden-Adams et al., 2008; Lehmann et al., 2001). However, none of these studies assessed whether exposure during early life resulted in evidence of increased susceptibility as compared to exposure during adulthood; this is an area for future research. See Section 4.6 for further information on immunotoxicity, and Section 4.8.3.3.5 for further discussion on developmental immunotoxicity.

4.10.1.1.3.2.5. Respiratory toxicity

Residents of Woburn, Massachusetts including 4,978 children were surveyed on residential and medical history to examine an association between observed adverse health outcomes and wells contaminated with TCE and other chemicals; among these children, an association was observed for lung and respiratory disorders (asthma, chronic bronchitis, or pneumonia) (Lagakos et al., 1986). Comparisons were not made for the adults living in this community. See Section 4.7 for further information on respiratory tract toxicity.

4.10.1.1.3.3. Susceptibility to cancer outcomes in early lifestages

The epidemiologic and experimental animal evidence is limited regarding susceptibility to cancer from exposure to TCE during early lifestages. The human epidemiological evidence is summarized above for cancer diagnosed during childhood (see Sections 4.8.2.1 and 4.8.3.3.6), including a discussion of childhood cancers of the nervous system including neuroblastoma and the immune system including leukemia (see Section 4.6.1.2). A current follow-up study of the Camp Lejeune cohort will examine childhood cancers and may provide additional insight (ATSDR, 2009; U.S. GAO, 2007b, a; ATSDR, 2003a). No studies of cancers in experimental animals in early lifestages have been observed.

4.10.1.1.3.3.1. Total childhood cancer

Total childhood cancers have been examined in relationship to TCE exposure (ATSDR, 2006a; Morgan and Cassady, 2002). Two studies examining total childhood cancer in relation to TCE in drinking water did not observe an association. A study in Endicott, New York contaminated by a number of VOCs, including ~~–t~~housands of gallons" of TCE observed fewer than six cases of cancer diagnosed between 1980 and 2001 in children aged 0–19 years, and did not exceed expected cases or types (ATSDR, 2006a). A California community exposed to TCE in drinking water from contaminated wells was examined for cancer, with a specific emphasis on childhood cancer (<15 years old); however, the incidence did not exceed those expected for the community (Morgan and Cassady, 2002). A third study of childhood cancer in relation to TCE in drinking water in Camp Lejeune, North Carolina is currently underway (U.S. GAO, 2007b, a).

4.10.1.1.3.3.2. Childhood leukemia

Childhood leukemia has been examined in relationship to TCE exposure (Costas et al., 2002; Shu et al., 1999; Cohn et al., 1994b; McKinney et al., 1991; Lowengart et al., 1987; Lagakos et al., 1986). In a study examining drinking water exposure to TCE in 75 New Jersey towns, childhood leukemia (including ALL) was significantly increased for girls (n = 6) diagnosed before age 20 years, but this was not observed for boys (Cohn et al., 1994b). A community in Woburn, Massachusetts with contaminated well water including TCE experienced 20 cases of childhood leukemia, significantly more than expected (Lagakos et al., 1986); however, the incidence of leukemia among children was not compared to the incidence rate among adults living in this community. Further analysis by Costas et al. (2002) also observed a greater than twofold increase over expected cases of childhood leukemia. Cases were more likely to be male (76%), <9 years old at diagnosis (62%), breast-fed (OR: 10.17, 95% CI: 1.22–84.50), and exposed during pregnancy (OR_{adj}: 8.33, 95% CI: 0.73–94.67). The highest risk was observed for exposure during pregnancy compared to preconception or postnatal exposure, and a dose-response was seen for exposure during pregnancy (Costas et al., 2002). In addition, family members of those diagnosed with childhood leukemia, including 13 siblings under age 19 at the time of exposure, had altered immune response, but an analysis looking at only these children was not done (Byers et al., 1988).

Case-control studies examined children diagnosed with ALL for parental occupational exposures and found a nonsignificant two- to fourfold increase of childhood leukemia risk for exposure to TCE during preconception, pregnancy, postnatally, or all developmental periods combined (Shu et al., 1999; McKinney et al., 1991; Lowengart et al., 1987). Some studies showed an elevated risk for maternal (Shu et al., 1999) or paternal exposure (McKinney et al., 1991; Lowengart et al., 1987), while others did not show an elevated risk for maternal (McKinney et al., 1991) or paternal exposure (Shu et al., 1999), possibly due to the small number of cases. No variability was observed in the developmental stages in Shu et al. (1999), although Lowengart et al. (1987) observed the highest risk to be paternal exposure to TCE after birth.

4.10.1.1.3.3.3. CNS tumors

In a case-control study of parental occupational exposures, paternal self-reported exposure to TCE was not significantly associated with neuroblastoma in the offspring (OR = 1.4, 95% CI: 0.7–2.9) (De Roos et al., 2001). Brain tumors have also been observed in the offspring of fathers exposed to TCE, but the OR could not be determined (Peters et al., 1985; Peters et al., 1981).

4.10.1.1.3.3.4. ADAFs

According to EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005e), there may be increased susceptibility to early-life

exposures for carcinogens with a mutagenic mode of action. Therefore, because the weight of evidence supports a mutagenic mode of action for TCE carcinogenicity in the kidney (see Section 4.4.7), the lack of data suggesting an absence of GSTT1 expression in neonates, and in the absence of chemical-specific data to evaluate differences in susceptibility, early-life susceptibility should be assumed and the ADAFs should be applied, in accordance with the *Supplemental Guidance*.

4.10.1.2. Later Lifestages

Few studies examine the differential effects of TCE exposure for elderly adults (>65 years old). These limited studies suggest that older adults may experience increased adverse effects than younger adults. However, there is no further evidence for elderly individuals exposed to TCE beyond these studies.

Toxicokinetics in later lifestages can be distinct from toxicokinetics in younger adults (Benedetti et al., 2007; Ginsberg et al., 2005), although there is limited evidence showing a possible age-related difference in CYP expression (Dorne et al., 2005; Parkinson et al., 2004; George et al., 1995b). GST expression has been observed to decrease with age in human lymphocytes, with the lowest expression in those aged 60–80 years old (van Lieshout and Peters, 1998).

Studies have examined the age differences in TK after exposure to a mixture of six VOCs including TCE for humans (Mahle et al., 2007) and rats (Mahle et al., 2007; Rodriguez et al., 2007). In humans, the blood:air partition coefficient for adult males (20–82 years) was significantly ($p \leq 0.05$) higher (11.7 ± 1.9) compared to male (11.2 ± 1.8) or female (11.0 ± 1.6) children (3–10 years) (Mahle et al., 2007); when the data was stratified for adults above and below 55 years of age, there was no significant difference observed between adults (20–55 years) and aged (56–82) (data not reported). In rats, the difference in tissue:air partition coefficients also increased from PND 10 to adult (2 months) to aged (2 years) rat (Mahle et al., 2007). TCE has also been measured in the brain of rats, with an increased level observed in older (2-year-old) rats compared to adult (2-month-old) rats (Rodriguez et al., 2007). It was also observed that aged rats reached steady state slower with higher concentrations compared to the adult rat; the authors suggest that the almost twofold greater percentage of body fat in the elderly is responsible for this response (Rodriguez et al., 2007).

One cohort of TCE exposed metal degreasers found an increase in psychoorganic syndrome and increased vibration threshold related to increasing age (Rasmussen et al., 1993b; Rasmussen et al., 1993c, d), although the age groups were ≤29, 30–39, and 40+ years, but the age ranged only from 18 to 68 years and did not examine >65 years as a separate category.

4.10.2. Other Susceptibility Factors

Aside from age, many other factors may affect susceptibility to TCE toxicity. A partial list of these factors includes gender, genetic polymorphisms, preexisting disease status, nutritional status, diet, and previous or concurrent exposures to other chemicals. The toxicity that results due to changes in multiple factors may be quite variable, depending on the exposed population and the type of exposure. Qualitatively, the presence of multiple susceptibility factors will increase the variability that is seen in a population response to TCE toxicity.

4.10.2.1. Gender

Individuals of different genders are physiologically, anatomically, and biochemically different. Males and females can differ greatly in many physiological parameters such as body composition, organ function, and ventilation rate, which can influence the toxicokinetics of chemicals and their metabolites in the body (Gochfeld, 2007; Gandhi et al., 2004).

4.10.2.1.1. Gender-specific toxicokinetics

Chapter 3 describes the toxicokinetics of TCE. Gender-specific information is described below for absorption, distribution, metabolism, and excretion, followed by available gender-specific PBPK models.

4.10.2.1.1.1. Absorption

As discussed in Section 3.1, exposure to TCE may occur via inhalation, ingestion, and skin absorption. Exposure via inhalation is proportional to the ventilation rate, duration of exposure, and concentration of expired air, and women have increased ventilation rates during exercise compared to men (Gochfeld, 2007). Percentage of body fat varies with gender (Gochfeld, 2007), which for lipophilic compounds such as TCE will affect absorption and retention of the absorbed dose. After experimental exposure to TCE, women were found to absorb a lower dose due to lower alveolar intake rates compared to men (Sato, 1993; Sato et al., 1991b).

4.10.2.1.1.2. Distribution

Both human and animal studies provide clear evidence that TCE distributes widely to all tissues of the body (see Section 3.2). The distribution of TCE to specific organs will depend on organ blood flow and the lipid and water content of the organ, which may vary between genders (Gochfeld, 2007). After experimental exposure to humans, higher distribution of TCE into fat tissue was observed in women leading to a greater blood concentration 16 hours after exposure compared to men (Sato, 1993; Sato et al., 1991b). In experimental animals, male rats generally have higher levels of TCE in tissues compared to female rats, likely due to gender differences in metabolism (Lash et al., 2006). In addition, TCE has been observed in the male reproductive organs (epididymis, vas deferens, testis, prostate, and seminal vesicle) (Zenick et al., 1984).

4.10.2.1.1.3. Metabolism

Section 3.3 describes the metabolic processes involved in the metabolism of TCE, including CYP and GST enzymes. In addition, the role of metabolism in male reproductive toxicity is discussed in Section 4.8.1.3.2.1. In general, there is some indication that TCE metabolism is different between males and females, with females more rapidly metabolizing TCE after oral exposure to rats (Lash et al., 2006), i.p. injections in rats (Verma and Rana, 2003), and in mouse, rat, and human liver microsomes (Elfarra et al., 1998).

In general, CYP expression may differ between genders (Gochfeld, 2007; Gandhi et al., 2004; Parkinson et al., 2004), although no gender-related difference in CYP2E1 activity is observed in the human liver microsomes (Parkinson et al., 2004; George et al., 1995a). After exposure to TCE, CYP2E1 was detected in the epididymis and testes of mice (Forkert et al., 2002), and CYP2E1 and GST-alpha has been detected in the ovaries of rats (Wu and Berger, 2008), indicating that metabolism of TCE can occur in both the male and female reproductive tracts. One study of TCE exposure in mice observed induced CYP2E1 expression in the liver of males only (Nakajima et al., 2000). Male rats have been shown to have higher levels of TCE metabolites in the liver (Lash et al., 2006), and lower levels of TCE metabolites in the kidney (Lash et al., 2006) compared to female rats. However, another study did not observe ant sex-related differences in the metabolism of TCE in rats (Nakajima et al., 1992b).

Unlike CYP-mediated oxidation, quantitative differences in the polymorphic distribution or activity levels of GST isoforms in humans are not presently known. However, the available data (Lash et al., 1999a; Lash et al., 1999b) do suggest that significant variation in GST-mediated conjugation of TCE exists in humans. One study observed that GSH conjugation is higher in male rats compared to female rats (Lash et al., 2000b); however, it has also been speculated that any gender difference may be due to a polymorphism in GSH conjugation of TCE rather than a true gender difference (Lash et al., 1999b). Also, induction of PPARα expression in male mice after TCE exposure was greater than that in females (Nakajima et al., 2000).

4.10.2.1.1.4. Excretion

The major processes of excretion of TCE and its metabolites are discussed in Section 3.4. Two human voluntary inhalation exposure studies observed the levels of TCE and its metabolites in exhaled breath and urine (Kimmerle and Eben, 1973a; Nomiyama and Nomiyama, 1971). Increased levels of TCE in exhaled breath in males were observed in one human voluntary inhalation exposure study of 250–380 ppm for 160 minutes (Nomiyama and Nomiyama, 1971), but no difference was observed in another study of 40 ppm for 4 hours or 50 ppm for 4 hours for 5 days (Kimmerle and Eben, 1973a).

After experimental exposure to TCE, women were generally found to excrete higher levels of TCE and TCA compared to men (Kimmerle and Eben, 1973a; Nomiyama and Nomiyama, 1971). However, other studies observed an increase in TCE in the urine of males (Inoue et al., 1989), an increase in TCA in the urine of males (Sato et al., 1991b), or no statistically significant ($p > 0.10$) gender difference for TCA in the urine (Inoue et al., 1989). Others found that the urinary elimination half-life of TCE metabolites is longer in women compared to men (Ikeda, 1977; Ikeda and Imamura, 1973).

In addition to excretion pathways that occur in both genders, excretion occurs uniquely in men and women. In both humans and experimental animals, it has been observed that females can excrete TCE and metabolites in breast milk (Fisher et al., 1997; Hamada and Tanaka, 1995; Fisher et al., 1990; Pellizzari et al., 1982), while males can excrete TCE and metabolites in seminal fluid (Forkert et al., 2003; Zenick et al., 1984).

4.10.2.1.1.5. PBPK models

Gender-specific differences in uptake and metabolism of TCE were incorporated into a PBPK model using human exposure data (Fisher et al., 1998). The chemical-specific parameters included cardiac output at rest, ventilation rates, tissue volumes, blood flow, and fat volume. This model found that gender differences for the toxicokinetics of TCE are minor.

4.10.2.1.2. Gender-specific effects

4.10.2.1.2.1. Gender susceptibility to noncancer outcomes

4.10.2.1.2.1.1. Liver toxicity

No gender susceptibility to noncancerous outcomes in the liver was observed. A detailed discussion of the studies examining the effects of TCE on the liver can be found in Section 4.5.

4.10.2.1.2.1.2. Kidney toxicity

A detailed discussion of the studies examining the noncancer effects of TCE on the kidney can be found in Section 4.4. A residential study found that females aged 55–64 years old had an elevated risk of kidney disease (RR = 4.57, 99% CI: 2.10–9.93) compared to males, although an elevated risk of urinary tract disorders was reported for both males and females (Burg et al., 1995). Additionally, a higher rate of diabetes in females compared to males exposed to TCE was reported in two studies (Davis et al., 2005; Burg et al., 1995). In rodents, however, kidney weights were increased more in male mice than in females (Kjellstrand et al., 1983a; Kjellstrand et al., 1983b), and male rats have exhibited increased renal toxicity to TCE compared to females (Lash et al., 2001b; Lash et al., 1998a).

4.10.2.1.2.1.3. Immunotoxicity

A detailed discussion of the studies examining the immunotoxic effects of TCE can be found in Section 4.6. Most of the immunotoxicity studies present data stratified by sex. The prevalence of exposure to TCE is generally lower in women compared with men. In men, the studies generally reported ORs between 2.0 and 8.0, and in women, the ORs were between 1.0 and 2.0 (Cooper et al., 2009). Based on small numbers of cases, an occupational study of TCE exposure found an increased risk for systemic sclerosis for men (OR: 4.75, 95% CI: 0.99–21.89) compared to women (OR: 2.10; 95% CI: 0.65–6.75) (Diot et al., 2002). Another study found similar results, with an elevated risk for men with a maximum intensity, cumulative intensity, and maximum probability of exposure to TCE compared to women (Nietert et al., 1998). These two studies, along with one focused exclusively on the risk of scleroderma to women (Garabrant et al., 2003), were included in a meta-analysis conducted by the EPA resulting in a combined estimate for "any" exposure, was OR = 2.5 (95% CI: 1.1, 5.4) for men and OR = 1.2 (95% CI: 0.58, 2.6) in women.

4.10.2.1.2.1.4. Respiratory toxicity

No gender susceptibility to noncancerous outcomes in the respiratory tract after TCE exposure was observed. A detailed discussion of the studies examining the respiratory effects of TCE can be found in Section 4.7.

4.10.2.1.2.1.5. Reproductive toxicity

A detailed discussion of the studies examining the gender-specific noncancer reproductive effects of TCE can be found in Section 4.8.1.

Studies examining males after exposure to TCE observed altered sperm morphology and hyperzoospermia (Chia et al., 1996), altered endocrine function (Goh et al., 1998; Chia et al., 1997), decreased sexual drive and function (Saihan et al., 1978; El Ghawabi et al., 1973; Bardodej and Vyskocil, 1956), and altered fertility to TCE exposure. Infertility was not associated with TCE exposure in other studies (Forkert et al., 2003; Sallmen et al., 1998), and sperm abnormalities were not observed in another study (Rasmussen et al., 1988).

There is more limited evidence for reproductive toxicity in females. There are epidemiological indicators of a possible effect of TCE exposure on female fertility (Sallmen et al., 1998), increased rate of miscarriage (ATSDR, 2001), and menstrual cycle disturbance (ATSDR, 2001; Zielinski, 1973; Bardodej and Vyskocil, 1956). In experimental animals, the effects on female reproduction include evidence of reduced in vitro oocyte fertilizability in rats (Wu and Berger, 2008, 2007; Berger and Horner, 2003). However, in other studies that assessed reproductive outcome in female rodents (Cosby and Dukelow, 1992; George et al., 1986; George et al., 1985; Manson et al., 1984), there was no evidence of adverse effects of TCE exposure on female reproductive function.

4.10.2.1.2.1.6. Developmental toxicity

A detailed discussion of the studies examining the gender-specific noncancer developmental effects of TCE can be found in Section 4.8.3. Only one study of contaminated drinking water exposure in Camp Lejeune, North Carolina observed a higher risk of SGA in males compared to females (Sonnenfeld et al., 2001; ATSDR, 1998a).

4.10.2.1.2.2. Gender susceptibility to cancer outcomes

A detailed discussion of the studies examining the carcinogenic effects of TCE can be found on the liver in Section 4.5, on the kidney in Section 4.4, in the immune system in Section 4.6, in the respiratory system in Section 4.7, and on the reproductive system in Section 4.8.2.

4.10.2.1.2.2.1. Liver cancer

An elevated risk of liver cancer was observed for females compared to males in both human (Raaschou-Nielsen et al., 2003) and rodent (Elfarra et al., 1998) studies. In addition, gallbladder cancer was significantly elevated for women compared to men (Raaschou-Nielsen et al., 2003). A detailed discussion of the studies examining the gender-specific liver cancer effects of TCE can be found in Section 4.5.

4.10.2.1.2.2.2. Kidney cancer

One study of occupational exposure to TCE observed an increase in RCC for women compared to men (Dosemeci et al., 1999), but no gender difference was observed in other studies (Raaschou-Nielsen et al., 2003; Pesch et al., 2000b). Blair et al. (1998) and Hansen et al. (2001) also present some results by sex, but both of these studies have too few cases to be informative about a sex difference for kidney cancer. Exposure differences between males and females in Dosemeci et al. (1999) may explain their finding. These studies, however, provide little information to evaluate susceptibility between sexes because of their lack of quantitative exposure assessment and lower statistical power. A detailed discussion of the studies examining the gender-specific kidney cancer effects of TCE can be found in Section 4.4.

4.10.2.1.2.2.3. Cancers of the immune system

Two drinking water studies suggest that there may be an increase of leukemia (Cohn et al., 1994b; Fagliano et al., 1990) and NHL (Cohn et al., 1994b) among females compared to males. An occupational study also observed an elevated risk of leukemia in females compared to males (Raaschou-Nielsen et al., 2003), although a study of contaminated drinking water in Woburn, Massachusetts observed an increased risk of childhood leukemia in males compared to

females (Costas et al., 2002). A detailed discussion of the studies examining the gender-specific cancers of the immune system following TCE exposure can be found in Section 4.6.

4.10.2.1.2.2.4. Respiratory cancers

One study observed significantly elevated risk of lung cancer following occupational TCE exposure for both men and women, although the risk was found to be higher for women compared to men (Raaschou-Nielsen et al., 2003). This same study observed a nonsignificant elevated risk in both men and women for laryngeal cancer, again with an increased risk for women compared to men (Raaschou-Nielsen et al., 2003). Conversely, a study of Iowa residents with TCE-contaminated drinking water observed a sevenfold increased annual age-adjusted incidence for males compared to females (Isacson et al., 1985). However, other studies did not observe a gender-related difference (ATSDR, 2002; Hansen et al., 2001; Blair et al., 1998). A detailed discussion of the studies examining the gender-specific respiratory cancers following TCE exposure can be found in Sections 4.7.

4.10.2.1.2.2.5. Reproductive cancers

Breast cancer in females and prostate cancer in males were reported after exposure to TCE in drinking water (Isacson et al., 1985). A statistically elevated risk for cervical cancer, but not breast, ovarian, or uterine cancer, was observed in women in another study (Raaschou-Nielsen et al., 2003). This study also did not observe elevated prostate or testicular cancer (Raaschou-Nielsen et al., 2003). A detailed discussion of the studies examining the gender-specific reproductive cancers following TCE exposure can be found in Section 4.8.2.

4.10.2.1.2.2.6. Other Cancers

Bladder and rectal cancer was increased in men compared to women after exposure to TCE in drinking water, but no gender difference was observed for colon cancer (Isacson et al., 1985). After occupational TCE exposure, bladder, stomach, colon, and esophageal cancer was nonsignificantly elevated in women compared to men (Raaschou-Nielsen et al., 2003).

4.10.2.2. Genetic Variability

Section 3.3 describes the metabolic processes involved in the metabolism of TCE. Human variation in response to TCE exposure may be associated with genetic variation. TCE is metabolized by both CYP and GST; therefore, it is likely that polymorphisms will alter the response to exposure (Garte et al., 2001; Nakajima and Aoyama, 2000), as well as exposure to other chemicals that may alter the metabolism of TCE (Lash et al., 2007) (see Section 4.10.2.6). It is important to note that even with a given genetic polymorphism, metabolic expression is not static, and depends on lifestage (see Section 4.10.1), obesity (see Section 4.10.2.4), and alcohol intake (see Section 4.10.2.5).

4.10.2.2.1. CYP genotypes

In general, variability in CYP expression occurs within humans (Dorne et al., 2005), and variability in CYP expression has been observed in experimental animals exposed to TCE (Nakajima et al., 1993). In particular, increased CYP2E1 activity may lead to increased susceptibility to TCE (Lipscomb et al., 1997). The CYP2E1*3 allele and the CYP2E1*4 allele were more common among those who developed scleroderma who were exposed to solvents including TCE (Povey et al., 2001). A PBPK model of CYP2E1 expression after TCE exposure has been developed for rats and humans (Yoon et al., 2007).

In experimental animals, toxicokinetics of TCE differed among CYP2E1 knockout and wild-type mice (Kim and Ghanayem, 2006). This study found that exhalation was more prevalent among the knockout mice, whereas urinary excretion was more prevalent among the wild-type mice. In addition, the dose was found to be retained to a greater degree by the knockout mice compared to the wild-type mice.

4.10.2.2.2. GST genotype

There is a possibility that GST polymorphisms could play a role in variability in toxic response to TCE (Caldwell and Keshava, 2006), but this has not been sufficiently tested (NRC, 2006). One study of renal cell cancer in workers exposed to TCE demonstrated a significant increased for those with GSTM1+ and GSTT1+ polymorphisms, compared to a negative risk for those with GSTM1– and GSTT1-polymorphisms (Brüning et al., 1997a). Another study of occupational TCE exposure found that RCC was significantly associated with the GSTT+ polymorphism but not with GSTT– (Moore et al., 2010). However, another study did not confirm this hypothesis, observing no clear relationship between GSTM1 and GSTT1 polymorphisms and RCC among TCE-exposed individuals, although they did see a possible association with the homozygous wild-type allele GSTP1*A (Wiesenhütter et al., 2007). Unrelated to TCE exposure, Sweeney et al. (2000) found GSTT1– to be associated with an increased risk of RCC, but no difference was seen for GSTM1 and GSTP1 alleles. The role of GST polymorphisms in the development of RCC is an area in need of future research.

4.10.2.2.3. Other genotypes

Other genetic polymorphisms could play a role in variability in toxic response, in particular TCE-related skin disorders. Studies have found that many TCE-exposed patients diagnosed with skin conditions exhibited the slow-acetylator NAT2 genotype (Nakajima et al., 2003; Huang et al., 2002), whereas there was no difference in NAT2 status for those diagnosed with RCC (Wiesenhütter et al., 2007). Other studies have found that many TCE-exposed patients diagnosed with skin conditions expressed variant HLA alleles (Li et al., 2007; Yue et al., 2007), in particular HLA-B*1301, which is more common in Asians compared to whites (Cao et

al., 2001; Williams et al., 2001), or TNF α-308 allele (Dai et al., 2004). Also, an in vitro study of human lung adenocarcinoma cells exposed to TCE varied in response based on their p53 status, with p53-wild-type cells resulting in severe cellular damage, but not the p53-null cells (Chen et al., 2002a).

4.10.2.3. Race/Ethnicity

Different racial or ethnic groups may express metabolic enzymes in different ratios and proportions due to genetic variability (Garte et al., 2001). In particular, ethnic variability in CYP (Dorne et al., 2005; Parkinson et al., 2004; McCarver et al., 1998; Shimada et al., 1994; Stephens et al., 1994) and GST (Nelson et al., 1995) expression has been reported.

It has been observed that the metabolic rate for TCE may differ between the Japanese and Chinese (Inoue et al., 1989). Also, body size varies among ethnic groups, and increased body size was related to increased absorption of TCE and urinary excretion of TCE metabolites (Sato et al., 1991b).

4.10.2.4. Preexisting Health Status

It is known that kidney and liver diseases can affect the clearance of chemicals from the body, and therefore, poor health may lead to increased half-lives for TCE and its metabolites. There are some data indicating that obesity/metabolic syndrome, diabetes, and hypertension may increase susceptibility to TCE exposure through altered toxicokinetics. In addition, some of these conditions lead to increased risk for adverse effects that have also been associated with TCE exposure, though the possible interaction between TCE and known risk factors for these effects is not understood.

4.10.2.4.1. Obesity

TCE is lipophilic and stored in adipose tissue; therefore, obese individuals may experience altered toxicokinetics of TCE compared to thin individuals. The absorption of TCE is increased in obese individuals compared to thin individuals (Clewell et al., 2000), as observed by lower blood concentrations immediately after exposure in obese men compared to thin men (Sato, 1993; Sato et al., 1991b). Once absorbed, obese individuals have increased storage of TCE in the adipose tissue compared to thin men (Clewell et al., 2000), which prolongs internal exposures (Lash et al., 2000b; Davidson and Beliles, 1991). Obesity also likely alters TCE metabolism, since increased CYP2E1 expression has been observed in obese individuals compared to thin individuals (McCarver et al., 1998). Finally, delayed excretion has been observed in obese individuals compared to thin individuals in both exhaled air (Monster, 1979) and urine (Sato, 1993; Sato et al., 1991b). In sum, obese individuals have altered toxicokinetics of TCE compared to thin individuals due to increased storage of TCE, increased CYP2E1 metabolism, and a slower rate of elimination.

In addition, individuals with high BMI are at increased risk of some of the same health effects associated with TCE exposure. For example, RCC, liver cancer, and prostate cancer may be positively associated with BMI or obesity (Wigle et al., 2008; El-Serag and Rudolph, 2007; Benichou et al., 1998; Asal et al., 1988a; Asal et al., 1988b). However, whether and how TCE interacts with known risk factors for such diseases is unknown, as existing epidemiologic studies have only examined these factors as possible confounders for effects associated with TCE, or vice versa (Krishnadasan et al., 2008; Charbotel et al., 2006).

4.10.2.4.2. Diabetes

A higher rate of diabetes in females compared to males exposed to TCE was reported in two studies (Davis et al., 2005; Burg et al., 1995). Whether the TCE may have caused the diabetes or the diabetes may have increased susceptibility to TCE is not clear. However, it has been observed that CYP2E1 expression is increased in obese Type II diabetics (Wang et al., 2003), and in poorly controlled Type I diabetics (Song et al., 1990), which may consequently alter the metabolism of TCE.

4.10.2.4.3. Hypertension

One study found no difference in risk for RCC among those diagnosed with hypertension among those living in an area with high TCE exposure; however, a slightly elevated risk was seen for those being treated for hypertension (OR: 1.57, 95% CI: 0.90–2.72) (Charbotel et al., 2006). Unrelated to TCE exposure, hypertension has been associated with increased risk of RCC in women compared to men (Benichou et al., 1998).

4.10.2.5. Lifestyle Factors and Nutrition Status

4.10.2.5.1. Alcohol intake

A number of studies have examined the interaction between TCE and ethanol exposure in both humans (McCarver et al., 1998; Sato, 1993; Sato et al., 1991a; Barret et al., 1984; Sato et al., 1981; 1975; Stewart et al., 1974b; Bardodej and Vyskocil, 1956) and experimental animals (Kaneko et al., 1994; Nakajima et al., 1992a; Okino et al., 1991; Nakajima et al., 1990; Larson and Bull, 1989; Nakajima et al., 1988; Sato and Nakajima, 1985; Sato et al., 1983; White and Carlson, 1981b; Sato et al., 1980).

The co-exposure causes metabolic inhibition of TCE in humans (Windemuller and Ettema, 1978; Muller et al., 1975), male rats (Kaneko et al., 1994; Okino et al., 1991; Nakajima et al., 1990; Larson and Bull, 1989; Nakajima et al., 1988; Sato and Nakajima, 1985; Sato et al., 1981; Nakanishi et al., 1978), and rabbits (White and Carlson, 1981b). Similarly, individuals exposed to TCE reported an increase in alcohol intolerance (Rasmussen and Sabroe, 1986; Bardodej and Vyskocil, 1956; Grandjean et al., 1955). Disulfiram, used to treat alcoholism, has also been found to decrease the elimination of TCE and TCA (Bartonicek and Teisinger, 1962).

A —degreasers flush" has been described, reflecting a reddening of the face of those working with TCE after drinking alcohol, and measured an elevated level of TCE in exhaled breath compared to nondrinkers exposed to TCE (Stewart et al., 1974a). This may be due to increased CYP2E1 expression in those that consume alcohol compared to nondrinkers, unrelated to TCE exposure (Caldwell et al., 2008b; Liangpunsakul et al., 2005; Lieber, 2004; Parkinson et al., 2004; McCarver et al., 1998; Perrot et al., 1989).

In experimental animals, male rats pretreated with ethanol experienced an induction of TCE metabolism (Nakajima et al., 1992a), although another study of male rats observed that pretreatment with ethanol did not decrease CYP activity (Okino et al., 1991). It is important to note that a further increased response of TCE and ethanol has been reported when also combined with low-fat or low-carbohydrate diets in male rats (Sato et al., 1983).

Since the liver is a target organ for both TCE and alcohol, decreased metabolism of TCE could be related to cirrhosis of the liver as a result of alcohol abuse (McCarver et al., 1998), and an in increase in clinical liver impairment along with degreasers flush has been observed (Barret et al., 1984).

The CNS may also be impacted by the co-exposure. Individuals exposed to TCE and ethanol reported an increase in altered mood states (Reif et al., 2003), decreased mental capacity as described as small increases in functional load (Windemuller and Ettema, 1978), and those exposed to TCE and tetrachloroethylene who consumed alcohol had an elevated color confusion index (Valic et al., 1997).

4.10.2.5.2. Tobacco smoking

Individuals who smoke tobacco may be at increased risk of the health effects from TCE exposure. One study examining those living in an area with high TCE exposure found an increasing trend of risk (p = 0.008) for RCC among smokers, with the highest OR among those with ≥40 pack-years (OR = 3.27, 95% CI: 1.48–7.19) (Charbotel et al., 2006). It has been shown that RCC is independently associated with smoking in a dose-response manner (Yuan et al., 1998), particularly in men (Benichou et al., 1998). While Charbotel et al. (2006) adjusted for smoking effects in analyses examining TCE exposure and RCC, this study provides no information on potential effect modification of TCE exposure by smoking.

A number of factors correlated to smoking (e.g., SES status, diet, alcohol consumption) may positively confound results if greater smoking rates were over-represented, as observed in an occupational cohort exposed to TCE (Raaschou-Nielsen et al., 2003). Absence of smoking information, on the other hand, could introduce a negative bias. In a drinking water study with exposures to TCE and perchlorate, Morgan and Cassidy (2002) noted that the relatively high education and high income levels as well as high access to health care of subjects in this study compared to the averages for the county as a whole likely leads to a lower smoking rate.

4.10.2.5.3. Nutritional status

Malnutrition may also increase susceptibility to TCE. Bioavailability of TCE after oral and i.v. exposure increased with fasting from approximately 63% in nonfasted rats to >90% in fasted rats, with blood levels in fasted rats were elevated two- to threefold, and increased half-life in the blood of fasted rats (D'Souza et al., 1985). Food deprivation (Sato and Nakajima, 1985) and carbohydrate restriction (Sato and Nakajima, 1985; Nakajima et al., 1982) enhanced metabolism of TCE in male rats, but this was not observed for dietary changes in protein or fat levels (Nakajima et al., 1982).

Vitamin intake may also alter susceptibility to TCE. An in vitro study of cultured normal human epidermal keratinocyte demonstrated an increased lipid peroxidation in a dose-dependent manner after exposure to TCE, which were then attenuated by exposure to Vitamin E (Ding et al., 2006).

4.10.2.5.4. Physical activity

Increased inhalation during physical activity increases TCE concentrations in the alveoli when compared to inhalation in a resting state (Astrand, 1975). Studies have examined the time course of inhaled TCE and metabolites in blood and urine in individuals with different workloads (Jakubowski and Wieczorek, 1988; Astrand and Ovrum, 1976; Monster et al., 1976; Vesterberg and Astrand, 1976; Vesterberg et al., 1976). These studies demonstrate that an increase in pulmonary ventilation increases the amount of TCE taken up during exposure (Sato, 1993; Jakubowski and Wieczorek, 1988; Astrand and Ovrum, 1976; Monster et al., 1976).

The Rocketdyne aerospace cohort exposed to TCE (and other chemicals) found a protective effect with high physical activity, but only after controlling for TCE exposure and SES status (OR = 0.55, 95% CI: 0.32–0.95, *p* trend = 0.04) (Krishnadasan et al., 2008). In general, physical activity may provide a protective effect for prostate cancer (Wigle et al., 2008) (see Section 4.8.2.1.1).

4.10.2.5.5. SES

SES can be an indicator for a number of co-exposures, such as increased tobacco smoking, poor diet, education, income, and health care access, which may play a role in the results observed in the health effects of TCE exposure (Morgan and Cassady, 2002).

Children‘s exposure to TCE was measured in a low SES community, as characterized by income, educational level, and receipt of free or reduced cost school meals (Sexton et al., 2005); however, this study did not compare data to a higher SES community, nor examine health effects.

An elevated risk of NHL and esophagus/adenocarcinoma after exposure to TCE was observed for blue-collar workers compared to white collar workers and workers with unknown

SES (Raaschou-Nielsen et al., 2003). Authors speculate that these results could be confounded due to other related factors than SES such as smoking.

4.10.2.6. Mixtures

TCE exposure often occurs concurrently with other chemical substances. In general, the effects of exposures to multiple chemicals is considered by EPA in the *Framework for Cumulative Risk Assessment* (U.S. EPA, 2003a). A summary of the interactive effects of TCE and other chemical co-exposures is addressed in Caldwell et al. (2008b) and in Chapter 10 of the NRC's report *Assessing the Human Health Risks of Trichloroethylene*: *Key Scientific Issues* (NRC, 2006).

Chapter 2 discusses that other parent compounds produce similar metabolites to TCE (see Table 2-1) or have similar properties or industrial uses (see Tables 2-3 and 2-14). The metabolic pathway of TCE in discussed in Section 3.3; due its metabolism into multiple compounds, exposure to TCE itself can be considered as exposure to a mixture (NRC, 2006). Many of the studies discussed above in Chapter 4 demonstrate that exposure to TCE and other chemical substances often occur together in both occupational and nonoccupational settings.

Co-exposures to other solvents may induce or saturate toxicokinetic pathways, altering the way in which TCE is metabolized and cleared from the body. The limited data summarized by the ATSDR in its interaction profile on TCE, 1,1,1-trichloroethane, 1,1-dichloroethane, and tetrachloroethylene suggest that additive joint action is plausible (ATSDR, 2004b; Pohl et al., 2003). Joint exposure to TCE and the fungicide fenarimol has been shown to alter TCE metabolism and genetic expression in mice (Hrelia et al., 1994). Joint exposure to TCE, benzene, and methyl mercury has been shown to induce genetic expression in the liver and the kidney of rats (Hendriksen et al., 2007). Metabolic competition was also observed for TCE and various agents in another study by Jakobson et al. (1986).

PBPK models have been developed demonstrating the interaction between 1,1-DCE and TCE (Andersen et al., 1987b) and the interaction between TCE, tetrachloroethylene, and 1,1,1-trichloroethane in rats (Dobrev et al., 2001) and humans (Dobrev et al., 2002). Other PBPK models also showed metabolic inhibition at higher doses for TCE and toluene (Thrall and Poet, 2000), and for TCE and chloroform (Isaacs et al., 2004). Another PBPK model of TCE and multiple VOCs showed metabolic inhibition and induction when exposure occurs concurrently (Haddad et al., 2000).

4.10.3. Uncertainty of Database and Research Needs for Susceptible Populations

There is some evidence that certain populations may be more susceptible to exposure to TCE. These populations include early and later lifestages, gender, genetic polymorphisms, race/ethnicity, preexisting health status, and lifestyle factors and nutrition status. In general, this

database would be improved by future epidemiologic and toxicological studies of TCE exposure that provide data on effect modification, including the factors discussed here.

Although the toxicokinetic variability has been characterized by population PBPK modeling (see Section 3.5), the available data are limited due to the relative small numbers of individuals ($n < 100$), their all being adults, and the fact that subjects were selected nonrandomly (healthy volunteers).

Although there is more information on early life exposure to TCE than on other potentially susceptible populations, there remain a number of uncertainties and data gaps regarding children's susceptibility. Improved PBPK modeling for using childhood parameters early lifestages as recommended by the NRC (2006), and validation of these models, will aid in determining how variations in metabolic enzymes affect TCE metabolism. In particular, the NRC states that it is prudent to assume children need greater protection than adults—unless sufficient data are available to justify otherwise (NRC, 2006).

More studies specifically designed to evaluate effects in early and later lifestages are needed in order to more fully characterize potential lifestage-related TCE toxicity. Because the neurological effects of TCE constitute the most sensitive endpoints of concern for noncancer effects, it is quite likely that the early lifestages may be more susceptible to these outcomes than are adults. Lifestage-specific neurotoxic effects, particularly in the developing fetus, need further evaluation. It is important to consider the use of age-appropriate testing for assessment of these and other outcomes, both for cancer and noncancer outcomes. Data specific to the carcinogenic effects of TCE exposure during the critical periods of development of experimental animals and humans also are sparse.

There is a need to better characterize the implications of TCE exposures to susceptible populations. There is suggestive evidence that there may be greater susceptibility for exposures to the elderly. Gender and race/ethnic differences in susceptibility are likely due to variation in physiology and exposure, and genetic variation likely has an effect on the toxicokinetics of TCE. In particular, the relationship between genetic variation and generalized hypersensitivity skin diseases is relevant for future study (see Sections 4.6.1.1.2 and 4.10.2.2). Diminished health status (e.g., impaired kidney liver or kidney), alcohol consumption, tobacco smoking, and nutritional status will likely affect an individual's ability to metabolize TCE. In addition, further evaluation of the effects due to co-exposures to other compounds with similar or different modes of action need to be evaluated. Future research should better characterize possible susceptibility for certain lifestages or populations.

4.11. HAZARD CHARACTERIZATION

4.11.1. Characterization of Noncancer Effects

4.11.1.1. Neurotoxicity

Both human and animal studies have associated TCE exposure with effects on several neurological domains. The strongest neurological evidence of hazard in humans is for changes in trigeminal nerve function or morphology and impairment of vestibular function. Fewer and more limited evidence exists in humans on delayed motor function, and changes in auditory, visual, and cognitive function or performance. Acute and subchronic animal studies show morphological changes in the trigeminal nerve, disruption of the peripheral auditory system leading to permanent function impairments and histopathology, changes in visual evoked responses to patterns or flash stimulus, and neurochemical and molecular changes. Additional acute studies reported structural or functional changes in hippocampus, such as decreased myelination or decreased excitability of hippocampal CA1 neurons, although the relationship of these effects to overall cognitive function is not established. Some evidence exists for motor-related changes in rats/mice exposed acutely/subchronically to TCE, but these effects have not been reported consistently across all studies.

Epidemiologic evidence supports a relationship between TCE exposure and trigeminal nerve function changes, with multiple studies in different populations reporting abnormalities in trigeminal nerve function in association with TCE exposure (Mhiri et al., 2004; Kilburn, 2002a; Kilburn and Warshaw, 1993a; Feldman et al., 1992; Ruijten et al., 1991; Feldman et al., 1988; Barret et al., 1987; Barret et al., 1984; Barret et al., 1982). Of these, two well-conducted occupational cohort studies, each including >100 TCE-exposed workers without apparent confounding from multiple solvent exposures, additionally reported statistically significant dose-response trends based on ambient TCE concentrations, duration of exposure, and/or urinary concentrations of the TCE metabolite TCA (1987; Barret et al., 1984). Limited additional support is provided by a positive relationship between prevalence of abnormal trigeminal nerve or sensory function and cumulative exposure to TCE (most subjects) or CFC113 (<25% of subjects) (Rasmussen et al., 1993a). Test for linear trend in this study was not statistically significant and may reflect exposure misclassification since some subjects included in this study did not have TCE exposure. The lack of association between TCE exposure and overall nerve function in three small studies (trigeminal (El Ghawabi et al., 1973); ulnar and medial (Triebig et al., 1983; 1982)) does not provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment because of limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. Laboratory animal studies have also shown TCE-induced changes in the trigeminal nerve. Although one study reported no significant changes in TSEP in rats exposed to TCE for 13 weeks (Albee et al., 2006), there is evidence of morphological changes in the trigeminal nerve following short-term exposures in rats (Barret et al., 1992; 1991).

Human chamber, occupational, geographic-based/drinking water, and laboratory animal studies clearly established TCE exposure causes transient impairment of vestibular function. Subjective symptoms such as headaches, dizziness, and nausea resulting from occupational (Liu et al., 1988; Rasmussen and Sabroe, 1986; Smith, 1970; Grandjean et al., 1955), environmental (Hirsch et al., 1996), or chamber exposures (Smith, 1970; Stewart et al., 1970) have been reported extensively. A few laboratory animal studies have investigated vestibular function, either by promoting nystagmus or by evaluating balance (Umezu et al., 1997; Niklasson et al., 1993; Tham et al., 1984; 1979).

In addition, mood disturbances have been reported in a number of studies, although these effects also tend to be subjective and difficult to quantify (Gash et al., 2008; Kilburn, 2002b, a; Kilburn and Warshaw, 1993a; Tröster and Ruff, 1990; McCunney, 1988; Rasmussen and Sabroe, 1986; Mitchell and Parsons-Smith, 1969), and a few studies have reported no effects from TCE on mood (Reif et al., 2003; Triebig et al., 1977a; Triebig et al., 1976). Few comparable mood studies are available in laboratory animals, although both Moser et al. (2003) and Albee et al. (2006) reported increases in handling reactivity among rats exposed to TCE. Finally, significantly increased number of sleep hours was reported by Arito et al. (1994) in rats exposed via inhalation to 50–300-ppm TCE for 8 hours/day for 6 weeks.

Four epidemiologic studies of chronic exposure to TCE observed disruption of auditory function. One large occupational cohort study showed a statistically significant difference in auditory function with cumulative exposure to TCE or CFC113 as compared to control groups after adjustment for possible confounders, as well as a positive relationship between auditory function and increasing cumulative exposure (Rasmussen et al., 1993c). Of the three studies based on populations from ATSDR's TCE Subregistry from the National Exposure Registry, more limited than Rasmussen et al. (1993c) due to inferior exposure assessment, Burg et al. (1995) and Burg and Gist (1999) reported a higher prevalence of self-reported hearing impairments. The third study reported that auditory screening revealed abnormal middle ear function in children <10 years of age, although a dose-response relationship could not be established and other tests did not reveal differences in auditory function (ATSDR, 2002). Further evidence for these effects is provided by numerous laboratory animal studies demonstrating that high dose subacute and subchronic TCE exposure in rats disrupts the auditory system leading to permanent functional impairments and histopathology.

Studies in humans exposed under a variety of conditions, both acutely and chronically, report impaired visual functions such as color discrimination, visuospatial learning tasks, and visual depth perception in subjects with TCE exposure. Abnormalities in visual depth perception were observed with a high acute exposure to TCE under controlled conditions (Vernon and Ferguson, 1969). Studies of lower TCE exposure concentrations also observed visuofunction effects. One occupational study (Rasmussen et al., 1993c) reported a statistically significant positive relationship between cumulative exposure to TCE or CFC113 and visual gestalts

learning and retention among Danish degreasers. Two studies of populations living in a community with drinking water containing TCE and other solvents furthermore suggested changes in visual function (Reif et al., 2003; Kilburn, 2002b, a). These studies used more direct measures of visual function as compared to Rasmussen et al. (1993c), but their exposure assessment is more limited because TCE exposure is not assigned to individual subjects (Kilburn 2002a), or because there are questions regarding control selection and exposure to several solvents (Reif et al., 2003; Kilburn, 2002b, a).

Additional evidence of effects of TCE exposure on visual function is provided by a number of laboratory animal studies demonstrating that acute or subchronic TCE exposure causes changes in visual evoked responses to patterns or flash stimulus (Boyes et al., 2005a; Boyes et al., 2003; Blain et al., 1994). Animal studies have also reported that the degree of some effects is correlated with simultaneous brain TCE concentrations (Boyes et al., 2005a; Boyes et al., 2003) and that, after a recovery period, visual effects return to control levels (Blain et al., 1994; Rebert et al., 1991). Overall, the human and laboratory animal data together suggest that TCE exposure can cause impairment of visual function, and some animal studies suggest that some of these effects may be reversible with termination of exposure.

Studies of human subjects exposed to TCE either acutely in chamber studies or chronically in occupational settings have observed deficits in cognition. Five chamber studies reported statistically significant deficits in cognitive performance measures or outcome measures suggestive of cognitive effects (Triebig et al., 1977a; Gamberale et al., 1976; Triebig et al., 1976; Stewart et al., 1970). Danish degreasers with high cumulative exposure to TCE or CFC113 had a high risk (OR = 13.7, 95% CI: 2.0–92.0) for psychoorganic syndrome characterized by cognitive impairment, personality changes, and reduced motivation, vigilance, and initiative compared to workers with low cumulative exposure. Studies of populations living in a community with contaminated groundwater also reported cognitive impairments0020(Kilburn, 2002b, a; Kilburn and Warshaw, 1993a), although these studies carry less weight in the analysis because TCE exposure is not assigned to individual subjects and their methodological design is weaker.

Laboratory studies provide some additional evidence for the potential for TCE to affect cognition, although the predominant effect reported has been changes in the time needed to complete a task, rather than impairment of actual learning and memory function (Umezu et al., 1997; Kishi et al., 1993; Kulig, 1987). In addition, in laboratory animals, it can be difficult to distinguish cognitive changes from motor-related changes. However, several studies have reported structural or functional changes in the hippocampus, such as decreased myelination (Isaacson et al., 1990; Isaacson and Taylor, 1989) or decreased excitability of hippocampal CA1 neurons (Ohta et al., 2001), although the relationship of these effects to overall cognitive function is not established.

Two studies of TCE exposure, one chamber study of acute exposure duration and one occupational study of chronic duration, reported changes in psychomotor responses. The chamber study of Gamberale et al. (1976) reported a dose-related decrease in performance in a CRT test in healthy volunteers exposed to 100 and 200 ppm TCE for 70 minutes as compared to the same subjects without exposure. Rasmussen et al. (1993a) reported a statistically significant association with cumulative exposure to TCE or CFC113 and dyscoordination trend among Danish degreasers. Observations in a third study (Gun et al., 1978) are difficult to judge given the author's lack of statistical treatment of data. In addition, Gash et al. (2008) reported that 14 out of 30 TCE-exposed workers exhibited significantly slower fine motor hand movements as measured through a movement analysis panel test. Studies of populations living in communities with TCE and other solvents detected in groundwater supplies reported significant delays in SRTs and CRTs in individuals exposed to TCE in contaminated groundwater as compared to referent groups (Kilburn, 2002b, a; Kilburn and Thornton, 1996; Kilburn and Warshaw, 1993a). Observations in these studies are more uncertain given questions of the representativeness of the referent population, lack of exposure assessment to individual study subjects, and inability to control for possible confounders including alcohol consumption and motivation. Finally, in a presentation of two case reports, decrements in motor skills as measured by the grooved pegboard and finger tapping tests were observed (Tröster and Ruff, 1990).

Laboratory animal studies of acute or subchronic exposure to TCE observed psychomotor effects, such as loss of righting reflex (Shih et al., 2001; Umezu et al., 1997) and decrements in activity, sensory-motor function, and neuromuscular function (2003; Moser et al., 1995; Kishi et al., 1993). However, two studies also noted an absence of significant changes in some measures of psychomotor function (Albee et al., 2006; Kulig, 1987). In addition, less consistent results have been reported with respect to locomotor activity in rodents. Some studies have reported increased locomotor activity after an acute i.p. dosage (Wolff and Siegmund, 1978) or decreased activity after acute or short-term gavage dosing (2003; Moser et al., 1995). No change in activity was observed following exposure through drinking water (Waseem et al., 2001), inhalation (Kulig, 1987), or orally during the neurodevelopment period (Fredriksson et al., 1993).

Several neurochemical and molecular changes have been reported in laboratory investigations of TCE toxicity. Kjellstrand et al. (1987) reported inhibition of sciatic nerve regeneration in mice and rats exposed continuously to 150 ppm TCE via inhalation for 24 days. Two studies have reported changes in GABAergic and glutamatergic neurons in terms of GABA or glutamate uptake (Briving et al., 1986) or response to GABAergic antagonistic drugs (Shih et al., 2001) as a result of TCE exposure, with the Briving et al. (1986) conducted at 50 ppm for 12 months. Although the functional consequences of these changes is unclear, Tham et al. (1984; 1979) described central vestibular system impairments as a result of TCE exposure that may be related to altered GABAergic function. In addition, several in vitro studies have demonstrated that TCE exposure alters the function of inhibitory ion channels such as receptors

for $GABA_A$ glycine, and serotonin (Lopreato et al., 2003; Beckstead et al., 2000; Krasowski and Harrison, 2000) or of voltage-sensitive calcium channels (Shafer et al., 2005).

4.11.1.2. Kidney Toxicity

There are few human data pertaining to TCE-related noncancer kidney toxicity. Observation of elevated excretion of urinary proteins in the available studies (Bolt et al., 2004; Green et al., 2004; Brüning et al., 1999a; Brüning et al., 1999b; Rasmussen et al., 1993b) indicates the occurrence of a toxic insult among TCE-exposed subjects compared to unexposed controls. Two studies are of subjects with previously diagnosed kidney cancer (Bolt et al., 2004; Brüning et al., 1999a), while subjects in the other studies are disease free. Urinary proteins are considered nonspecific markers of nephrotoxicity and include α1-microglobulin, albumin, and NAG (Lybarger et al., 1999; 1999; Price et al., 1996). Four studies measure α1-microglobulin with elevated excretion observed in the German studies (Bolt et al., 2004; Brüning et al., 1999a; Brüning et al., 1999b) but not Green et al. (2004). However, Rasmussen et al. (1993b) reported a positive relationship between increasing urinary NAG, another nonspecific marker of tubular toxicity, and increasing exposure duration; and Green et al. (2004) found statistically significant group mean differences in NAG. Observations in Green et al. (2004) provide evidence of tubular damage among workers exposed to TCE at current occupational levels. Elevated excretion of NAG has also been observed with acute TCE poisoning (Carrieri et al., 2007). Some support for TCE nephrotoxicity in humans is provided by a study of ESRD in a cohort of workers at Hill Air Force Base (Radican et al., 2006), although subjects in this study were exposed to hydrocarbons, JP-4 gasoline, and solvents in addition to TCE, including 1,1,1-tri-chloroethane, and a second reporting a twofold elevated risk for progression of glomerulonephritis to ESRD with TCE exposure (Jacob et al., 2007).

Laboratory animal and in vitro data provide additional support for TCE nephrotoxicity. Multiple studies with both gavage and inhalation exposure show that TCE causes renal toxicity in the form of cytomegaly and karyomegaly of the renal tubules in male and female rats and mice (summarized in Section 4.4.4). Further studies with TCE metabolites have demonstrated a potential role for DCVC, TCOH, and TCA in TCE-induced nephrotoxicity. Of these, available data suggest that DCVC-induced renal effects most like those of TCE and is formed in sufficient amounts following TCE exposure to account for these effects. TCE or DCVC have also been shown to be cytotoxic to primary cultures of rat and human renal tubular cells (Cummings and Lash, 2000; Cummings et al., 2000a; Cummings et al., 2000c).

Overall, multiple lines of evidence support the conclusion that TCE causes nephrotoxicity in the form of tubular toxicity, mediated predominantly through the TCE GSH conjugation product DCVC.

4.11.1.3. Liver Toxicity

Few studies on liver toxicity and TCE exposure are found in humans. Of these, three studies reported significant changes in serum liver function tests, widely used in clinical settings in part to identify patients with liver disease, in metal degreasers whose TCE exposure was assessed using urinary trichloro-compounds as a biomarker (Xu et al., 2009; Nagaya et al., 1993; Rasmussen et al., 1993b). Two additional studies reported plasma or serum bile acid changes (Neghab et al., 1997; Driscoll et al., 1992). One study of subjects from the TCE subregistry of ATSDR's National Exposure Registry is suggestive of liver disorders but limitations preclude inferences whether TCE caused these conditions is not possible given the study's limitations (Davis et al., 2005). Furthermore, a number of case reports exist of liver toxicity including hepatitis accompanying immune-related generalized skin diseases described as a variation of erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis patients, and hypersensitivity syndrome (Kamijima et al., 2007) in addition to jaundice, hepatomegaly, hepatosplenomegaly, and liver failure in TCE-exposed workers (Huang et al., 2002; Thiele et al., 1982). Cohort studies have examined cirrhosis mortality and either TCE exposure (Radican et al., 2008; Boice et al., 2006b; ATSDR, 2004a; Boice et al., 1999; Ritz, 1999a; 1998; Morgan et al., 1998; Blair et al., 1989; Garabrant et al., 1988) or solvent exposure (Leigh and Jiang, 1993), but are greatly limited by their use of death certificates where there is a high degree (up to 50%) of underreporting (Blake et al., 1988), so these null findings do not rule out an effect of TCE on cirrhosis. Overall, while some evidence exists of liver toxicity as assessed from liver function tests, the data are inadequate for making conclusions regarding causality.

In laboratory animals, TCE exposure is associated with a wide array of hepatotoxic endpoints. Like humans, laboratory animals exposed to TCE have been observed to have increased serum bile acids (Neghab et al., 1997; Bai et al., 1992b), although the toxicological importance of this effect is unclear. Most other effects in laboratory animals have not been studied in humans, but nonetheless provide evidence that TCE exposure leads to hepatotoxicity. These effects include increased liver weight, small transient increases in DNA synthesis, cytomegaly in the form of "swollen" or enlarged hepatocytes, increased nuclear size probably reflecting polyploidization, and proliferation of peroxisomes. Liver weight increases proportional to TCE dose are consistently reported across numerous studies and appear to be accompanied by periportal hepatocellular hypertrophy (Laughter et al., 2004; Nunes et al., 2001; Nakajima et al., 2000; Tao et al., 2000; Berman et al., 1995; Dees and Travis, 1993; Goel et al., 1992; Merrick et al., 1989; Goldsworthy and Popp, 1987; Melnick et al., 1987; Buben and O'Flaherty, 1985; Elcombe et al., 1985; Kjellstrand et al., 1983a; Kjellstrand et al., 1983b; Tucker et al., 1982; Kjellstrand et al., 1981b). There is also evidence of increased DNA synthesis in a small portion of hepatocytes at around 10 days in vivo exposure (Channel et al., 1998; Dees and Travis, 1993; Mirsalis et al., 1989; Elcombe et al., 1985). The lack of correlation of hepatocellular mitotic figures with whole-liver DNA synthesis or DNA synthesis

observed in individual hepatocytes (Dees and Travis, 1993; Elcombe et al., 1985) supports the conclusions that cellular proliferation is not the predominant cause of increased DNA synthesis and that nonparenchymal cells may also contribute to such synthesis. Indeed, nonparenchymal cell activation or proliferation has been noted in several studies (Goel et al., 1992; Kjellstrand et al., 1983a). Moreover, the histological descriptions of TCE-exposed livers are consistent with and, in some cases, specifically note increased polyploidy (Buben and O'Flaherty, 1985). Interestingly, changes in TCE-induced hepatocellular ploidy, as indicated by histological changes in nuclei, have been noted to remain after the cessation of exposure (Kjellstrand et al., 1983a). In regard to apoptosis, TCE has been reported either to have no effect or to cause a slight increase at high doses (Channel et al., 1998; Dees and Travis, 1993). Some studies have also noted effects from dosing vehicle alone (such as corn oil, in particular) not only on liver pathology, but also on DNA synthesis (Channel et al., 1998; Merrick et al., 1989). Available data also suggest that TCE does not induce substantial cytotoxicity, necrosis, or regenerative hyperplasia, as only isolated, focal necroses and mild to moderate changes in serum and liver enzyme toxicity markers have been reported (Channel et al., 1998; Dees and Travis, 1993; Elcombe et al., 1985). Data on peroxisome proliferation, along with increases in a number of associated biochemical markers, show effects in both mice and rats (Channel et al., 1998; Goldsworthy and Popp, 1987; Elcombe et al., 1985). These effects are consistently observed across rodent species and strains, although the degree of response at a given mg/kg/day dose appears to be highly variability across strains, with mice on average appearing to be more sensitive.

While it is likely that oxidative metabolism is necessary for TCE-induced effects in the liver, the specific metabolite or metabolites responsible is less clear. TCE, TCA, and DCA exposures have all been associated with induction of changes in liver weight, DNA synthesis, and peroxisomal enzymes. The available data strongly support TCA ***not*** being the sole or predominant active moiety for TCE-induced liver effects, particularly with respect to hepatomegaly. In particular, TCE and TCA dose-response relationships are quantitatively inconsistent, for TCE leads to greater increases in liver/body weight ratios that expected from predicted rates of TCA production (see analysis in Section 4.5.6.2.1). In fact, above a certain dose of TCE, liver/body weight ratios are greater than that observed under any conditions studied so far for TCA. Histological changes and effects on DNA synthesis are generally consistent with contributions from either TCA or DCA, with a degree of polyploidization, rather than cell proliferation, likely to be significant for TCE, TCA, and DCA.

Overall, TCE, likely through its oxidative metabolites, clearly leads to liver toxicity in laboratory animals, with mice appearing to be more sensitive than other laboratory animal species, but there is only limited epidemiologic evidence of hepatotoxicity being associated with TCE exposure.

4.11.1.4. Immunotoxicity

Studies in humans provide evidence of associations between TCE exposure and a number of immunotoxicological endpoints. The relation between systemic autoimmune diseases, such as scleroderma, and occupational exposure to TCE has been reported in several recent studies. A meta-analysis of scleroderma studies (Garabrant et al., 2003; Diot et al., 2002; Nietert et al., 1998) conducted by the EPA resulted in a statistically significant combined OR for any exposure in men (OR: 2.5, 95% CI: 1.1, 5.4), with a lower RR seen in women (OR: 1.2, 95% CI: 0.58, 2.6). The incidence of systemic sclerosis among men is very low (approximately 1 per 100,000 per year), and is approximately 10 times lower than the rate seen in women (Cooper and Stroehla, 2003). Thus, the human data at this time do not allow determination of whether the difference in effect estimates between men and women reflects the relatively low background risk of scleroderma in men, gender-related differences in exposure prevalence or in the reliability of exposure assessment (Messing et al., 2003), a gender-related difference in susceptibility to the effects of TCE, or chance. Changes in levels of inflammatory cytokines were reported in an occupational study of degreasers exposed to TCE (Iavicoli et al., 2005) and a study of infants exposed to TCE via indoor air (2002; Lehmann et al., 2001).

Experimental studies provide additional support for these effects. Numerous studies have demonstrated accelerated autoimmune responses in autoimmune-prone mice (Cai et al., 2008; Blossom et al., 2007; Blossom et al., 2004; Griffin et al., 2000a; Griffin et al., 2000b). With shorter exposure periods, effects include changes in cytokine levels similar to those reported in human studies. More severe effects, including autoimmune hepatitis, inflammatory skin lesions, and alopecia, were manifest at longer exposure periods, and interestingly, these effects differ somewhat from the "normal" expression in these mice. Immunotoxic effects, including increases in anti-dsDNA antibodies in adult animals, decreased thymus weights, and decreased PFC response with prenatal and neonatal exposure, have been also reported in $B6C3F_1$ mice, which do not have a known particular susceptibility to autoimmune disease (Keil et al., 2009; Peden-Adams et al., 2006; Gilkeson et al., 2004). Recent mechanistic studies have focused on the roles of various measures of oxidative stress in the induction of these effects by TCE (Wang et al., 2008; Wang et al., 2007b).

There have been a large number of case reports of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis, associated with occupational exposure to TCE, with prevalences as high as 13% of workers in the same location (2008; Kamijima et al., 2007). Evidence of a treatment-related increase in delayed hypersensitivity response accompanied by hepatic damage has been observed in guinea pigs following intradermal injection (Tang et al., 2008; Tang et al., 2002), and hypersensitivity response was also seen in mice exposed via drinking water pre- and postnatally (GD 0 through to 8 weeks of age) (Peden-Adams et al., 2006).

Human data pertaining to TCE-related immunosuppression resulting in an increased risk of infectious diseases is limited to the report of an association between reported history of bacteria of viral infections in Woburn, Massachusetts (Lagakos et al., 1986). Evidence of localized immunosuppression, as measured by pulmonary response to bacterial challenge (i.e., risk of Streptococcal pneumonia-related mortality and clearance of *Klebsiella* bacteria) was seen in an acute exposure study in CD-1 mice (Aranyi et al., 1986). A 4-week inhalation exposure in Sprague-Dawley rats reported a decrease in PFC response at exposures of 1,000 ppm (Woolhiser et al., 2006).

Overall, the human and animal studies of TCE and immune-related effects provide strong evidence for a role of TCE in autoimmune disease and in a specific type of generalized hypersensitivity syndrome, while there are less data pertaining to immunosuppressive effects.

4.11.1.5. Respiratory Tract Toxicity

There are very limited human data on pulmonary toxicity and TCE exposure. Two recent reports of a study of gun manufacturing workers reported asthma-related symptoms and lung function decrements associated with solvent exposure (Saygun et al., 2007; Cakmak et al., 2004), but these studies are limited by multiple solvent exposures and the significant effect of smoking on pulmonary function. Laboratory studies in mice and rats have shown toxicity in the bronchial epithelium, primarily in Clara cells, following acute exposures to TCE by inhalation (see Section 4.7.2.1.1). A few studies of longer duration have reported more generalized toxicity, such as pulmonary fibrosis 90 days after a single 2,000 mg/kg i.p. dose in mice and pulmonary vasculitis after 13-week gavage exposures to 2,000 mg/kg-day in rats (Forkert and Forkert, 1994; NTP, 1990). However, respiratory tract effects were not reported in other longer-term studies. Acute pulmonary toxicity appears to be dependent on oxidative metabolism, although the particular active moiety is not known. While earlier studies implicated chloral produced in situ by CYP enzymes in respiratory tract tissue was responsible for toxicity (reviewed in Green, 2000), the evidence is inconsistent, and several other possibilities are viable. First, substantial —accumulation" of chloral is unlikely, as it is likely either to be rapidly converted to TCOH in respiratory tract tissue or to diffuse rapidly into blood and be converted to TCOH in erythrocytes or the liver. Conversely, a role for systemically produced oxidative metabolites cannot be discounted, as CH and TCOH in blood have both been reported following inhalation dosing in mice. In addition, a recent study reported DCAC protein adducts in the lungs of mice to which TCE was administered by i.p. injection, suggesting DCAC, which is not believed to be derived from chloral, may also contribute to TCE respiratory toxicity. Although humans appear to have lower overall capacity for enzymatic oxidation in the lung relative to mice, CYP enzymes do reside in human respiratory tract tissue, suggesting that, qualitatively, the respiratory tract toxicity observed in rodents is biologically plausible in humans. However, quantitative estimates of differential sensitivity across species due to respiratory metabolism are highly uncertain due to

limited data. Therefore, overall, data are suggestive of TCE causing respiratory tract toxicity, based primarily on short-term studies in mice and rats, and no data suggest that such hazards would be biologically precluded in humans.

4.11.1.6. Reproductive Toxicity

Reproductive toxicity related to TCE exposure has been evaluated in human and experimental animal studies for effects in males and females. Only a limited number of studies have examined whether TCE causes female reproductive toxicity. Epidemiologic studies have identified possible associations of TCE exposure with effects on female fertility (ATSDR, 2001; Sallmén et al., 1995) and with menstrual cycle disturbances (ATSDR, 2001; Sagawa et al., 1973; Zielinski, 1973; Bardodej and Vyskocil, 1956). Reduced in vitro oocyte fertilizability has been reported as a result of TCE exposure in rats (Wu and Berger, 2007; Berger and Horner, 2003), but a number of other laboratory animal studies did not report adverse effects on female reproductive function (Cosby and Dukelow, 1992; George et al., 1986; George et al., 1985; Manson et al., 1984). Overall, there are inadequate data to conclude whether adverse effects on human female reproduction are caused by TCE.

By contrast, a number of human and laboratory animal studies suggest that TCE exposure has the potential for male reproductive toxicity. In particular, human studies have reported TCE exposure to be associated, in several cases statistically-significantly, with increased sperm density and decreased sperm quality (Chia et al., 1996; Rasmussen et al., 1988), altered sexual drive or function (Saihan et al., 1978; El Ghawabi et al., 1973; Bardodej and Vyskocil, 1956), or altered serum endocrine levels (Goh et al., 1998; Chia et al., 1997). In addition, three studies that reported measures of fertility did not or could not report changes associated with TCE exposure (Forkert et al., 2003; ATSDR, 2001; Sallmen et al., 1998), although the statistical power of these studies is quite limited. Further evidence of similar effects is provided by several laboratory animal studies that reported effects on sperm (Kumar et al., 2001b; Veeramachaneni et al., 2001; Kumar et al., 2000a; Kumar et al., 2000b; George et al., 1985; Land et al., 1981), libido/copulatory behavior (Veeramachaneni et al., 2001; George et al., 1986; Zenick et al., 1984), and serum hormone levels (Veeramachaneni et al., 2001; Kumar et al., 2000a). As with the human database, some studies that assessed sperm measures did not report treatment-related alterations (Xu et al., 2004; Cosby and Dukelow, 1992; George et al., 1986; Zenick et al., 1984). Additional adverse effects on male reproduction have also been reported, including histopathological lesions in the testes or epididymides (Kan et al., 2007; Forkert et al., 2002; Kumar et al., 2001b; Kumar et al., 2000b; George et al., 1986) and altered in vitro sperm-oocyte binding or in vivo fertilization due to TCE or metabolites (DuTeaux et al., 2004a; Xu et al., 2004). While reduced fertility in rodents was only observed in one study (George et al., 1986), this is not surprising given the redundancy and efficiency of rodent reproductive capabilities. Furthermore, while George et al. (1986) proposed that the adverse male reproductive outcomes

observed in rats were due to systemic toxicity, the database as a whole suggests that TCE does induce reproductive toxicity independent of systemic effects. Therefore, overall, the human and laboratory animal data together support the conclusion that TCE exposure poses a potential hazard to the male reproductive system.

4.11.1.7. Developmental Toxicity

The relationship between TCE exposure (direct or parental) and adverse developmental outcomes has been investigated in a number of epidemiologic and laboratory animal studies. Prenatal effects examined include death (spontaneous abortion, perinatal death, pre- or postimplantation loss, resorptions), decreased growth (low birth weight, SGA, IUGR, decreased postnatal growth), and congenital malformations, in particular eye and cardiac defects. Postnatal developmental outcomes examined include growth and survival, developmental neurotoxicity, developmental immunotoxicity, and childhood cancers.

A few epidemiological studies have reported associations between parental exposure to TCE and spontaneous abortion or perinatal death (ATSDR, 2001; Taskinen et al., 1994; Windham et al., 1991), although other studies reported mixed or null findings (ATSDR, 2008b, 2006a; Bove, 1996; Bove et al., 1995; Goldberg et al., 1990; Lindbohm et al., 1990; Taskinen et al., 1989; Lagakos et al., 1986). Studies examining associations between TCE exposure and decreased birth weight or SGA have reported small, often nonstatistically significant, increases in risk for these effects (ATSDR, 2008b, 2006a; Windham et al., 1991). However, other studies observed mixed or no association (Rodenbeck et al., 2000; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). While comprising both occupational and environmental exposures, these studies are overall not highly informative due to their small numbers of cases and limited exposure characterization or to the fact that exposures to mixed solvents were involved. However, a number of laboratory animal studies show analogous effects of TCE exposure in rodents. In particular, pre- or postimplantation losses, increased resorptions, perinatal death, and decreased birth weight have been reported in multiple well-conducted studies in rats and mice (Kumar et al., 2000b; Narotsky and Kavlock, 1995; Narotsky et al., 1995; George et al., 1986; George et al., 1985; Healy et al., 1982). Interestingly, the rat studies reporting these effects used F344 or Wistar rats, while several other studies, all of which used Sprague-Dawley rats, reported no increased risk in these developmental measures (Carney et al., 2006; Hardin et al., 1981; Schwetz et al., 1975). Overall, based on weakly suggestive epidemiologic data and fairly consistent laboratory animal data, it can be concluded that TCE exposure poses a potential hazard for prenatal losses and decreased growth or birth weight of offspring.

Epidemiologic data provide some support for the possible relationship between maternal TCE exposure and birth defects in offspring, in particular cardiac defects. Other developmental outcomes observed in epidemiology and experimental animal studies include an increase in total birth defects (ATSDR, 2001; Flood, 1988), CNS defects (ATSDR, 2001; Bove, 1996; Bove et

al., 1995; Lagakos et al., 1986), oral cleft defects (Lorente et al., 2000; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986), eye/ear defects (Narotsky and Kavlock, 1995; Narotsky et al., 1995; Lagakos et al., 1986), kidney/urinary tract disorders (Lagakos et al., 1986), musculoskeletal birth anomalies (Lagakos et al., 1986), lung/respiratory tract disorders (Das and Scott, 1994; Lagakos et al., 1986), and skeletal defects (Healy et al., 1982). Occupational cohort studies, while not consistently reporting positive results, are generally limited by the small number of observed or expected cases of birth defects (Lorente et al., 2000; Taskinen et al., 1989; Tola et al., 1980).

While only one of the epidemiological studies specifically reported observations of eye anomalies (Lagakos et al., 1986), studies in rats have identified increases in the incidence of fetal eye defects following oral exposures during the period of organogenesis with TCE (Narotsky and Kavlock, 1995; Narotsky et al., 1995) or its oxidative metabolites, DCA and TCA (Warren et al., 2006; Smith et al., 1992; Smith et al., 1989). No other developmental or reproductive toxicity studies identified abnormalities of eye development following TCE exposures, which may have been related to the administered dose or other aspects of study design (e.g., level of detail applied to fetal ocular evaluation). Overall, the study evidence suggests a potential for the disruption of ocular development by exposure to TCE and its oxidative metabolites.

The epidemiological studies, while individually limited, as a whole show relatively consistent elevations, some of which were statistically significant, in the incidence of cardiac effects in TCE-exposed populations compared to reference groups (ATSDR, 2008b, 2006a; Yauck et al., 2004; ATSDR, 2001; Bove, 1996; Bove et al., 1995; Goldberg et al., 1990). Interestingly, Goldberg et al. (1990) noted that the OR for congenital heart disease in offspring declined from threefold to no difference as compared to controls after TCE-contaminated drinking water wells were closed, suggestive of a causal relationship. However, this study reported no significant differences in cardiac lesions between exposed and nonexposed groups (Goldberg et al., 1990). One additional community study reported that, among the five cases of cardiovascular anomalies, there was no significant association with TCE (Lagakos et al., 1986), but due to the small number of cases, this does not support an absence of effect. In laboratory animal models, avian studies were the first to identify adverse effects of TCE exposure on cardiac development, and the initial findings have been confirmed multiple times (Rufer et al., 2008; Drake et al., 2006a; Drake et al., 2006b; Mishima et al., 2006; Boyer et al., 2000; Loeber et al., 1988; Bross et al., 1983). Additionally, administration of TCE and TCE metabolites TCA and DCA in maternal drinking water during gestation has been reported to induce cardiac malformations in rat fetuses (Johnson et al., 2005, 2003; Johnson et al., 1998b; Johnson et al., 1998a; Dawson et al., 1993; Epstein et al., 1992; Smith et al., 1992; Dawson et al., 1990; Smith et al., 1989). However, it is notable that a number of other studies, several of which were well conducted, did not report induction of cardiac defects in rats or rabbits from TCE administered by inhalation (Carney et al., 2006; Healy et al., 1982; Hardin et al., 1981; Dorfmueller et al.,

1979; Schwetz et al., 1975) or in rats and mice by gavage (Fisher et al., 2001; Narotsky and Kavlock, 1995; Narotsky et al., 1995; Cosby and Dukelow, 1992).

The potential importance of these effects warrants a more detailed discussion of possible explanations for the apparent inconsistencies in the laboratory animal studies. Many of the studies that did not identify cardiac anomalies used a traditional free-hand section technique on fixed fetal specimens (Healy et al., 1982; Hardin et al., 1981; Dorfmueller et al., 1979; Schwetz et al., 1975). Detection of such anomalies can be enhanced through the use of a fresh dissection technique as described by Staples (1974) and Stuckhardt and Poppe (1984) and this was the technique used in the study by Dawson et al. (1990) with further refinement of the technique used in the positive studies by Dawson et al. (1993) and Johnson et al. (2005, 2003). However, two studies that used the same or similar fresh dissection technique did not report cardiac anomalies (Carney et al., 2006; Fisher et al., 2001), although it has been suggested that differences in experimental design (e.g., inhalation versus gavage versus drinking water route of administration, exposure during organogenesis versus the entire gestational period, or varied dissection or evaluation procedures) may have been contributing factors to the differences in observed response. A number of other limitations in the studies by Dawson et al. (1993) and Johnson et al. (2005, 2003) have been suggested (Watson et al., 2006; Hardin et al., 2005). One concern is the lack of clear dose-response relationship for the incidence of any specific cardiac anomaly or combination of anomalies, a disparity for which no reasonable explanation has been put forth. In addition, analyses on a fetal- rather than litter-basis and the pooling of data collected over an extended period, including nonconcurrent controls, have been criticized. With respect to the first issue, the study authors provided individual litter incidence data to EPA for analysis (see Chapter 5, Dose-Response Assessment), and, in response to the second issue, the study authors provided further explanation as to their experimental procedures (Johnson et al., 2004). In sum, while the studies by Dawson et al. (1993) and Johnson et al. (2005, 2003) have significant limitations, there is insufficient reason to dismiss their findings.

Finally, mechanistic studies, particularly based on the avian studies mentioned above, provide additional support for TCE-induced fetal cardiac malformation, particularly with respect to defects involving septal and valvular morphogenesis. As summarized by NRC (2006), there is substantial concordance in the stages and events of cardiac valve formation between mammals and birds. While quantitative extrapolation of findings from avian studies to humans is not possible without appropriate kinetic data for these experimental systems, the treatment-related alterations in endothelial cushion development observed in avian in ovo and in vitro studies (Mishima et al., 2006; Ou et al., 2003; Boyer et al., 2000) provide a plausible mechanistic basis for defects in septal and valvular morphogenesis observed in rodents, and consequently support the plausibility of cardiac defects induced by TCE in humans.

Postnatal developmental outcomes examined after TCE prenatal and/or postnatal exposure in both humans and experimental animals include developmental neurotoxicity,

developmental immunotoxicity, and childhood cancer. Effects on the developing nervous system included a broad array of structural and behavioral alterations in humans (Windham et al., 2006; Laslo-Baker et al., 2004; ATSDR, 2002; Till et al., 2001a; Burg and Gist, 1997; White et al., 1997, abstract; Burg et al., 1995; Bernad et al., 1987; Beppu, 1968) and animals (Blossom et al., 2008; Narotsky and Kavlock, 1995; Fredriksson et al., 1993; Isaacson and Taylor, 1989; George et al., 1986; Noland-Gerbec et al., 1986; Taylor et al., 1985; Westergren et al., 1984). Adverse immunological findings in humans following developmental exposures to TCE were reported by Lehmann et al. (2002) and Byers et al. (1988). In mice, alterations in T-cell subpopulations, spleen and/or thymic cellularity, cytokine production, autoantibody levels (in an autoimmune-prone mouse strain), and/or hypersensitivity response were observed after exposures during development (Blossom et al., 2008; Peden-Adams et al., 2008; Blossom and Doss, 2007; Peden-Adams et al., 2006). Childhood cancers included leukemia and NHL (Costas et al., 2002; Morgan and Cassady, 2002; Shu et al., 1999; Flood, 1997a; MDPH, 1997a; Cohn et al., 1994b; McKinney et al., 1991; ADHS, 1990; Kioski et al., 1990a; Kioski et al., 1990b; Flood, 1988; Lowengart et al., 1987; Cutler et al., 1986; Lagakos et al., 1986), CNS tumors (Morgan and Cassady, 2002; De Roos et al., 2001; Flood, 1997a; ADHS, 1990; Kioski et al., 1990a; Flood, 1988; Peters et al., 1985; Peters et al., 1981), and total cancers (ATSDR, 2006a; Flood, 1997a; Porter, 1993; ADHS, 1990; Flood, 1988). These outcomes are discussed in the other relevant sections for neurotoxicity, immunotoxicity, and carcinogenesis.

4.11.2. Characterization of Carcinogenicity

Following EPA (2005b) *Guidelines for Carcinogen Risk Assessment*, TCE is characterized as "carcinogenic to humans" by all routes of exposure. This conclusion is based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer. The kidney cancer association cannot be reasonably attributed to chance, bias, or confounding. The human evidence of carcinogenicity from epidemiologic studies of TCE exposure is strong for NHL but less convincing than for kidney cancer, and more limited for liver and biliary tract cancer. In addition to the body of evidence pertaining to kidney cancer, NHL, and liver cancer, the available epidemiologic studies also provide more limited evidence of an association between TCE exposure and other types of cancer, including bladder, esophageal, prostate, cervical, breast, and childhood leukemia. Differences between these sets of data and the data for kidney cancer, NHL, and liver cancer are observations from fewer numbers of studies, a mixed pattern of observed risk estimates, and the general absence of exposure-response data from the studies using a quantitative TCE-specific exposure measure.

There are several lines of supporting evidence for TCE carcinogenicity in humans. First, TCE induces multiple types of cancer in rodents given TCE by gavage and inhalation, including cancers in the same target tissues identified in the epidemiologic studies – kidney, liver, and lymphoid tissues. Second, toxicokinetic data indicate that TCE absorption, distribution,

metabolism, and excretion are qualitatively similar in humans and rodents. Finally, there is sufficient weight of evidence to conclude that a mutagenic mode of action is operative for TCE-induced kidney tumors, and this mode of action is clearly relevant to humans. Modes of action have not been established for other TCE-induced cancers in rodents, and no mechanistic data indicate that any hypothesized key events are biologically precluded in humans.

4.11.2.1. Summary Evaluation of Epidemiologic Evidence of TCE and Cancer

The available epidemiologic studies provide convincing evidence of a causal association between TCE exposure and cancer. The strongest epidemiologic evidence consists of reported increased risks of kidney cancer, with more limited evidence for NHL and liver cancer, in several well-designed cohort and case-control studies (discussed below). The summary evaluation below of the evidence for causality is based on guidelines adapted from Hill (1965) by EPA (2005b), and focuses on evidence related to kidney cancer, NHL, and liver cancer.

4.11.2.1.1. (a) Consistency of observed association

Elevated risks for kidney cancer have been observed across many independent studies. Twenty-four studies in which there was a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis were identified in a systematic review of the epidemiologic literature. Of the 15 of these 24 studies reporting risks of kidney cancer (Moore et al., 2010; Radican et al., 2008; Charbotel et al., 2006; Zhao et al., 2005; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Pesch et al., 2000b; Boice et al., 1999; Dosemeci et al., 1999; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991), most estimated RRs between 1.1 and 1.9 for overall exposure to TCE. Six of these 15 studies reported statistically significant increased risks either for overall exposure to TCE (Moore et al., 2010; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Dosemeci et al., 1999) or for one of the highest TCE exposure groups (Moore et al., 2010; Charbotel et al., 2006; Zhao et al., 2005; Raaschou-Nielsen et al., 2003). Thirteen other cohort, case-control, and geographic-based studies were given less weight because of their lesser likelihood of TCE exposure and other study design limitations that would decrease statistical power and study sensitivity (see Sections 4.1 and 4.4.2).

The consistency of the association between TCE exposure and kidney cancer is further supported by the results of the meta-analyses of the 15 cohort and case-control studies of sufficient quality and with high probability of TCE exposure to individual subjects. These analyses observed a statistically significant increased RRm estimate for kidney cancer of 1.27 (95% CI: 1.13, 1.43) for overall TCE. The RRms were robust and did not change appreciably with the removal of any individual study or with the use of alternate RR estimates from individual studies. In addition, there was no evidence for heterogeneity or publication bias.

The consistency of increased kidney cancer RR estimates across a large number of independent studies of different designs and populations from different countries and industries argues against chance, bias or confounding as the basis for observed associations. This consistency thus provides substantial support for a causal effect between kidney cancer and TCE exposure.

Some evidence of consistency is found between TCE exposure and NHL and liver cancer. In a weight-of-evidence review of the NHL studies, 17 studies in which there was a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis were identified. These studies generally reported excess RR estimates for NHL between 0.8 and 3.1 for overall TCE exposure. Statistically significant elevated RR estimates for overall exposure were observed in two cohort studies (Raaschou-Nielsen et al., 2003; Hansen et al., 2001) and one case-control study (Hardell et al., 1994). The other 14 identified studies reported elevated RR estimates with overall TCE exposure that were not statistically significant (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009; Radican et al., 2008; Miligi et al., 2006; Zhao et al., 2005; Boice et al., 1999; Persson and Fredrikson, 1999; Morgan et al., 1998; Nordström et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Siemiatycki, 1991). Fifteen additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease study power and sensitivity (Sinks et al., 1992)(see Sections 4.1 and 4.6.1.2). The observed lack of association with NHL in these studies likely reflects study design and exposure assessment limitations and is not considered inconsistent with the overall evidence on TCE and NHL.

Consistency of the association between TCE exposure and NHL is further supported by the results of meta-analyses. These meta-analyses found a statistically significant increased RRm estimate for NHL of 1.23 (95% CI: 1.07, 1.42) for overall TCE exposure. This result and its statistical significance were not overly influenced by most individual studies. Some heterogeneity was observed across the 17 studies of overall exposure, although it was not statistically significant ($p = 0.16$). Analyzing the cohort and case-control studies separately resolved most of the heterogeneity, but the result for the summary case-control studies was only about a 7% increased RR estimate and was not statistically significant. The sources of heterogeneity are uncertain but may be the result of some bias associated with exposure assessment and/or disease classification, or from differences between cohort and case-control studies in average TCE exposure. In addition, there is some evidence of potential publication bias in this data set; however, it is uncertain that this is actually publication bias rather than an association between SE and effect size resulting for some other reason (e.g., a difference in study populations or protocols in the smaller studies). Furthermore, if there is publication bias in this data set, it does not appear to account completely for the finding of an increased NHL risk.

There are fewer studies on liver cancer than for kidney cancer and NHL. Of nine studies, all of them cohort studies, in which there was a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review (Radican et al., 2008; Boice et al., 2006b; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Morgan et al., 1998; Anttila et al., 1995; Axelson et al., 1994; Greenland et al., 1994), most reported RR estimates for liver and gallbladder cancer between 0.5 and 2.0 for overall exposure to TCE. RR estimates were generally based on small numbers of cases or deaths, with the result of wide CIs on the estimates, except for one study (Raaschou-Nielsen et al., 2003). This study reported almost 6 times more cancer cases than the next largest study and observed a statistically significant elevated liver and gallbladder cancer risk with overall TCE exposure (RR = 1.35 [95% CI: 1.03, 1.77]). Ten additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease statistical power and study sensitivity (see Sections 4.1 and 4.5.2).

Consistency of the association between TCE exposure and liver cancer is further supported by the results of meta-analyses. These meta-analyses found a statistically significant increased RRm estimate for liver and biliary tract cancer of 1.29 (95% CI: 1.07, 1. 56) with overall TCE exposure. Although there was no evidence of heterogeneity or publication bias and the summary estimate was fairly insensitive to the use of alternative RR estimates, the statistical significance of the summary estimate depends heavily on the one large study by Raaschou-Nielsen et al. (2003). However, there were fewer adequate studies available for meta-analysis of liver cancer (9 vs. 17 for NHL and 15 for kidney), leading to lower statistical power, even with pooling. Moreover, liver cancer is comparatively rarer, with age-adjusted incidences roughly half or less those for kidney cancer or NHL; thus, fewer liver cancer cases are generally observed in individual cohort studies.

4.11.2.1.2. (b) Strength of the observed association

In general, the observed associations between TCE exposure and cancer are modest, with RRs or ORs for overall TCE exposure generally <2.0 and higher RRs or ORs for high exposure categories. Among the highest statistically significant RRs were those reported for kidney cancer in the studies by Henschler et al. (1995) (7.97 [95% CI: 2.59, 8.59]) and Vamvakas et al. (1998) (10.80 [95% CI: 3.36, 34.75]). As discussed in Section 4.4.2.2.1, risk magnitude in both studies is highly uncertain due, in part, to possible selection biases, and neither was included in the meta-analyses. However, the findings of these studies were corroborated, though with lower reported RRs, by later studies, which overcame many of their deficiencies, such as Brüning et al. (2003) (2.47 [95% CI: 1.36, 4.49]), Charbotel et al. (2006) (2.16 [95% CI: 1.02, 4.60] for the high cumulative exposure group), and Moore et al. (2010) (2.05 [95% CI: 1.13, 3.73] for high confidence assessment of TCE). In addition, the very high apparent exposure in the subjects of

Henschler et al. (1995) and Vamvakas et al. (1998) may have contributed to their reported RRs being higher than those in other studies. Exposures in most population case-control studies are of lower overall TCE intensity compared to exposures in Brüning et al. (2003) and Charbotel et al. (2006), and, as would be expected, observed RR estimates are lower (1.24 [95% CI: 1.03, 1.49]), Pesch et al. (2000b); 1.30 [95% CI: 0.9, 1.9], Dosemeci et al. (1999)). A few high-quality cohort and case-control studies reported statistically significant RRs of approximately 2.0 with highest exposure, including Zhao et al. (2005) (4.9 [95% CI: 1.23, 19.6] for high TCE score), Raaschou-Nielsen et al. (2003) (1.7 [95% CI: 1.1, 2.4] for ≥5 year exposure duration, subcohort with higher exposure]), Charbotel et al. (2006) (2.16 [95% CI: 1.02, 4.60] for high cumulative exposure and 2.73 [95% CI: 1.06, 7.07] for high cumulative exposure plus peaks) and Moore et al. (2010) (2.23 [95% CI: 1.07, 4.64] for high cumulative exposure and 2.41 [95% CI: 1.05, 5.56] for high average intensity TCE exposure).

Among the highest statistically significant RRs reported for NHL were those of Hansen et al. (2001) (3.1 [95% CI: 1.3, 6.1]), Hardell et al. (1994) (7.2 [95% CI: 1.3, 42]), the latter a case-control study whose magnitude of risk is uncertain because of self-reported occupational TCE exposure. A similar magnitude of risk was reported in Purdue et al. (2011) for highest exposure (3.3 [95% CI: 1.1, 10.1], >234,000 ppm-hour, and 7.9 [95% CI: 1.8, 34.3], >360 ppm-hour/week). Observed RR estimates for liver cancer and overall TCE exposure are generally more modest.

The strength of association between TCE exposure and cancer is modest with overall TCE exposure. Large RR estimates are considered strong evidence of causality; however, a modest risk does not preclude a causal association and may reflect a lower level of exposure, an agent of lower potency, or a common disease with a high background level (U.S. EPA, 2005b). Modest RR estimates have been observed with several well-established human carcinogens such as benzene and secondhand smoke. Chance cannot explain the observed association between TCE and cancer; statistically significant associations were found in a number of the studies that contribute greater weight to the overall evidence, given their design and statistical analysis approaches. In addition, other known or suspected risk factors cannot fully explain the observed elevations in kidney cancer RRs. All kidney cancer case-control studies included adjustment for possible confounding effects of smoking, and some studies included BMI, hypertension, and co-exposure to other occupational agents such as cutting or petroleum oils. Cutting and petroleum oils, known as metalworking fluids, have not been associated with kidney cancer (Mirer, 2010; NIOSH, 1998), and potential confounding by this occupational co-exposure is unable to explain the observed association with TCE. Additionally, the associations between kidney cancer and TCE exposure remained in these studies after statistical adjustment for possible known and suspected confounders. Charbotel et al. (2006) observed a nonstatistically significantly kidney cancer risk with exposure to TCE adjusted for cutting or petroleum oil exposures (1.96 [95% CI:

0.71, 5.37] for the high-cumulative exposure group and 2.63 [95% CI: 0.79, 8,83] for high-exposure group with peaks).

All kidney cancer case-control studies adjusted for smoking except the Moore et al. (2010) study, which reported that smoking did not significantly change the overall association with TCE exposure. Although direct examination of smoking and other suspected kidney cancer risk factors is usually not possible in cohort studies, confounding is less likely in Zhao et al. (2005), given their use of an internal referent group and adjustment for SES status, an indirect surrogate for smoking, and other occupational exposures. In addition, the magnitude of the lung cancer risk in Raaschou-Nielsen et al. (2003) suggests that a high smoking rate is unlikely and cannot explain their finding on kidney cancer. Last, a meta-analysis of the nine cohort studies that reported kidney cancer risks found an RRm estimate for lung cancer of 0.96 (95% CI: 0.76, 1.21) for overall TCE exposure and 0.96 (95% CI: 0.72, 1.27) for the highest exposure group. These observations suggest that confounding by smoking is not an alternative explanation for the kidney cancer meta-analysis results.

Few risk factors are recognized for NHL, with the exception of viruses and suspected factors such as immunosuppression or smoking, which are associated with specific NHL subtypes. Associations between NHL and TCE exposure are based on groupings of several NHL subtypes. Three of the seven NHL case-control studies adjusted for age, sex, and smoking in statistical analyses (Wang et al., 2009; Miligi et al., 2006) two others adjusted for age, sex, and education (Purdue et al., 2011; Cocco et al., 2010), and the other three case-control studies adjusted for age only or age and sex (Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994). Like for kidney cancer, direct examination of possible confounding in cohort studies is not possible. The use of internal controls in some of the cohort studies is intended to reduce possible confounding related to lifestyle differences, including smoking habits, between exposed and referent subjects.

Heavy alcohol use and viral hepatitis are established risk factors for liver cancer, with severe obesity and diabetes characterized as a metabolic syndrome associated with liver cancer. Only cohort studies for liver cancer are available, and they were not able to consider these possible risk factors.

4.11.2.1.3. (c) Specificity of the observed association

Specificity is generally not as relevant as other aspects for judging causality. As stated in the EPA *Guidelines for Carcinogen Risk Assessment* (2005b), based on our current understanding that many agents cause cancer at multiple sites and that cancers have multiple causes, the absence of specificity does not detract from evidence for a causal effect. Evidence for specificity could be provided by a biological marker in tumors that was specific to TCE exposure. There is some evidence suggesting that particular *VHL* mutations in kidney tumors may be caused by TCE, but uncertainties in these data preclude a definitive conclusion.

4.11.2.1.4. (d) Temporal relationship of the observed association

Each cohort study was evaluated for the adequacy of the follow-up period to account for the latency of cancer development. The studies with the greatest weight based on study design characteristics (e.g., those used in the meta-analysis) all had adequate follow-up to assess associations between TCE exposure and cancer. Therefore, the findings of those studies are consistent with a temporal relationship.

4.11.2.1.5. (e) Biological gradient (exposure-response relationship)

Exposure-response relationships are examined in the TCE epidemiologic studies only to a limited extent. Many studies examined only overall "exposed" vs. "unexposed" groups and did not provide exposure information by level of exposure. Others do not have adequate exposure assessments to confidently distinguish between levels of exposure. For example, many studies used duration of employment as an exposure surrogate; however, this is a poor exposure metric given subjects may have differing exposure intensity with similar exposure duration (NRC, 2006).

Three studies of kidney cancer reported a statistically significant trend of increasing risk with increasing TCE exposure, Zhao et al. (2005) (p = 0.023 for trend with TCE score), Charbotel et al. (2006) (p = 0.04 for trend with cumulative TCE exposure) and Moore et al. (2010) (p = 0.02 for trend with cumulative TCE exposure). Charbotel et al. (2006) was specifically designed to examine TCE exposure and had a high-quality exposure assessment, and the Moore et al. (2010) exposure assessment considered detailed information on jobs using solvents. Zhao et al. (2005) also had a relatively well-designed exposure assessment. A positive trend was also observed in one other study (Raaschou-Nielsen et al. (2003), with employment duration).

Biological gradient is further supported by meta-analyses for kidney cancer using only the highest exposure groups and accounting for possible reporting bias, which yielded a higher RRm estimate (1.58 [95% CI: 1.28, 1.96]) than for overall TCE exposure (1.27 [95% CI: 1.13, 1.43]). Although this analysis uses a subset of studies in the overall TCE exposure analysis, the finding of higher risk in the highest exposure groups, where such groups were available, is consistent with a trend of increased risk with increased exposure.

The NHL case-control study of Purdue et al. (2011) reported a statistically significant trend with TCE exposure (p = 0.02 for trend with average-weekly TCE exposure), and NHL risk in Boice et al. (1999) appeared to increase with increasing exposure duration (p = 0.20 for routine-intermittent exposed subjects). The borderline trend with TCE intensity in the case-control studies of Wang et al. (2009) (p = 0.06) and Purdue et al. (2011) (p = 0.08 for trend with cumulative TCE exposure) is consistent with their findings for average weekly TCE exposure. As with kidney cancer, further support was provided by meta-analyses using only the highest exposure groups, which yielded a higher RRm estimate (1.43 [95% CI: 1.13, 1.82]) than for

overall TCE exposure (1.23 [95% CI: 1.07, 1.42]). For liver cancer, the meta-analyses using only the highest exposure groups yielded a lower, and nonstatistically significant, RRm estimate (1.28 [95% CI: 0.93, 1.77]) than for overall TCE exposure (1.29 [95% CI: 1.07, 1.56]). There were no case-control studies on liver cancer and TCE, and the cohort studies generally had few liver cancer cases, making it more difficult to assess exposure-response relationships. The one large study (Raaschou-Nielsen et al., 2003) used only duration of employment, which is an inferior exposure metric.

4.11.2.1.6. (f) Biological plausibility

TCE metabolism is similar in humans, rats, and mice and results in reactive metabolites. TCE is metabolized in multiple organs and metabolites are systemically distributed. Several oxidative metabolites produced primarily in the liver, including CH, TCA and DCA, are rodent hepatocarcinogens. Two other metabolites, DCVC and DCVG, which can be produced and cleared by the kidney, have shown genotoxic activity, suggesting the potential for carcinogenicity. Kidney cancer, NHL, and liver cancer have all been observed in rodent bioassays (see below). The laboratory animal data for liver and kidney cancer are the most robust, and are corroborated in multiple studies, sexes, and strains, although each has only been reported in a single species and the incidences of kidney cancer are quite low. Lymphomas were only reported to be statistically significantly elevated in a single study in mice, but one additional mouse study reported elevated lymphoma incidence and one rat study reported elevated leukemia incidence. In addition, there is some evidence both in humans and laboratory animals for kidney, liver and immune system noncancer toxicity from TCE exposure. Several hypothesized modes of action have been presented for the rodent tumor findings, and the available evidence does not preclude the relevance of the hypothesized modes of action to humans. Activation of macrophages, NK cells, and cytokine production (e.g., tumor necrosis factor) may also play an etiologic role in carcinogenesis, and thus, the immune-related effects of TCE should also be considered. In addition, the decreased in lymphocyte counts and subsets, including CD4+ T cells, and decreased lymphocyte activation seen in TCE-exposed workers (Lan et al., 2010) also support the biological plausibility of a role of TCE exposure in NHL.

4.11.2.1.7. (g) Coherence

Coherence is defined as consistency with the known biology. As discussed under biological plausibility, the observance of kidney and liver cancer and NHL in humans is consistent with the biological processing and toxicity of TCE.

4.11.2.1.8. (h) Experimental evidence (from human populations)

Few experimental data from human populations are available on the relationship between TCE exposure and cancer. The only study of a "natural experiment" (i.e., observations of a temporal change in cancer incidence in relation to a specific event) notes that childhood leukemia cases appeared to be more evenly distributed throughout Woburn, Massachusetts, after closure of the two wells contaminated with TCE and other organic solvents (MDPH, 1997c).

4.11.2.1.9. (i) Analogy

Exposure to structurally related chlorinated solvents such as tetrachloroethylene and dichloromethane have also been associated with kidney, lymphoid, and liver tumors in human, although the evidence for TCE is considered stronger.

4.11.2.1.10. Conclusion

In conclusion, based on the weight-of-evidence analysis for kidney cancer and in accordance with EPA guidelines, TCE is characterized as "carcinogenic to humans." This hazard descriptor is used when there is convincing epidemiologic evidence of a causal association between human exposure and cancer. Convincing evidence is found in the consistency of the kidney cancer findings. The consistency of increased kidney cancer relative risk estimates across a large number of independent studies of different designs and populations from different countries and industries provides compelling evidence given the difficulty, a priori, in detecting effects in epidemiologic studies when the relative risks are modest, the cancers are relatively rare, and therefore, individual studies have limited statistical power. This strong consistency argues against chance, bias, and confounding as explanations for the elevated kidney cancer risks. In addition, statistically significant exposure-response trends are observed in high-quality studies. These studies were designed to examine kidney cancer in populations with high TCE exposure intensity. These studies addressed important potential confounders and biases, further supporting the observed associations with kidney cancer as causal. In a meta-analysis of the 15 studies that met the inclusion criteria, a statistically significant RRm estimate was observed for overall TCE exposure (RRm: 1.27 [95% CI: 1.13, 1.43]). The RRm estimate was greater for the highest TCE exposure groups (RRm: 1.58 [95% CI: 1.28, 1.96]; n = 13 studies). Meta-analyses investigating the influence of individual studies and the sensitivity of the results to alternate risk estimate selections found the RRm estimates to be highly robust. Furthermore, there was no indication of publication bias or significant heterogeneity. It would require a substantial amount of negative data from informative studies (i.e., studies having a high likelihood of TCE exposure in individual study subjects and which meet, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review) to contradict this observed association.

The evidence is less convincing for NHL and liver cancer. While the evidence is strong for NHL, issues of (nonstatistically significant) study heterogeneity, potential publication bias, and weaker exposure-response results contribute greater uncertainty. The evidence is more limited for liver cancer mainly because only cohort studies are available and most of these studies have small numbers of cases. In addition to the body of evidence described above pertaining to kidney cancer, NHL, and liver cancer, the available epidemiologic studies also provide suggestive evidence of an association between TCE exposure and other types of cancer, including bladder, esophageal, prostate, cervical, breast, and childhood leukemia, breast. Differences between these sets of data and the data for kidney cancer, NHL, and liver cancer are observations are from fewer numbers of studies, a mixed pattern of observed risk estimates and the general absence of exposure-response data from the studies using a quantitative TCE-specific cumulative exposure measure.

4.11.2.2. Summary of Evidence for TCE Carcinogenicity in Rodents

Additional evidence of TCE carcinogenicity consists of increased incidences of tumors reported in multiple chronic bioassays in rats and mice. In total, this database identifies some of the same target tissues of TCE carcinogenicity also seen in epidemiological studies, including the kidney, liver, and lymphoid tissues.

Of particular note is the site-concordant finding of TCE-induced kidney cancer in rats. In particular, low, but biologically and sometimes statistically significant, increases in the incidence of kidney tumors were observed in multiple strains of rats treated with TCE by either inhalation or corn oil gavage (NTP, 1990; Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986). For instance, Maltoni et al. (1986) reported that although only 4/130 renal adenocarcinomas were noted in rats in the highest dose group, these tumors had never been observed in over 50,000 Sprague-Dawley rats (untreated, vehicle-treated, or treated with different chemicals) examined in previous experiments in the same laboratory. In addition, the gavage study by NCI (1976) and two inhalation studies by Henschler et al. (1980), and Fukuda et al. (1983) each observed one renal adenoma or adenocarcinoma in some dose groups and none in controls. The largest (but still small) incidences were observed in treated male rats, only in the highest dose groups. However, given the small numbers, an effect in females cannot be ruled out. Several studies in rats were limited by excessive toxicity, accidental deaths, or deficiencies in reporting (NTP, 1990, 1988; NCI, 1976). Individually, therefore, these studies provide only suggestive evidence of renal carcinogenicity. Overall, given the rarity of these types of tumors in the rat strains tested and the repeated similar results across experiments and strains, these studies taken together support the conclusion that TCE is a kidney carcinogen in rats, with males being more sensitive than females. No other tested laboratory species (i.e., mice and hamsters) have exhibited increased kidney tumors, although high incidences of kidney toxicity have been reported in mice (NTP, 1990; Maltoni et al., 1988; Maltoni et al., 1986; NCI, 1976). The GSH-conjugation-

derived metabolites suspected of mediating TCE-induced kidney carcinogenesis have not been tested in a standard 2-year bioassay, so their role cannot be confirmed definitively. However, it is clear that GSH conjugation of TCE occurs in humans and that the human kidney contains the appropriate enzymes for bioactivation of GSH conjugates. Therefore, the production of the active metabolites thought to be responsible for kidney tumor induction in rats likely occurs in humans.

Statistically significant increases in TCE-induced liver tumors have been reported in multiple inhalation and gavage studies with male Swiss mice and B6C3F$_1$ mice of both sexes (Bull et al., 2002; Anna et al., 1994; NTP, 1990; Maltoni et al., 1988; Herren-Freund et al., 1987; Maltoni et al., 1986; NCI, 1976). On the other hand, in female Swiss mice, Fukuda et al. (1983) (CD-1 [ICR, Swiss-derived] mice) and Maltoni et al. (1988; 1986) both reported small, nonsignificant increases at the highest dose by inhalation. Henschler et al. (1984; 1980) reported no increases in either sex of Han:NMRI (also Swiss-derived) mice exposed by inhalation and ICR/HA (Swiss) mice exposed by gavage. However, the inhalation study (Henschler et al., 1980) had only 30 mice per dose group and the gavage study (Henschler et al., 1984) had dosing interrupted due to toxicity. Studies in rats (NTP, 1990; Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986; Henschler et al., 1980; NCI, 1976) and hamsters (Henschler et al., 1980) did not report statistically significant increases in liver tumor induction with TCE treatment. However, several studies in rats were limited by excessive toxicity or accidental deaths (NTP, 1990, 1988; NCI, 1976), and the study in hamsters only had 30 animals per dose group. These data are inadequate for concluding that TCE lacks hepatocarcinogenicity in rats and hamsters, but are indicative of a lower potency in these species. Moreover, it is notable that a few studies in rats reported low incidences (too few for statistical significance) of very rare biliary- or endothelial-derived tumors in the livers of some treated animals (Maltoni et al., 1988; Maltoni et al., 1986; Fukuda et al., 1983; Henschler et al., 1980). Further evidence for the hepatocarcinogenicity of TCE is derived from chronic bioassays of the TCE oxidative metabolites CH, TCA, and DCA in mice (e.g., DeAngelo et al., 2008; Leakey et al., 2003a; Leakey et al., 2003b; George et al., 2000; DeAngelo et al., 1999; DeAngelo et al., 1996; Bull et al., 1990), all of which reported hepatocarcinogenicity. Very limited testing of these TCE metabolites has been done in rats, with a single experiment reported in both Richmond et al. (1995) and DeAngelo et al. (1996) finding statistically significant DCA-induced hepatocarcinogenicity. With respect to TCA, DeAngelo et al. (1997), often cited as demonstrating lack of hepatocarcinogenicity in rats, actually reported elevated adenoma multiplicity and carcinoma incidence from TCA treatment. However, statistically, the role of chance could not be confidently excluded because of the low number of animals per dose group (20–24 per treatment group at final sacrifice). Overall, TCE and its oxidative metabolites are clearly carcinogenic in mice, with males more sensitive than females and the B6C3F$_1$ strain appearing to be more sensitive than the Swiss strain. Such strain and sex differences are not unexpected, as they appear to parallel, qualitatively, differences in

background tumor incidence. Data in other laboratory animal species are limited. Thus, except for DCA, which is carcinogenic in rats, inadequate evidence exists to evaluate the hepatocarcinogenicity of these compounds in rats or hamsters. However, to the extent that there is hepatocarcinogenic potential in rats, TCE is clearly less potent in the strains tested in this species than in $B6C3F_1$ and Swiss mice.

Additionally, there is more limited evidence for TCE-induced lymphohematopoietic cancers in rats and mice, lung tumors in mice, and testicular tumors in rats. With respect to lymphomas, Henschler et al. (1980) reported statistically significant increases in lymphomas in female Han:NMRI mice treated via inhalation. While Henschler et al. (1980) suggested that these lymphomas were of viral origin specific to this strain, subsequent studies reported increased lymphomas in female $B6C3F_1$ mice treated via corn oil gavage (NTP, 1990) and leukemias in male Sprague-Dawley and female August rats (Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986). However, these tumors had relatively modest increases in incidence with treatment, and were not reported to be increased in other studies. With respect to lung tumors, rodent bioassays have demonstrated a statistically significant increase in pulmonary tumors in mice following chronic inhalation exposure to TCE (Maltoni et al., 1988; Maltoni et al., 1986; Fukuda et al., 1983). Pulmonary tumors were not reported in other species tested (i.e., rats and hamsters; (Maltoni et al., 1988; Maltoni et al., 1986; Fukuda et al., 1983; Henschler et al., 1980)). Chronic oral exposure to TCE led to a nonstatistically significant increase in pulmonary tumors in mice but, again, not in rats or hamsters (NTP, 1990; Maltoni et al., 1988; NTP, 1988; Maltoni et al., 1986; Henschler et al., 1984; Van Duuren et al., 1979; NCI, 1976). A lower response via oral exposure would be consistent with a role of respiratory metabolism in pulmonary carcinogenicity. Finally, increased testicular (interstitial cell and Leydig cell) tumors have been observed in rats exposed by inhalation and gavage (NTP, 1990, 1988; Maltoni et al., 1986). Statistically significant increases were reported in Sprague-Dawley rats exposed via inhalation (Maltoni et al., 1988; Maltoni et al., 1986) and Marshall rats exposed via gavage (NTP, 1988). In three rat strains, ACI, August, and F344/N, a high (>75%) control rate of testicular tumors was observed, limiting the ability to detect a treatment effect (NTP, 1990, 1988).

In summary, there is clear evidence for TCE carcinogenicity in rats and mice, with multiple studies showing TCE to cause different kinds of cancers. The apparent lack of site concordance across laboratory animal species may be due to limitations in design or conduct in a number of rat bioassays and/or genuine interspecies differences in sensitivity. Nonetheless, these studies have shown carcinogenic effects across different strains, sexes, and routes of exposure, and site-concordance is not necessarily expected for carcinogens. Of greater import is the finding that there is site-concordance between the main cancers observed in TCE-exposed humans and those observed in rodent studies—in particular, cancers of the kidney, liver, and lymphoid tissues.

4.11.2.3. Summary of Additional Evidence on Biological Plausibility

Additional evidence from toxicokinetic, toxicity, and mechanistic studies supports the biological plausibility of TCE carcinogenicity in humans.

4.11.2.3.1. Toxicokinetics

As described in Chapter 3, there is no evidence of major qualitative differences across species in TCE absorption, distribution, metabolism, and excretion. In particular, available evidence is consistent with TCE being readily absorbed via oral, dermal, and inhalation exposures, and rapidly distributed to tissues via systemic circulation. Extensive in vivo and in vitro data show that mice, rats, and humans all metabolize TCE via two primary pathways: oxidation by CYPs and conjugation with GSH via GSTs. Several metabolites and excretion products from both pathways, including TCA, DCA, TCOH, TCOG, NAcDCVC, and DCVG, have been detected in blood and urine from exposed humans was well as from at least one rodent species. In addition, the subsequent distribution, metabolism, and excretion of TCE metabolites are qualitatively similar among species. Therefore, humans possess the metabolic pathways that produce the TCE metabolites thought to be involved in the induction of rat kidney and mouse liver tumors, and internal target tissues of both humans and rodents experience a similar mix of TCE and metabolites.

As addressed in further detail elsewhere (see Chapters 3 and 5), examples of quantitative interspecies differences in toxicokinetics include differences in partition coefficients, metabolic capacity and affinity in various tissues, and plasma binding of the metabolite TCA. These and other differences are addressed through PBPK modeling, which also incorporates physiological differences among species (see Section 3.5), and are accounted for in the PBPK model-based dose-response analyses (see Chapter 5). Importantly, these quantitative differences affect only interspecies extrapolations of carcinogenic potency, and do not affect inferences as to the carcinogenic hazard for TCE. In addition, available data on toxicokinetic differences do not appear sufficient to explain interspecies differences in target sites of TCE carcinogenicity (discussed further in Chapter 5: Dose-Response Assessment).

4.11.2.3.2. Toxicity and mode of action

Many different modes of action have been proposed for TCE-induced carcinogenesis. With respect to genotoxicity, although it appears unlikely that TCE, as a pure compound, causes point mutations, there is evidence for TCE genotoxicity with respect to other genetic endpoints, such as micronucleus formation (see Section 4.2.1.4.4). In addition, as discussed further below, several TCE metabolites have tested positive in genotoxicity assays. The mode-of-action conclusions for specific target organs in laboratory animals are summarized below. Only in the case of the kidney is it concluded that the data are sufficient to support a particular mode of

action being operative. However, the available evidence do not indicate that qualitative differences between humans and test animals would preclude any of the hypothesized key events in rodents from occurring in humans.

For the kidney, the predominance of positive genotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular DCVC, see Section 4.2.5), together with toxicokinetic data consistent with their systemic delivery to and in situ formation in the kidney, supports the conclusion that a mutagenic mode of action is operative in TCE-induced kidney tumors (see Section 4.4.7.1). Relevant data include demonstration of genotoxicity in available in vitro assays of GSH conjugation metabolites and reported kidney-specific genotoxicity after in vivo administration of TCE or DCVC. Mutagenicity is a well-established cause of carcinogenicity. While supporting the biological plausibility of this hypothesized mode of action, available data on the *VHL* gene in humans or transgenic animals do not conclusively elucidate the role of *VHL* mutation in TCE-induced renal carcinogenesis. Cytotoxicity and compensatory cell proliferation, also presumed to be mediated through metabolites formed after GSH-conjugation of TCE, have also been suggested to play a role in the mode of action for renal carcinogenesis, as high incidences of nephrotoxicity have been observed in animals at doses that also induce kidney tumors. Human studies have reported markers for nephrotoxicity at current occupational exposures, although data are lacking at lower exposures. Toxicity is observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but kidney tumors are only observed at low incidences in rats at the highest tested doses. Therefore, nephrotoxicity alone appears to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis, since maximal levels of toxicity are reached before the onset of tumors. In addition, nephrotoxicity has not been shown to be necessary for kidney tumor induction by TCE in rodents. In particular, there is a lack of experimental support for causal links, such as compensatory cellular proliferation or clonal expansion of initiated cells, between nephrotoxicity and kidney tumors induced by TCE. Furthermore, it is not clear if nephrotoxicity is one of several key events in a mode of action, if it is a marker for an "upstream" key event (such as oxidative stress) that may contribute independently to both nephrotoxicity and renal carcinogenesis, or if it is incidental to kidney tumor induction. Therefore, although the data are consistent with the hypothesis that cytotoxicity and regenerative proliferation contribute to TCE-induced kidney tumors, the weight of evidence is not as strong as the support for a mutagenic mode of action. Moreover, while toxicokinetic differences in the GSH conjugation pathway, along with their uncertainty, are addressed through PBPK modeling, no data suggest that any of the proposed key events for TCE-induced kidney tumors rats are precluded in humans. Therefore, TCE-induced rat kidney tumors provide additional support for the convincing human evidence of TCE-induced kidney cancer, with mechanistic data supportive of a mutagenic mode of action.

The strongest data supporting the hypothesis of a mutagenic mode of action in either the lung or the liver are those demonstrating the genotoxicity of CH (see Section 4.2.4), which is produced in these target organs as a result of oxidative metabolism of TCE. It has been suggested that CH mutagenicity is unlikely to be the cause of TCE hepatocarcinogenicity because the concentrations required to elicit these responses are several orders of magnitude higher that achieved in vivo (Moore and Harrington-Brock, 2000). However, it is not clear how much of a correspondence is to be expected from concentrations in genotoxicity assays in vitro and concentrations in vivo, as reported in vivo CH concentrations are in whole liver homogenate while in vitro concentrations are in culture media. The use of i.p. administration, which leads to an inflammatory response, in many other in vivo genotoxicity assays in the liver and lung complicates the comparison with carcinogenicity data. Also, it is difficult with the available data to assess the contributions from genotoxic effects of CH along with those from the genotoxic and nongenotoxic effects of other oxidative metabolites (e.g., DCA and TCA). Therefore, while data are insufficient to conclude that a mutagenic mode of action mediated by CH is operant, a mutagenic mode of action in the liver or lung, either mediated by CH or by some other oxidative metabolite of TCE, cannot be ruled out.

A second mode-of-action hypothesis for TCE-induced liver tumors involves activation of the PPARα receptor. Clearly, in vivo administration of TCE leads to activation of PPARα in rodents and likely does so in humans as well (based on in vitro data for TCE and its oxidative metabolites). However, the evidence as a whole does not support the view that PPARα is the sole operant mode of action mediating TCE hepatocarcinogenesis. Although metabolites of TCE activate PPARα, the data on the subsequent elements in the hypothesized mode of action (e.g., gene regulation, cell proliferation, apoptosis, and selective clonal expansion), while limited, indicate significant differences between PPARα agonists such as Wy-14643 and TCE or its metabolites. For example, compared with other agonists, TCE induces transient as opposed to persistent increases in DNA synthesis; increases (or is without effect on), as opposed to decreases, apoptosis; and induces a different H-ras mutation frequency or spectrum. These data support the view that mechanisms other than PPARα activation may contribute to these effects; besides PPARα activation, the other hypothesized key events are nonspecific, and available data (e.g., using knockout mice) do not indicate that they are solely or predominantly dependent on PPARα. A second consideration is whether certain TCE metabolites (e.g., TCA) that activate PPARα are the sole contributors to its carcinogenicity. As summarized above (see Section 4.11.1.3), TCA is not the only metabolite contributing to the observed noncancer effects of TCE in the liver. Other data also suggest that multiple metabolites may also contribute to the hepatic carcinogenicity of TCE. Liver phenotype experiments, particularly those utilizing immunostaining for c-Jun, support a role for both DCA and TCA in TCE-induced tumors, with strong evidence that TCA cannot solely account for the characteristics of TCE-induced tumors (e.g., Bull et al., 2002). In addition, H-ras mutation frequency and spectrum of TCE-induced

tumors more closely resembles that of spontaneous tumors or of those induced by DCA, and were less similar in comparison to that of TCA-induced tumors. The heterogeneity of TCE-induced tumors is similar to that observed to be induced by a diversity carcinogens including those that do not activate PPARα, and to that observed in human liver cancer. Taken together, the available data indicate that, rather than being solely dependent on a single metabolite (TCA) and/or molecular target (PPARα), multiple TCE metabolites and multiple toxicity pathways contribute to TCE-induced liver tumors.

Other considerations as well as new data published since the NRC (2006) review are also pertinent to the liver tumor mode of action conclusions. It is generally acknowledged that, qualitatively, there are no data to support the conclusion that effects mediated by the PPARα receptor that contribute to hepatocarcinogenesis would be biologically precluded in humans (NRC, 2006; Klaunig et al., 2003). It has, on the other hand, been argued that due to quantitative toxicokinetic and toxicodynamic differences, the hepatocarcinogenic effects of chemicals activating this receptor are "unlikely" to occur in humans (NRC, 2006; Klaunig et al., 2003); however, several lines of evidence strongly undermine the confidence in this assertion. With respect to toxicokinetics, as discussed above, quantitative differences in oxidative metabolism are accounted for in PBPK modeling of available in vivo data, and do not support interspecies differences of a magnitude that would preclude hepatocarcinogenic effects based on toxicokinetics alone. With respect to the mode of action proposed by Klaunig et al. (2003), recent experiments have demonstrated that PPARα activation and the sequence of key events in the hypothesized mode of action are not sufficient to induce hepatocarcinogenesis (Yang et al., 2007). Moreover, the demonstration that the PPARα agonist DEHP induces tumors in PPARα-null mice supports the view that the events comprising the hypothesized mode of action are not necessary for liver tumor induction in mice by this PPARα agonist (Ito et al., 2007). Therefore, several lines of evidence, including experiments published since the NRC (2006) review, call into question the scientific validity of using the PPARα mode-of-action hypothesis as the basis for evaluating the relevance to human carcinogenesis of rodent liver tumors (Guyton et al., 2009).

In summary, available data support the conclusion that the mode of action for TCE-induced liver tumors in laboratory animals is not known. However, a number of qualitative similarities exist between observations in TCE-exposed mice and what is known about the etiology and induction of human HCCs. Polyploidization, changes in glycogen storage, inhibition of GST-zeta, and aberrant DNA methylation status, which have been observed in studies of mice exposed to TCE or its oxidative metabolites, are all either clearly related to human carcinogenesis or are areas of active research as to their potential roles (PPARα activation is discussed below). The mechanisms by which TCE exposure may interact with known risk factors for human HCCs are not known. However, available data do not suggest that TCE exposure to mice results in liver tumors that are substantially different in terms of their

phenotypic characteristics either from human HCCs or from rodent liver tumors induced by other chemicals.

Comparing various other, albeit relatively nonspecific, tumor characteristics between rodent species and humans provides additional support to the biologic plausibility of TCE carcinogenicity. For example, in the kidney and the liver, the higher incidences of background and TCE-induced tumors in male rats and mice, respectively, as compared to females parallels the observed higher human incidences in males for these cancers (Ries et al., 2008). For the liver, while there is a lower background incidence of liver tumors in humans than in rodents, in the United States, there is an increasing occurrence of liver cancer associated with several factors, including viral hepatitis, higher survival rates for cirrhosis, and possibly diabetes (reviewed in El-Serag, 2007). In addition, Leakey et al. (2003a) reported that increased body weight in B6C3F$_1$ mice is strongly associated with increased background liver tumor incidences, although the mechanistic basis for this risk factor in mice has not been established. Nonetheless, it is interesting that recent epidemiologic studies have suggested obesity, in addition to associated disorders such as diabetes and metabolic syndrome, as a risk factor for human liver cancer (El-Serag, 2007; El-Serag and Rudolph, 2007). Furthermore, the phenotypic and morphologic heterogeneity of tumors seen in the human liver is qualitatively similar to descriptions of mouse liver tumors induced by TCE exposure, as well as those observed from exposure to a variety of other chemical carcinogens. These parallels suggest similar pathways (e.g., for cell signaling) of carcinogenesis may be active in mice and humans and support the qualitative relevance of mouse models of liver to human liver cancer.

For mouse lung tumors, mode-of-action hypotheses have centered on TCE metabolites produced via oxidative metabolism in situ. As discussed above, the hypothesis that the mutagenicity of reactive intermediates or metabolites (e.g., CH) generated during CYP metabolism contributes to lung tumors cannot be ruled out, although available data are inadequate to conclusively support this mode of action. An alternative mode of action has been posited involving other effects of such oxidative metabolites, particularly CH, including cytotoxicity and regenerative cell proliferation. Experimental support for this alternative hypothesis remains limited, with no data on proposed key events in experiments ≥2 weeks in duration. While the data are inadequate to support this mode-of-action hypothesis, the data also do not suggest that any proposed key events would be biologically plausible in humans. Furthermore, the focus of the existing mode-of-action hypothesis involving cytotoxicity has been CH, and, as summarized above (see Section 4.11.1.5), other metabolites may contribute to respiratory tract noncancer toxicity or carcinogenicity. In sum, the mode of action for mouse lung tumors induced by TCE is not known.

A mode of action subsequent to in situ oxidative metabolism, whether involving mutagenicity, cytotoxicity, or other key events, may also be relevant to other tissues where TCE would undergo CYP metabolism. For instance, CYP2E1, oxidative metabolites, and protein

adducts have been reported in the testes of rats exposed to TCE, and, in some rat bioassays, TCE exposure increased the incidence of rat testicular tumors. However, inadequate data exist to adequately define a mode-of-action hypothesis for this tumor site.

4.11.3. Characterization of Factors Impacting Susceptibility

As discussed in more detail in Section 4.10, there is some evidence that certain populations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status, and lifestyle factors and nutrition status.

Examination of early lifestages includes exposures such as transplacental transfer (Ghantous et al., 1986; Withey and Karpinski, 1985; Laham, 1970; Beppu, 1968; Helliwell and Hutton, 1950) and breast milk ingestion (Fisher et al., 1997; Hamada and Tanaka, 1995; Fisher et al., 1990; Pellizzari et al., 1982), early lifestage-specific toxicokinetics, PBPK models (Fisher et al., 1990, 1989), and differential outcomes in early lifestages such as developmental cardiac defects. Although there is more information on susceptibility to TCE during early lifestages than on susceptibility during later lifestages or for other populations with potentially increased susceptibility, there remain a number of uncertainties and data gaps regarding children's susceptibility. Improved PBPK modeling for using childhood parameters for early lifestages as recommended by the NRC (2006), and validation of these models will aid in determining how variations in metabolic enzymes affect TCE metabolism. In particular, the NRC states that it is prudent to assume children need greater protection than adults, unless sufficient data are available to justify otherwise (NRC, 2006). Because the weight of evidence supports a mutagenic mode of action for TCE carcinogenicity in the kidney (see Section 4.4.7), and there is an absence of chemical-specific data to evaluate differences in carcinogenic susceptibility, early-life susceptibility should be assumed and the ADAFs should be applied, in accordance with the Supplemental Guidance (discussed further in Chapter 5).

Fewer data are available on later lifestages, although there is suggestive evidence to indicate that older adults may experience increased adverse effects than younger adults (Mahle et al., 2007; Rodriguez et al., 2007). In general, more studies specifically designed to evaluate effects in early and later lifestages are needed in order to more fully characterize potential lifestage-related TCE toxicity.

Examination of gender-specific susceptibility includes toxicokinetics, PBPK models (Fisher et al., 1998), and differential outcomes. Gender differences observed are likely due to variation in physiology and exposure.

Genetic variation likely has an effect on the toxicokinetics of TCE. In particular, differences in CYP2E1 activity may affect susceptibility of TCE due to effects on production of toxic metabolites (Yoon et al., 2007; Kim and Ghanayem, 2006; Povey et al., 2001; Lipscomb et al., 1997). GST polymorphisms could also play a role in variability in toxic response

(Wiesenhütter et al., 2007; Brüning et al., 1997a), as well as other genotypes, but these have not been sufficiently tested. Differences in genetic polymorphisms related to the metabolism of TCE have also been observed among various race/ethnic groups (Sato et al., 1991b; Inoue et al., 1989).

Preexisting diminished health status may alter the response to TCE exposure. Individuals with increased body mass may have an altered toxicokinetic response (Clewell et al., 2000; Lash et al., 2000a; McCarver et al., 1998; Sato, 1993; Davidson and Beliles, 1991; Sato et al., 1991b; Monster et al., 1979a), resulting in changes the internal concentrations of TCE or in the production of toxic metabolites. Other conditions, including diabetes and hypertension, are risk factors for some of the same health effects that have been associated with TCE exposure, such as RCC. However, the interaction between TCE and known risk factors for human diseases is not known, and further evaluation of the effects due to these factors is needed.

Lifestyle and nutrition factors examined include alcohol consumption, tobacco smoking, nutritional status, physical activity, and SES status. In particular, alcohol intake has been associated with metabolic inhibition (altered CYP2E1 expression) of TCE in both humans and experimental animals (McCarver et al., 1998; Kaneko et al., 1994; Sato, 1993; Nakajima et al., 1992a; Okino et al., 1991; Sato et al., 1991a; Nakajima et al., 1990; Larson and Bull, 1989; Nakajima et al., 1988; Sato and Nakajima, 1985; Barret et al., 1984; Sato et al., 1983, 1981; White and Carlson, 1981a; Sato et al., 1980; Muller et al., 1975; Stewart et al., 1974a; Bardodej and Vyskocil, 1956). In addition, such factors have been associated with increased baseline risks for health effects associated with TCE, such as kidney cancer (e.g., smoking) and liver cancer (e.g., alcohol consumption). However, the interaction between TCE and known risk factors for human diseases is not known, and further evaluation of the effects due to these factors is needed.

In sum, there is some evidence that certain populations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status, and lifestyle factors and nutrition status. However, except in the case of toxicokinetic variability characterized using the PBPK model described in Section 3.5, there are inadequate chemical-specific data to quantify the degree of differential susceptibility due to such factors.

5. DOSE-RESPONSE ASSESSMENT

5.1. DOSE-RESPONSE ANALYSES FOR NONCANCER ENDPOINTS

Because of the large number of noncancer health effects associated with TCE exposure and the large number of studies reporting on these effects, a screening process, described below, was used to reduce the number of endpoints and studies to those that would best inform the selection of the critical effects for the inhalation RfC and oral RfD.[16] The screening process helped identify the more sensitive endpoints for different types of effects within each health effect domain (e.g., different target systems) and provided information on the exposure levels that could contribute to the most sensitive effects, used for the RfC and RfD, as well as to additional noncancer effects as exposure increases. These more sensitive endpoints were also used to investigate the impacts of pharmacokinetic uncertainty and variability.

The general process used to derive the RfD and RfC was as follows (see Figure 5-1):

(1) Consider all studies described in Chapter 4 that reported adverse noncancer health effects or markers for such effects and provide quantitative dose-response data[17].

(2) Consider for each study/endpoint possible points of departure (PODs) on the basis of applied dose, with the order of preference being first a BMD[18] derived from empirical modeling of the dose-response data, then a NOAEL, and lastly a LOAEL.

(3) Adjust each POD by endpoint/study-specific ~~"u~~ncertainty factors" (UFs), accounting for uncertainties and adjustments in the extrapolation from the study conditions to conditions of human exposure, to derive candidate RfCs (cRfCs) or RfDs (cRfDs) intended to be protective for each endpoint (individually) on the basis of applied dose.

(4) Array the cRfCs and cRfDs across the following health effect domains: (1) neurotoxic effects; (2) systemic (body weight) and organ toxicity (kidney, liver) effects; (3) immunotoxic effects; (4) reproductive effects; and (5) developmental effects.

(5) Select as candidate critical effects those endpoints with the lowest cRfCs or cRfDs for each species (where appropriate), within each of these effect domains, taking into account the confidence in each estimate. When there are alternative estimates available for a particular endpoint, preference is given to studies whose design characteristics (e.g., species, statistical power, exposure level(s) and duration, endpoint measures) are better suited for determining the most sensitive human health effects of chronic TCE exposure.

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[16]In U.S. EPA noncancer health assessments, the RfC (RfD) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation (daily oral) exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark concentration (dose), with uncertainty factors generally applied to reflect limitations of the data used.

[17]Adequate dose-response data comprise, at a minimum, one exposure group and an appropriate control group, from which one can derive a LOAEL (or a NOAEL, if evidence of the effect is available from some other comparable study).

[18]More precisely, it is the BMDL, i.e., the (one-sided) 95% lower confidence bound on the dose corresponding to the benchmark response for the effect that is used as the POD.

(6) For each candidate critical effect selected in step 5, use, to the extent possible, the PBPK model developed in Section 3.5 to calculate an internal dose POD (idPOD) for plausible internal dose-metrics that were selected on the basis of what is understood about the role of different TCE metabolites in toxicity and the mode of action for toxicity. Effects within the same health effect domain were generally assumed to have the same relevant internal dose-metrics; thus, screening for the effects with the lowest cRfCs and cRfDs for each species within health effect domains on the basis of applied dose should capture the same endpoints which would have the lowest candidate reference values on the basis of an appropriate dose-metric.

(7) For each idPOD for each candidate critical effect, use the PBPK model to estimate interspecies and within-human pharmacokinetic variability (or just within-human variability for human-based PODs). The results of this calculation are 99th percentile estimates of the human equivalent concentration and human equivalent dose (HEC_{99} and HED_{99}) for each candidate critical effect.[19]

(8) Adjust each HEC_{99} or HED_{99} by endpoint-/study-specific UFs (which, due to the use of the PBPK model, may differ from the UFs used in step 3) to derive a PBPK model-based candidate RfCs (p-cRfC) and RfD (p-cRfD) for each candidate critical effect.

(9) Characterize the uncertainties in the cRfCs, cRfDs, p-cRfCs, and p-cRfDs, with the inclusion of quantitative uncertainty analyses of pharmacokinetic uncertainty and variability as derived from the Bayesian population analysis using the PBPK model.

(10) Evaluate the most sensitive cRfCs, p-cRfCs, cRfDs, and p-cRfDs, taking into account the confidence in the estimates, to arrive at an RfC and RfD for TCE. Except for candidate critical effects for which the PBPK model could not be used, the candidate reference values considered in the final selection process were those based on the most plausible internal dose-metric on the basis of the metabolism and mode-of-action considerations for each candidate critical effect.

[19]The choice of the 99th percentile is discussed in Section 5.1.3.2.

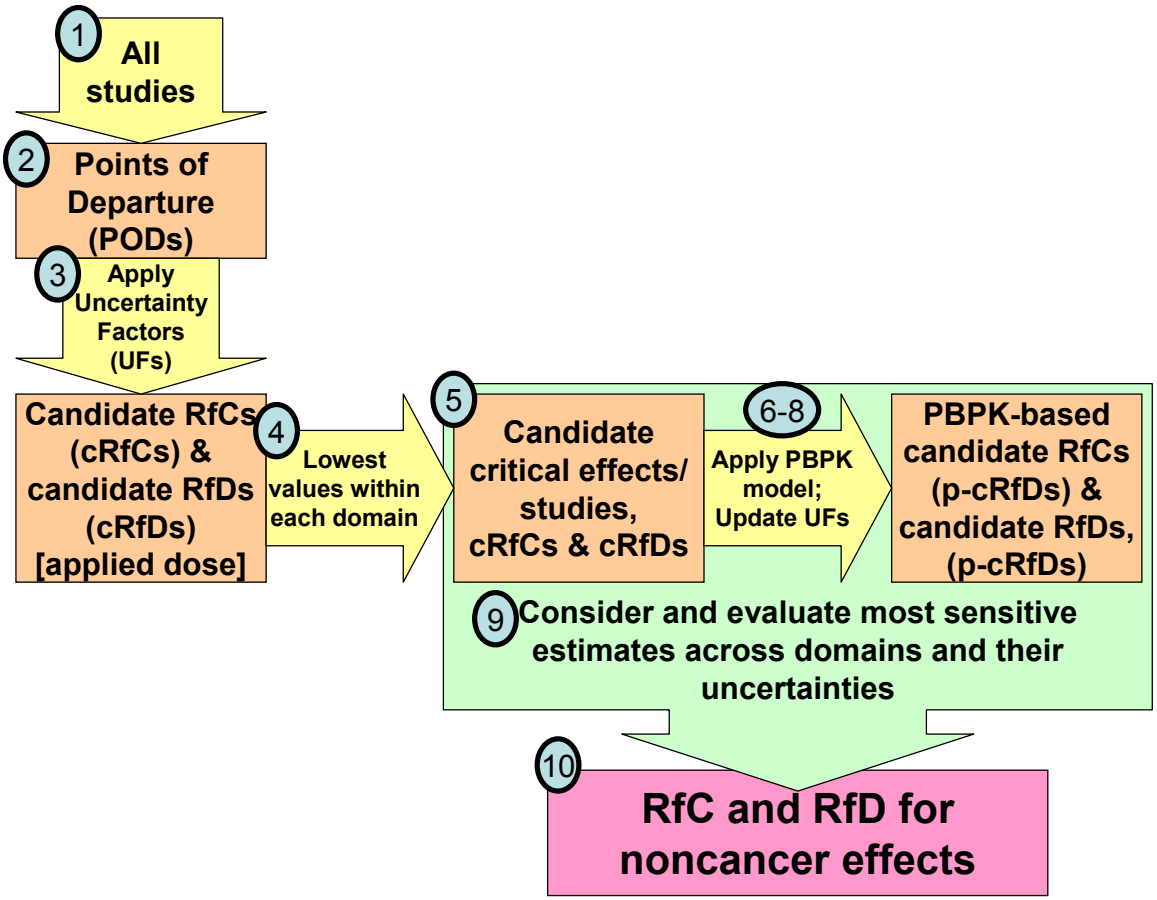

Figure 5-1. Flow-chart of the process used to derive the RfD and RfC for noncancer effects.

In contrast to the approach used in most previous assessments, in which the RfC and RfD are each based on a single critical effect, the final RfC and RfD for TCE were based on multiple critical effects that resulted in very similar candidate RfC and RfD values at the low end of the full range of values. This approach was taken here as it was considered to provide more robust estimates of the RfC and RfD and because it highlights the multiple effects that are yielding very similar candidate values. This approach is also consistent with recommendations from *A Review of the Reference Dose and Reference Concentration Process* (U.S. EPA, 2002b), which proposes that reference values be based on consideration of all relevant and appropriate endpoints carried through to the derivation of sample (or —andidate") reference values. The results of this process are summarized in the sections below, with technical details presented in Appendix F.

5.1.1. Modeling Approaches and UFs for Developing Candidate Reference Values Based on Applied Dose

This section summarizes the general methodology used with all of the TCE studies and endpoints for developing cRfCs and cRfDs on the basis of applied dose. A detailed discussion of

the application of these approaches to the studies and endpoints for each health effect domain follows in the next section (see Section 5.1.2).

Standard adjustments[20] were made to the applied doses to obtain continuous inhalation exposures and daily average oral doses over the study exposure period (see Appendix F for details), except for effects for which there was sufficient evidence that the effect was more closely associated with administered exposure level (e.g., changes in visual function). The PODs based on applied dose in the following sections and in Appendix F are presented in terms of the adjusted doses (except where noted).

As described above, wherever possible,[21] BMD modeling was conducted to obtain benchmark dose lower bounds (BMDLs) to serve as PODs for the cRfCs and cRfDs. Note that not all quantitative dose-response data are amenable to BMD modeling. For example, while nonnumerical data (e.g., data presented in line or bar graphs rather than in tabular form) were considered for developing LOAELs or NOAELs, they were not used for BMD modeling. In addition, sometimes, the available models used do not provide an adequate fit to the data. For the BMD modeling for this assessment, the EPA's BenchMark Dose Software (BMDS), which is freely available at www.epa.gov/ncea/bmds, was used. For dichotomous responses, the log-logistic, multistage, and Weibull models were fitted. This subset of BMDS dichotomous models was used to reduce modeling demands, and these particular models were selected because, as a group, they have been found to be capable of describing the great majority of dose-response data sets, and specifically for some TCE data sets (Filipsson and Victorin, 2003). For continuous responses, the distinct models available in BMDS—the power, polynomial, and Hill models—were fitted. For some reproductive and developmental data sets, two nested models (the nested logistic and the Rai and Van Ryzin models in BMDS[22]) were fitted to examine and account for potential intralitter correlations. Models with unconstrained power parameters <1 were considered when the dose-response relationship appeared supralinear, but these models often yield very low BMDL estimates and there was no situation in which an unconstrained model with a power parameter <1 was selected for the data sets modeled here. In most cases, a constrained model or the Hill model provided an adequate fit to such a dose-response relationship. In a few cases, the highest dose group was dropped to obtain an improved fit to the lower dose groups. See Appendix F for further details on model fitting and parameter constraints.

[20]Discontinuous exposures (e.g., gavage exposures once a day, 5 days/week, or inhalation exposures for 5 days/week, 6 hours/day) were adjusted to the continuous exposure yielding the same cumulative exposure. For inhalation studies, these adjustments are equivalent to those recommended by U.S. EPA (1994a) for deriving a human equivalent concentration for a Category 3 gas for which the blood:air partition coefficient in laboratory animals is greater than that in humans (The posterior population median estimate for the TCE blood:air partition coefficient was 14 in the mouse [Table 3-37], 19 in the rat [Table 3-38], and 9.2 in the human [Table 3-39]).

[21]An exception was for the systemic effect of decreased body weight, which was observed in multiple chronic studies. Dose-response data were available, but the resources were not invested into modeling these data because the endpoint appeared a priori to be less sensitive than others and was not expected to be a critical effect.

[22]The BMDS v1.4 module for the National Center for Toxicological Research model failed with the TCE data sets.

After fitting these models to the data sets, the following procedure for model selection was applied. First, models were rejected if the *p*-value for goodness of fit was <0.10.[23] Second, models were rejected if they did not appear to adequately fit the low-dose region of the dose-response relationship, based on an examination of graphical displays of the data and scaled residuals. If the BMDL estimates from the remaining models were "sufficiently close" (with a criterion of within twofold for "sufficiently close"), then the model with the lowest Akaike's Information Criteria (AIC) was selected.[24] If the BMDL estimates from the remaining models are not sufficiently close, some model dependence is assumed. With no clear biological or statistical basis to choose among them, the lowest BMDL was chosen as a reasonable conservative estimate, unless the lowest BMDL appeared to be an outlier, in which case, further judgments were made. Additionally, for continuous models, constant variance models were used for model parsimony unless the *p*-value for the test of homogenous variance was <0.10, in which case the modeled variance models were considered.

For BMR selection, statistical and biological considerations were taken into account. For dichotomous responses, our general approach was to use 10% extra risk as the BMR for borderline or minimally adverse effects and either 5 or 1% extra risk for adverse effects, with 1% reserved for the most severe effects. For continuous responses, the preferred approach for defining the BMR is to use a preestablished cut-point for the minimal level of change in the endpoint at which the effect is generally considered to become biologically significant (e.g., there is substantial precedence for using a 10% change in weight for organ and body weights and a 5% change in weight for fetal weight). In the absence of a well-established cut-point, a BMR of 1 (control) SD change from the control mean, or 0.5 SD for effects considered to be more serious, was generally selected. For one neurological effect (traverse time), a doubling (i.e., twofold change) was selected because the control SD appeared unusually small.

After the PODs were determined for each study/endpoint, UFs were applied to obtain the cRfCs and cRfDs. UFs are used to address differences between study conditions and conditions of human environmental exposure (U.S. EPA, 2002b). These include:

(a) *Extrapolating from laboratory animals to humans:* If a POD is derived from experimental animal data, it is divided by an UF to reflect pharmacokinetic and pharmacodynamic differences that may make humans more sensitive than laboratory animals. For oral exposures, the standard value for the interspecies UF is 10, which breaks down (approximately) to a factor of 3 for pharmacokinetic differences (which is removed if the PBPK model is used) and a factor of 3 for pharmacodynamic

[23]In a few cases in which none of the models fit the data with $p > 0.10$, linear models were selected on the basis of an adequate visual fit overall.

[24]Akaike's Information Criteria—a measure of information loss from a dose-response model that can be used to compare a set of models. Among a specified set of models, the model with the lowest AIC is considered the "best." If two or more models share the lowest AIC, an average of the BMDLs could be used, but averaging was not used in this assessment because for the one occasion in which models shared the lowest AIC, a selection was made based on visual fit.

differences. For inhalation exposures, ppm equivalence across species is generally assumed or other cross-species scaling is performed, in accordance with U.S. EPA (1994a) inhalation dosimetry guidance, in which case, residual pharmacokinetic differences are considered to be negligible, and the standard value used for the interspecies UF is 3, which is ascribed to pharmacodynamic differences. These standard values were used for all of the cRfCs and cRfDs based on laboratory animal data in this assessment.

(b) *Human (intraspecies) variability:* RfCs and RfDs apply to the human population, including sensitive subgroups, but studies rarely examine sensitive humans. Sensitive humans could be adversely affected at lower exposures than a general study population; consequently, PODs from general-population studies are divided by an UF to address sensitive humans. Similarly, the animals used in most laboratory animal studies are considered to be "typical" or "average" responders, and the human (intraspecies) variability UF is also applied to PODs from such studies to address sensitive subgroups. The standard value for the human variability UF is 10, which breaks down (approximately) to a factor of 3 for pharmacokinetic variability (which is removed if the PBPK model is used) and a factor of 3 for pharmacodynamic variability. This standard value was used for all of the PODs in this assessment with the exception of the PODs for a few immunological effects that were based on data from a sensitive (autoimmune-prone) mouse strain; for those PODs, an UF of 3 was used for human variability.

(c) *Uncertainty in extrapolating from subchronic to chronic exposures:*[25] RfCs and RfDs apply to lifetime exposure, but sometimes the best (or only) available data come from less-than-lifetime studies. Lifetime exposure can induce effects that may not be apparent or as large in magnitude in a shorter study; consequently, a dose that elicits a specific level of response from a lifetime exposure may be less than the dose eliciting the same level of response from a shorter exposure period. Thus, PODs based on subchronic exposure data are generally divided by a subchronic-to-chronic UF, which has a standard value of 10. If there is evidence suggesting that exposure for longer time periods does not increase the magnitude of an effect, a lower value of 3 or one might be used. For some reproductive and developmental effects, chronic exposure is that which covers a specific window of exposure that is relevant for eliciting the effect, and subchronic exposure would correspond to an exposure that is notably less than the full window of exposure.

(d) *Uncertainty in extrapolating from LOAELs to NOAELs:* PODs are intended to be estimates of exposure levels without appreciable risk under the study conditions so that, after the application of appropriate UFs for interspecies extrapolation, human variability, and/or duration extrapolation, the absence of appreciable risk is conveyed to the RfC or RfD exposure level to address sensitive humans with lifetime exposure. Under the NOAEL/LOAEL approach to determining a POD, however, adverse effects are sometimes observed at all study doses. If the POD is a LOAEL, then it is divided by an UF to better estimate a NOAEL. The standard value for the LOAEL-to-NOAEL UF is 10, although a value of 3 is sometimes used if the effect is considered minimally adverse at the response level observed at the LOAEL or is an

[25]Rodent studies exceeding 90 days of exposure are considered chronic, and rodent studies with 4 weeks to 90 days of exposure are considered subchronic (see http://www.epa.gov/iris/help_gloss.htm).

early marker for an adverse effect. For one POD in this assessment, a value of 30 was used for the LOAEL-to-NOAEL UF because the incidence rate for the adverse effect was ≥90% at the LOAEL.

(e) *Additional database uncertainties*: A database UF of 1, 3, or 10 is used to reflect the potential for deriving an underprotective toxicity value as a result of an incomplete characterization of the chemical's toxicity. No database UF was used in this assessment. See Section 5.1.4.1 for additional discussion of the uncertainties associated with the overall database for TCE.

(Note that UF values of "3" actually represent √10, and, when 2 such values are multiplied together, the result is 10 rather than 9.)

5.1.2. Candidate Critical Effects by Effect Domain

A large number of endpoints and studies were considered within each of the five health effect domains. A comprehensive list of all endpoints/studies that were considered for developing cRfCs and cRfDs is shown in Tables 5-1–5-5. These tables also summarize the PODs for the various study endpoints, the UFs applied, and the resulting cRfCs or cRfDs. Inhalation and oral studies are presented together so that the extent of the available data, as well as concordance, or lack thereof, in the responses across routes of exposure, is evident. In addition, the PBPK model developed in Section 3.5 will be applied to each candidate critical effect to develop an idPOD; and subsequent extrapolation of the idPOD to pharmacokinetically sensitive humans is performed for both inhalation and oral human exposures, regardless of the route of exposure in the original study.

The sections below discuss the cRfCs and cRfDs developed from the effects and studies identified in the hazard characterization (see Chapter 4) that were suitable for the derivation of reference values (i.e., that provided quantitative dose-response data). Because the general approach for applying UFs was discussed above, the sections below only discuss the selection of particular UFs when there are study characteristics that require additional judgment as to the appropriate UF values and possible deviations from the standard values usually assigned.

5.1.2.1. Candidate Critical Neurological Effects on the Basis of Applied Dose

As summarized in Section 4.11.1.1, both human and experimental animal studies have associated TCE exposure with effects on several neurological domains. The strongest neurological evidence of hazard is for changes in trigeminal nerve function or morphology and impairment of vestibular function. There is also evidence for effects on motor function; changes in auditory, visual, and cognitive function or performance; structural or functional changes in the brain; and neurochemical and molecular changes. Studies with numerical dose-response information are summarized in Table 5-1, with their corresponding cRfCs or cRfDs shown in Table 5-2. Because impairment of vestibular function occurs at higher exposures, such changes were not considered candidate critical effects; however, the other neurological effect domains are

represented. For trigeminal nerve effects, cRfC estimates based on two human studies are in a similar range of 0.4–0.5 ppm (Mhiri et al., 2004; Ruijten et al., 1991). There remains some uncertainty as to the exposure characterization, as shown by the use of an alternative POD for Mhiri et al. (2004) based on urinary TCA resulting in a fivefold smaller cRfC. However, the overall confidence in these estimates is increased by the fact that they are based on humans exposed under chronic or nearly chronic conditions. Other human studies (e.g., Barret et al., 1984), while indicative of hazard, did not have adequate exposure information for quantitative estimates of an inhalation POD. A cRfD of 0.2 mg/kg/day was developed from the only oral study demonstrating trigeminal nerve changes, a subchronic study in rats (Barret et al., 1992). This estimate required multiple extrapolations with a composite UF of 10,000.[26]

For auditory effects, a high confidence cRfC of about 0.7 ppm was developed based on BMD modeling of data from Crofton and Zhao (1997); and cRfCs developed from two other auditory studies (Albee et al., 2006; Rebert et al., 1991) were within about fourfold. No oral data were available for auditory effects. For psychomotor effects, the available human studies (e.g., Rasmussen et al., 1993a; Rasmussen et al., 1993b; Rasmussen et al., 1993d) did not have adequate exposure information for quantitative estimates of an inhalation POD. However, a relatively high confidence cRfC of 0.5 ppm was developed from a study in rats (Waseem et al., 2001). Two cRfDs within a narrow range of 0.7–1.7 mg/kg/day were developed based on two oral studies reporting psychomotor effects (Nunes et al., 2001; Moser et al., 1995), although varying in degree of confidence.

[26]U.S. EPA's report on the RfC and RfD processes (U.S. EPA, 2002b) recommends not deriving reference values with a composite UF of >3,000; however, composite UFs exceeding 3,000 are considered here because the derivation of the cRfCs and cRfDs is part of a screening process and the subsequent application of the PBPK model for candidate critical effects will reduce the values of some of the individual UFs.

Table 5-1. Summary of studies of neurological effects suitable for dose-response assessment

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Trigeminal nerve effects				**Section 4.3.1**
Mhiri et al. (2004)	Human phosphate industry workers (23 exposed, 23 controls)	Inhalation: Exposure ranged from 50 to 150 ppm, for 6 hrs/d for at least 2 yrs	Increased TSEP latency.	Table 4-20
Ruijten et al. (1991)	Human mail printing workers (31 exposed, 28 controls)	Inhalation: Mean cumulative exposure: 704 ppm × yrs; mean exposure duration: 16 yrs	Increased latency in masseter reflex.	Table 4-20
Barret et al. (1992)	Rat, Sprague-Dawley, female, 7/group	Oral: 0 and 2,500 mg/kg; 1 dose/d, 5 d/wk, 10 wks	Increased internode length and fiber diameter in class A fibers of the trigeminal nerve observed with TCE treatment; changes in fatty acid composition.	Table 4-21
Auditory effects				**Section 4.3.2**
Rebert et al. (1991)	Rat, Long-Evans, male, 10/group	Inhalation: 0, 1,600, and 3,200 ppm; 12 hrs/d, 12 wks	Significant decreases in BAER amplitude and an increase in latency of appearance of the initial peak (P1).	Table 4-23
Albee et al. (2006)	Rat, F344, male and female, 10/sex/group	Inhalation: 0, 250, 800, and 2,500 ppm; 6 hrs/d, 5 d/wk, 13 wks	Mild frequency specific hearing deficits; focal loss of cochlear hair cells.	Table 4-23
Crofton and Zhao (1997)	Rat, Long-Evans, male, 8–10/group	Inhalation: 0, 800, 1,600, 2,400, and 3,200 ppm; 6 hrs/d, 5 d/wk, 13 wks	Increased auditory thresholds as measured by BAERs for the 16 kHz tone.	Table 4-23

Table. 5-1 Summary of studies of neurological effects suitable for dose-response assessment (continued)

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Psychomotor effects				**Section 4.3.6**
Waseem et al. (2001)	Rat, Wistar, male, 8/group	Inhalation: 0 and 376 ppm for up to 180 d; 4 hrs/d, 5 d/wk	Changes in locomotor activity.	Table 4-31
Nunes et al. (2001)	Rat, Sprague-Dawley, male, 10/group	Oral: 0 and 2,000 mg/kg/d; 7 d	Increased foot splay.	Table 4-30
Moser et al. (1995)	Rat, F344, female, 8/dose	Oral: 0, 150, 500, 1,500, and 5,000 mg/kg, 1 dose	Neuro-muscular impairment.	Table 4-30
		0, 50, 150, 500, and 1,500 mg/kg/d, 14 d	Increased rearing activity.	Table 4-30
Visual function effects				**Section 4.3.4**
Blain et al. (1994)	Rabbit, New Zealand albino, male, 6–8/group	Inhalation: 0, 350, 700 ppm; 4 hrs/d, 4 d/wk, 12 wks	Weekly ERGs and OPs.	Table 4-26
Cognitive effects				**Sections 4.3.5 and 4.3.6**
Kulig et al. (1987)	Rat, Wistar, male, 8/dose	Inhalation: 0, 500, 1,000, and 1,500 ppm; 16 hrs/d, 5 d/wk, 18 wks	Increased time in two-choice visual discrimination test.	Table 4-31
Isaacson et al. (1990)	Rat, Sprague-Dawley, male weanlings, 12/dose	Oral: (1) 0 mg/kg/d, 8 wks (2) 47 mg/kg/d, 4 wks + 0 mg/kg/d, 4 wks (3) 47 mg/kg/d, 4 wks + 0 mg/kg/d, 2 wks + 24 mg/kg/d, 2 wks	Demyelination of hippocampus	Table 4-28
Mood and sleep disorders				**Section 4.3.7**
Albee et al. (2006)	Rat, F344, male and female, 10/sex/group	Inhalation: 0, 250, 800, and 2,500 ppm; 6 hrs/d, 5 d/wk, 13 wks	Increased handling reactivity.	Table 4-33

Table. 5-1 Summary of studies of neurological effects suitable for dose-response assessment (continued)

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Arito et al. (1994)	Rat, Wistar, male, 5/group	Inhalation: 0, 50, 100, and 300 ppm; 8 hrs/d, 5 d/wk, for 6 wks	Significant decreases in wakefulness.	Table 4-33
Other neurological effects				**Section 4.3.9**
Kjellstrand et al. (1987)	Rat, Sprague-Dawley, female	0 and 300 ppm, 24 hrs/d, 24 d	Sciatic nerve regeneration was inhibited.	Table 4-36
	Mouse, NMRI, male	0, 150, or 300 ppm, 24 hrs/d, 24 d	Sciatic nerve regeneration was inhibited.	Table 4-36
Gash et al. (2008)	Rat, F344, male, 9/group	Oral: 0 and 1,000 mg/kg; 5 d/wk, 6 wks	Degeneration of dopamine-containing neurons in substantia nigra.	Table 4-35

Table 5-2. Neurological effects in studies suitable for dose-response assessment, and corresponding cRfCs and cRfDs

Effect type Supporting studies[a]	Species	POD type	POD[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC (ppm)	cRfD (mg/kg/d)	Effect; comments
Trigeminal nerve effects												
Mhiri et al. (2004)	Human	LOAEL	40	1	1	10	10	1	100	0.40		Abnormal TSEPs; preferred POD based on middle of reported range of 50–150 ppm.
	Human	LOAEL	6	1	1	10	10	1	100	0.06		Alternate POD based on U-TCA and Ikeda et al. (1972).
Ruijten et al. (1991)	Human	LOAEL	14	1	1	10	3	1	30	0.47		Trigeminal nerve effects; POD based on mean cumulative exposure and mean duration, UF_L = 3 due to early marker effect and minimal degree of change.
Barret et al. (1992)	Rat	LOAEL	1,800	10	10	10	10	1	10,000[d]		0.18	Morphological changes; uncertain adversity; some effects consistent with demyelination.
Auditory effects												
Rebert et al. (1991)	Rat	NOAEL	800	10	3	10	1	1	300	2.7		
Albee et al. (2006)	Rat	NOAEL	140	10	3	10	1	1	300	0.47		
Crofton and Zhao (1997)	Rat	BMDL	274	10	3	10	1	1	300	0.91		Preferred, due to better dose-response data, amenable to BMD modeling. BMR = 10 dB absolute change.
Psychomotor effects												
Waseem et al. (2001)	Rat	LOAEL	45	1	3	10	3	1		0.45		Changes in locomotor activity; transient, minimal degree of adversity; no effect reported in same study for oral exposures (210 mg/kg/d).
Nunes et al. (2001)	Rat	LOAEL	2,000	10	10	10	3	1	3,000		0.67	↑ Foot splaying; minimal adversity.

Table 5-2. Neurological effects in studies suitable for dose-response assessment, and corresponding cRfCs and cRfDs (continued)

Effect type Supporting studies[a]	Species	POD type	POD[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC (ppm)	cRfD (mg/kg/d)	Effect; comments
Psychomotor effects (continued)												
Moser et al. (1995)	Rat	BMDL	248	3	10	10	1	1	300		0.83	↑ # rears (standing on hindlimbs); BMR = 1 SD change.
	Rat	NOAEL	500	3	10	10	1	1	300		1.7	↑ Severity score for neuromuscular changes.
Visual function effects												
Blain et al. (1994)	Rabbit	LOAEL	350	10	3	10	10	1	3,000	0.12		POD not adjusted to continuous exposure because visual effects more closely associated with administered exposure.
Cognitive effects												
Kulig et al. (1987)	Rat	NOAEL	500	1	3	10	1	1	30	17		↑ time in 2-choice visual discrimination test; test involves multiple systems but largely visual so not adjusted to continuous exposure.
Isaacson et al. (1990)	Rat	LOAEL	47	10	10	10	10	1	10,000[d]		0.0047	Demyelination in hippocampus.
Mood and sleep disorders												
Albee et al. (2006)	Rat	NOAEL	140	10	3	10	1	1	300	0.47		Hyperactivity.
Arito et al. (1994)	Rat	LOAEL	12	3	3	10	10	1	1,000	0.012		Changes in wakefulness.
Other neurological effects												
Kjellstrand et al. (1987)	Rat	LOAEL	300	10	3	10	10	1	3,000	0.10		↓ regeneration of sciatic nerve.
	Mouse	LOAEL	150	10	3	10	10	1	3,000	0.050		↓ regeneration of sciatic nerve.
Gash et al. (2008)	Rat	LOAEL	710	10	10	10	10	1	10,000[d]		0.071	Degeneration of dopaminergic neurons.

[a]Shaded studies/endpoints were selected as candidate critical effects/studies.
[b]Adjusted to continuous exposure unless otherwise noted. For inhalation studies, adjustments yield a POD that is a HEC as recommended for a Category 3 gas in U.S. EPA (1994a) in the absence of PBPK modeling. Same units as cRfC (ppm) or cRfD (mg/kg/day).
[c]Product of individual UFs.
[d]EPA's report on the RfC and RfD processes (U.S. EPA, 2002b) recommends not deriving reference values with a composite UF of >3,000; however, composite UFs exceeding 3,000 are considered here because the derivation of the cRfCs and cRfDs is part of a screening process and the subsequent application of the PBPK model for candidate critical effects will reduce the values of some of the individual UFs.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

For the other neurological effects, the estimated cRfCs and cRfDs were more uncertain, as there were fewer studies available for any particular endpoint, and the PODs from several studies required more adjustment to arrive at a cRfC or cRfD. However, the endpoints in these studies also tended to be indicative of more sensitive effects and, therefore, they need to be considered. The lower cRfCs fall in the range 0.01–0.1 ppm and were based on effects on visual function in rabbits (Blain et al., 1994), wakefulness in rats (Arito et al., 1994), and regeneration of the sciatic nerve in mice and rats (Kjellstrand et al., 1987). Of these, altered wakefulness (Arito et al., 1994) has both the lowest POD and the lowest cRfC. There is relatively high confidence in this study, as it shows a clear dose-response trend, with effects persisting postexposure. For the subchronic-to-chronic UF, a value of 3 was used because, even though it was just a 6-week study, there was no evidence of a greater impact on wakefulness following 6 weeks of exposure than there was following 2 weeks of exposure at the LOAEL, although there was an effect of repeated exposure on the postexposure period impacts of higher exposure levels. The cRfDs, in the range 0.005−0.07, were based on demyelination in the hippocampus (Isaacson et al., 1990) and degeneration of dopaminergic neurons (Gash et al., 2008), both in rats. In both of these cases, adjusting for study design characteristics led to a composite uncertainty factor of 10,000,[27] so the confidence in these cRfDs is lower. However, no other studies of these effects are available.

In summary, although there is high confidence both in the hazard and in the cRfCs and cRfDs for trigeminal nerve, auditory, or psychomotor effects, the available data suggest that the more sensitive indicators of TCE neurotoxicity are changes in wakefulness, regeneration of the sciatic nerve, demyelination in the hippocampus, and degeneration of dopaminergic neurons. Therefore, these more sensitive effects are considered the candidate critical effects for neurotoxicity, albeit with more uncertainty in the corresponding cRfCs and cRfDs. Of these more sensitive effects, for the reasons discussed above, there is greater confidence in the changes in wakefulness reported by Arito et al. (1994). In addition, trigeminal nerve effects are considered a candidate critical effect because this is the only type of neurological effect for which human data are available, and the POD for this effect is similar to that from the most sensitive rodent study (Arito et al., 1994, for changes in wakefulness). Between the two human studies of trigeminal nerve effects, Ruijten et al. (1991) is preferred for deriving noncancer reference values because its exposure characterization is considered more reliable.

5.1.2.2. Candidate Critical Kidney Effects on the Basis of Applied Dose

As summarized in Section 4.11.1.2, multiple lines of evidence support TCE nephrotoxicity in the form of tubular toxicity, mediated predominantly through the GSH

[27]U.S. EPA's report on the RfC and RfD processes (U.S. EPA, 2002b) recommends not deriving reference values with a composite UF of >3,000; however, composite UFs exceeding 3,000 are considered here because the derivation of the cRfCs and cRfDs is part of a screening process and the subsequent application of the PBPK model for candidate critical effects will reduce the values of some of the individual UFs.

conjugation product DCVC. Available human studies, while providing evidence of hazard, did not have adequate exposure information for quantitative estimates of PODs. Several studies in rodents, some of chronic duration, have shown histological changes, nephropathy, or increased kidney/body weight ratios. Studies with numerical dose-response information are summarized in Table 5-3, with their corresponding cRfCs or cRfDs shown in Table 5-4.

The cRfCs developed from three suitable inhalation studies, one reporting meganucleocytosis in rats (Maltoni et al., 1986), and two others reporting increased kidney weights in mice (Kjellstrand et al., 1983a) and rats (Woolhiser et al., 2006),[28] are in a narrow range of 0.5–1.3 ppm. All three utilized BMD modeling and, thus, take into account statistical limitations of the Woolhiser et al. (2006) and Kjellstrand et al. (1983a) studies, such as variability in responses or the use of low numbers of animals in the experiment. The response used for kidney weight increases was the organ weight as a percentage of body weight, to account for any commensurate decreases in body weight, although the results did not generally differ much when absolute weights were used instead. Although the two studies reporting kidney weight changes were subchronic, longer-term experiments by Kjellstrand et al. (1983a) did not report increased severity, so no subchronic-to-chronic UF was used in the derivation of the cRfC. The high response level of 73% at the lowest dose for meganucleocytosis in the chronic study of Maltoni et al. (1986) implies more uncertainty in the low-dose extrapolation. However, it is the only inhalation study that includes histopathological analysis, and it uses relatively high numbers of animals per dose group.

[28]Woolhiser et al. (2006) is an Organisation for Economic Co-operation and Development guideline immunotoxicity study performed by the Dow Chemical Company, certified by Dow as conforming to Good Laboratory Practices as published by the U.S. EPA for the Toxic Substances Control Act.

Table 5-3. Summary of studies of kidney, liver, and body weight effects suitable for dose-response assessment

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Histological changes in kidney				**Section 4.4.4**
Maltoni et al. (1986)	Rat, Sprague-Dawley, M, 116–124/group	Inhalation: 0, 100, 300, and 600 ppm, 7 hrs/d, 5 d/wk, 104 wks exposure, observed for lifespan	Meganucleocytosis	Table 4-49, Table 4-43
NTP (1990)	Rat, F344/*N*, male and female, 48–50/group	Oral: 0, 500, and 1,000 mg/kg/d, 5 d/wk, 103 wks	Cytomegaly and karyomegaly	Table 4-45, Table 4-44
NCI (1976)	Mouse, $B6C3F_1$, female, 20–50/group	Oral: 0, 869, and 1,739 mg/kg/d, 5 d/wk, TWA during exposure period (78 wks), observed for 90 wks	Toxic nephrosis	Table 4-46, Table 4-44
NTP (1988)	Rat, Marshall, F, 44–50/group	Oral: 0, 500, and 1,000 mg/kg/d, 5 d/wk, 104 wks	Toxic nephropathy	Table 4-47, Table 4-44
↑ kidney/body weight ratio				**Section 4.4.4**
Kjellstrand et al. (1983a)	Mouse, NMRI, M, 10–20/group	Inhalation: 0 (air), 37, 75, 150, 225, 300, 450, 900, 1,800, and 3,600 ppm; continuous and intermittent exposures for 30–120 d	Increased kidney/body weight ratio	Table 4-43
Woolhiser et al. (2006)	Rat, Sprague-Dawley, F, 16/group	Inhalation: 0, 100, 300, and 1,000 ppm, 6 hr/d, 5 d/wk, for 4 wks	Increased kidney/body weight ratio	Table 4-43

Table 5-3. Summary of studies of kidney, liver, and body weight effects suitable for dose-response assessment (continued)

Effect type Study reference	Species, strain (if applicable), sex, number used for dose-response assessment	Exposure(s) used for dose-response assessment	Endpoint(s) used for dose-response assessment	Chapter 4 Section/Table
↑ liver/body weight ratio				**Section 4.5.4.1**
Kjellstrand et al. (1983a)	Mouse, NMRI, M, 10–20/group	Inhalation: 0 (air), 37, 75, 150, 225, 300, 450, 900, 1,800, and 3,600 ppm; continuous and intermittent exposures for 30–120 d	Increased liver/body weight ratio	Table 4-59
Woolhiser et al. (2006)	Rat, Sprague-Dawley, F, 16/group	Inhalation: 0, 100, 300, and 1,000 ppm, 6 hr/d, 5 d/wk, for 4 wks	Increased liver/body weight ratio	Table 4-59
Buben and O'Flaherty (1985)	Mouse, Swiss-Cox, 12–15/group	Oral: 0, 100, 200, 400, 800, 1,600, 2,400, and 3,200 mg/kg/d, 5 d/wk for 6 wks	Increased liver/body weight ratio	Table 4-58
Decreased body weight				
NTP (1990)	Mouse, $B6C3F_1$, M, 48–50/group	Oral: 0 and 1,000 mg/kg/d, 5 d/wk, 103 wks	Decreased body weight.	NA
NCI (1976)	Rat, Osborne-Mendel, M and F, 20–50/group	Oral: 0, 549, and 1,097 mg/kg/d, 5 d/wk, TWA during exposure period (78 wks), observed at 110 wks	Decreased body weight.	NA

Table 5-4. Kidney, liver, and body weight effects in studies suitable for dose-response assessment, and corresponding cRfCs and cRfDs

Effect type Supporting studies[a]	Species	POD type	POD[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC (ppm)	cRfD (mg/kg/d)	Effect; comments
Histological changes in kidney												
Maltoni (1986)	Rat	BMDL	40.2	1	3	10	1	1	30	1.3		meganucleocytosis; BMR = 10% extra risk
Maltoni (1986)	Rat	BMDL	34	1	10	10	1	1	100		0.34	meganucleocytosis; BMR = 10% extra risk
NTP (1990)	Rat	LOAEL	360	1	10	10	10	1	1,000		0.36	cytomegaly and karyomegaly; considered minimally adverse, but UF_L = 10 due to high response rate (≥98%) at LOAEL; also in mice, but use NCI (1976) for that species
NCI (1976)	Mouse	LOAEL	620	1	10	10	30	1	3,000		0.21	toxic nephrosis; UF_L = 30 due to >90% response at LOAEL for severe effect
NTP (1988)	Rat	BMDL	9.45	1	10	10	1	1	100		0.0945	toxic nephropathy; female Marshall (most sensitive sex/strain); BMR = 5% extra risk
↑ kidney/body weight ratio												
Kjellstrand et al. (1983a)	Mouse	BMDL	34.7	1	3	10	1	1	30	1.2		BMR = 10% increase; 30 d, but 120 d @ 120 ppm not more severe so UF_S = 1; results are for males, which were slightly more sensitive, and yielded better fit to variance model
Woolhiser et al. (2006)	Rat	BMDL	15.7	1	3	10	1	1	30	0.52		BMR = 10% increase; UF_S = 1 based on Kjellstrand et al. (1983a) result
↑ liver/body weight ratio												
Kjellstrand et al. (1983a)	Mouse	BMDL	21.6	1	3	10	1	1	30	0.72		BMR = 10% increase; UF_S = 1 based on not more severe at 4 months
Woolhiser et al. (2006)	Rat	BMDL	25.2	1	3	10	1	1	30	0.84		BMR = 10% increase; UF_S = 1 based on Kjellstrand et al. (1983a) result
Buben and O'Flaherty (1985)	Mouse	BMDL	81.5	1	10	10	1	1	100		0.82	BMR = 10% increase; UF_S = 1 based on Kjellstrand et al. (1983a) result

Table 5-4. Kidney, liver, and body weight effects in studies suitable for dose-response assessment, and corresponding cRfCs and cRfDs (continued)

Effect type Supporting studies	Species	POD type	POD[a]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[b]	cRfC (ppm)	cRfD (mg/kg/d)	Effect; comments
Histological changes in kidney												
NTP (1990)	Mouse	LOAEL	710	1	10	10	10	1	1,000		0.71	
NCI (1976)	Rat	LOAEL	360	1	10	10	10	1	1,000		0.36	Reflects several, but not all, strains/sexes.

[a]Shaded studies/endpoints were selected as candidate critical effects/studies.
[b]Adjusted to continuous exposure unless otherwise noted. For inhalation studies, adjustments yield a POD that is a HEC as recommended for a Category 3 gas in U.S. EPA (1994a) in the absence of PBPK modeling. Same units as cRfC (ppm) or cRfD (mg/kg/day).
[c]Product of individual UFs.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

The suitable oral studies give cRfDs within a narrow range of 0.09–0.4 mg/kg/day, as shown in Table 5-4, although the degree of confidence in the cRfDs varies considerably. For cRfDs based on NTP (NTP, 1990) and NCI (NCI, 1976) chronic studies in rodents, extremely high response rates of >90% precluded BMD modeling. An UF of 10 was applied for extrapolation from a LOAEL to a NOAEL in the NTP (1990) study because the effect (cytomegaly and karyomegaly), although minimally adverse, was observed at such a high incidence. An UF of 30 was applied for extrapolation from a LOAEL to a NOAEL in the NCI (1976) study because of the high incidence of a clearly adverse effect (toxic nephrosis). There is more confidence in the cRfDs based on meganucleocytosis reported in Maltoni et al. (1986) and toxic nephropathy NTP (1988), as BMD modeling was used to estimate BMDLs. Because these two oral studies measured somewhat different endpoints, but both were sensitive markers of nephrotoxic responses, they were considered to have similarly strong weight from a hazard perspective. For meganucleocytosis, a BMR of 10% extra risk was selected because the effect was considered to be minimally adverse. For toxic nephropathy, a BMR of 5% extra risk was used because toxic nephropathy is a severe toxic effect. This BMR required substantial extrapolation below the observed responses (about 60%); however, the response level seemed warranted for this type of effect and the ratio of the BMD to the BMDL was not large (1.56). Thus, from a dose-response extrapolation perspective, there is more confidence in Maltoni et al. (1986). However, the effect observed in NTP (1988) is more severe and therefore also merits consideration.

In summary, there is high confidence in the hazard and moderate confidence in the cRfCs and cRfDs for histopathological and weight changes in the kidney. These effects are considered to be candidate critical effects for several reasons. First, they appear to be the most sensitive indicators of toxicity that are available for the kidney. In addition, as discussed in Section 3.5, some pharmacokinetic data indicate substantially more production of GSH-conjugates thought to mediate TCE kidney effects in humans relative to rats and mice, although there is uncertainty in these data due to possible analytic errors. As discussed above, several studies are considered reliable for developing cRfCs and cRfDs for these endpoints. For histopathological changes, in general, the most sensitive were selected as candidate critical studies. These include the only available inhalation study (Maltoni et al., 1986), the Maltoni et al. (1986) and NTP (1988) oral studies in rats, and the NCI (1976) oral study in mice. For oral studies in rats, Maltoni et al. (1986) was considered in addition to NTP (1988), despite its having a higher cRfD, because of the much greater degree of low-dose extrapolation necessary for NTP (1988) and the excessive mortality present in that study. While the NCI (1976) study has even greater uncertainty, as discussed above, with a high response incidence at the POD that necessitates greater low-dose extrapolation, it is included to add a second species to the set of candidate critical effects. For kidney weight changes, both available studies were chosen as candidate critical studies.

5.1.2.3. Candidate Critical Liver Effects on the Basis of Applied Dose

As summarized in Section 4.11.1.3, while there is only limited epidemiologic evidence of TCE hepatotoxicity, TCE clearly leads to liver toxicity in laboratory animals, likely through its oxidative metabolites. Available human studies contribute to the overall weight of evidence of hazard, but did not have adequate exposure information for quantitative estimates of PODs. In rodent studies, TCE causes a wide array of hepatotoxic endpoints: increased liver weight, small transient increases in DNA synthesis, changes in ploidy, cytomegaly, increased nuclear size, and proliferation of peroxisomes. Increased liver weight (hepatomegaly, or specifically increased liver/body weight ratio) has been the most studied endpoint across a range of studies in both sexes of rats and mice, with a variety of exposure routes and durations. Hepatomegaly was selected as the critical liver effect for multiple reasons. First, it has been consistently reported in multiple studies in rats and mice following both inhalation and oral routes of exposure. In addition, it appears to accompany the other hepatic effects at the doses tested, and hence constitutes a hepatotoxicity marker of similar sensitivity to the other effects. Finally, in several studies, there are good dose-response data for BMD modeling.

As shown in Table 5-4, cRfCs for hepatomegaly developed from the two most suitable subchronic inhalation studies (Woolhiser et al., 2006; Kjellstrand et al., 1983a), while in different species (rats and mice, respectively), are both based on similar PODs derived from BMD modeling, have the same composite UF of 30, and result in similar cRfC estimates of about 0.8 ppm. The cRfD for hepatomegaly developed from the oral study of Buben and O'Flaherty (1985) in mice also was based on a POD derived from BMD modeling and resulted in a cRfD estimate of 0.8 mg/kg/day. Among the studies reporting liver weight changes (reviewed in Section 4.5 and Appendix E), this study had by far the most extensive dose-response data. The response used in each case was the liver weight as a percentage of body weight, to account for any commensurate decreases in body weight, although the results did not generally differ much when absolute weights were used instead.

There is high confidence in all of these candidate reference values. BMD modeling takes into account statistical limitations such as variability in response or low numbers of animals and standardizes the response rate at the POD. Although the studies were subchronic, hepatomegaly occurs rapidly with TCE exposure, and the degree of hepatomegaly does not increase with chronic exposure (Kjellstrand et al., 1983a), so no subchronic-to-chronic UF was used.

In summary, there is high confidence both in the hazard and the cRfCs and cRfDs for hepatomegaly. Hepatomegaly also appears to be the most sensitive indicator of toxicity that is available for the liver and is therefore considered a candidate critical effect. As discussed above, several studies are considered reliable for developing cRfCs and cRfDs for this endpoint, and, since they all indicated similar sensitivity but represented different species and/or routes of exposure, they were all considered candidate critical studies.

5.1.2.4. Candidate Critical Body Weight Effects on the Basis of Applied Dose

The chronic oral bioassays, NCI (1976) and NTP (1990), reported decreased body weight with TCE exposure, as shown in Table 5-4. However, the lowest doses in these studies were quite high, even on an adjusted basis (see PODs in Table 5-4). These were not considered critical effects because they are not likely to be the most sensitive noncancer endpoints, and were not considered candidate critical effects.

5.1.2.5. Candidate Critical Immunological Effects on the Basis of Applied Dose

As summarized in Section 4.11.1.4, the human and experimental animal studies of TCE and immune-related effects provide strong evidence for a role of TCE in autoimmune disease and in a specific type of generalized hypersensitivity syndrome, while there are fewer data pertaining to immunosuppressive effects. Available human studies, while providing evidence of hazard, did not have adequate exposure information for quantitative estimates of PODs. Several studies in rodents were available on autoimmune and immunosuppressive effects that were adequate for deriving cRfCs and cRfDs. Studies with numerical dose-response information are summarized in Table 5-5, with their corresponding cRfCs or cRfDs summarized in Table 5-6.

For decreased thymus weights, a cRfD from the only suitable study (Keil et al., 2009) is 0.00035 mg/kg/day based on results from nonautoimmune-prone $B6C3F_1$ mice, with a composite UF of 1,000 for a POD that is a LOAEL (the dose-response relationship is sufficiently supralinear that attempts at BMD modeling did not result in adequate fits to these data). Thymus weights were not significantly affected in autoimmune prone mice in the same study, consistent with the results reported by Kaneko et al. (2000) in autoimmune-prone mice. In addition, Keil et al. (2009) and Peden-Adams et al. (2008) reported that for several immunotoxicity endpoints associated with TCE, the autoimmune-prone strain appeared to be less sensitive than the nonautoimmune prone $B6C3F_1$ strain. In rats, Woolhiser et al. (2006) reported no significant change in thymus weights in the Sprague-Dawley strain. These data are consistent with normal mice being sensitive to this effect as compared to autoimmune-prone mice or Sprague-Dawley rats, so the results of Keil et al. (2009) are not necessarily discordant with the other studies.

Table 5-5. Summary of studies of immunological effects suitable for dose-response assessment

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
↓ thymus weight				**Section 4.6.2.3**
Keil et al. (2009)	Mouse, B6C3F$_1$, Female, 10/group	Oral: 0, 1,400, or 14,000 ppb TCE (0, 0.35, or 3.5 mg/kg/d), 27 wks	Decreased thymus weights; decrease in thymus cellularity	Table 4-78
Autoimmunity				**Section 4.6.2.3**
Kaneko et al. (2000)	5/group	Inhalation: 0, 500, 1,000, or 2,000 ppm TCE, 4 hrs/d, 6 d/wk, 8 wks	Liver inflammation, splenomegaly and hyperplasia of lymphatic follicles	Table 4-78
Keil et al. (2009)	Mouse, B6C3F$_1$, Female, 10/group	Oral: 0, 1,400, or 14,000 ppb TCE (0, 0.35, or 3.5 mg/kg/d), 27 wks	Increased anti-dsDNA and anti-ssDNA antibodies	Table 4-78
Griffin et al. (2000b)	Mouse, MRL +/+, Female, 8/group	Oral: 0, 21, 100, or 400 mg/kg/d, 32 wks	Various signs of autoimmune hepatitis (serology, ex vivo assays of cultured splenocytes, clinical and histopathologic findings)	Table 4-78
Cai et al. (2008)	Mouse, MRL +/+, Female, 5/group	Oral: 0 or 60 mg/kg/d, 48 wks	Hepatic necrosis; hepatocyte proliferation; leukocyte infiltrate in the liver, lungs, and kidneys	Table 4-78

Table 5-5. Summary of studies of immunological effects suitable for dose-response assessment (continued)

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Immunosuppression				**Section 4.6.2.1**
Woolhiser et al. (2006)	Rat, Sprague-Dawley, female, 16/group	Inhalation: 0, 100, 300, or 1,000 ppm, 6 hrs/d, 5 d/wk, 4 wks	Decreased PFC assay response	Table 4-76
Sanders et al. (1982b)	Mouse, CD-1, Female, 7–25/group	Oral: 0, 0.1, 1.0, 2.5, or 5.0 mg/mL (0, 18, 217, 393, or 660 mg/kg/d, from Tucker et al., 1982), 4 or 6 mo	Decreased humoral immunity, cell-mediated immunity, and bone marrow stem cell colonization	Table 4-76

Table 5-6. Immunological effects in studies suitable for dose-response assessment, and corresponding cRfCs and cRfDs

Effect type Supporting studies[a]	Species	POD type	POD[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC (ppm)	cRfD (mg/kg/d)	Effect; comments
↓ thymus weight												
Keil et al. (2009)	Mouse	LOAEL	0.35	1	10	10	10	1	1,000		0.00035	↓ thymus weight; corresponding decrease in total thymic cellularity reported at 10 × higher dose
Autoimmunity												
Kaneko et al., (2000)	Mouse (MRL-lpr/lpr)	LOAEL	70	10	3	3	10	1	1,000	0.070		Changes in immunoreactive organs—liver (incl. sporadic necrosis in hepatic lobules), spleen; $UF_H = 3$ due to autoimmune-prone mouse
Keil et al. (2009)	Mouse	LOAEL	0.35	1	10	10	3	1	300		0.0012	↑ anti-dsDNA and anti-ssDNA Abs (early markers for autoimmune disease) ($B6C3F_1$ mouse); $UF_L = 3$ due to early marker
Griffin et al. (2000b)	Mouse (MRL+/+)	BMDL	13.4	1	10	3	1	1	30		0.45	Various signs of autoimmune hepatitis; BMR = 10% extra risk for > minimal effects
Cai et al. (2008)	Mouse (MRL+/+)	LOAEL	60	1	10	3	10	1	300		0.20	Inflammation in liver, kidney, lungs, and pancreas indicative of autoimmune disease; hepatic necrosis; $UF_H = 3$ due to autoimmune-prone mouse
Immunosuppression												
Woolhiser et al. (2006)	Rat	BMDL	31.2	10	3	10	1	1	300	0.10		↓ PFC response; BMR = 1 SD change
Sanders et al. (1982b)	Mouse	NOAEL	190	1	10	10	1	1	100		1.9	↓ humoral response to SRBC; largely transient during exposure
Sanders et al. (1982b)	Mouse	LOAEL	18	1	10	10	10	1	1,000		0.018	↓ cell-mediated response to SRBC (largely transient during exposure) and ↓ stem cell bone marrow recolonization (sustained); females more sensitive; $UF_L = 10$ since multiple immunotoxicity effects were observed

[a]Shaded studies/endpoints were selected as candidate critical effects/studies.
[b]Adjusted to continuous exposure unless otherwise noted. For inhalation studies, adjustments yield a POD that is a HEC as recommended for a Category 3 gas in U.S. EPA (1994a) in the absence of PBPK modeling. Same units as cRfC (ppm) or cRfD (mg/kg/day).
[c]Product of individual UFs.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

For autoimmune effects, the cRfC from the only suitable inhalation study (Kaneko et al., 2000) is 0.07 ppm. This study reported changes in immunoreactive organs (i.e., liver and spleen) in autoimmune-prone mice. BMD modeling was not feasible, so a LOAEL was used as the POD. The standard value of 10 was used for the LOAEL-to-NOAEL UF because the inflammation was reported to include sporadic necrosis in the hepatic lobules at the LOAEL, so this was considered an adverse effect. A value of 3 was used for the human (intraspecies) variability UF because the effect was induced in autoimmune-prone mice, a sensitive mouse strain for such an effect. The cRfDs from the oral studies (Keil et al., 2009; Cai et al., 2008; Griffin et al., 2000b) spanned over a 100-fold range from 0.001 to 0.5 mg/kg/day. Each of the studies used different markers for autoimmune effects, which may explain the over 100-fold range of PODs (0.4–60 mg/kg/day). The most sensitive endpoint, reported by Keil et al. (2009), was increases in anti-dsDNA and anti-ssDNA antibodies in B6C3F_1mice exposed to the lowest tested dose of 0.35 mg/kg/day. These markers of autoimmune responsiveness were not accompanied by evidence of inflammation or kidney disease in a similar dose- and time-dependent manner. In accordance with the interpretation of these measures as an early, subclinical or pre-clinical marker of disease, a LOAEL-to-NOAEL UF of 3 was used, and the resulting cRfD was 0.001 mg/kg/day. The results of Keil et al. (2009) are not discordant with the higher PODs and cRfDs derived from the other oral studies that examined leukocyte infiltration and tissue damage in autoimmune-prone mice (Cai et al., 2008; Griffin et al., 2000a). Cai et al. (2008) noted that the autoimmune nephritis together with multi-organ involvement and an increased level of antinuclear antibodies observed in their study suggested the induction of autoimmune disease.

For immunosuppressive effects, the only suitable inhalation study (Woolhiser et al., 2006) gave a cRfC of 0.08 ppm. The cRfDs from the only suitable oral study (Sanders et al., 1982b) ranged from 0.06 to 2 mg/kg/day, based on different markers for immunosuppression. Woolhiser et al. (2006) reported decreased PFC response in rats. Data from Woolhiser et al. (2006) were amenable to BMD modeling, but there is notable uncertainty in the modeling. First, it is unclear what should constitute the cut-point for characterizing the change as minimally biologically significant, so a BMR of 1 control SD change was used. In addition, the dose-response relationship is supralinear, and the highest exposure group was dropped to improve the fit to the low-dose data points. Nonetheless, the uncertainty in the BMD modeling is no greater than the uncertainty inherent in the use of a LOAEL or NOAEL. The more sensitive endpoints reported by Sanders et al. (1982b), both of which were in female mice exposed to a LOAEL of 18 mg/kg/day TCE in drinking water for 4 months, were decreased cell-mediated response to SRBC and decreased stem cell bone recolonization, a sign of impaired bone marrow function. The cRfD based on these endpoints is 0.02 mg/kg/day, with a LOAEL-to-NOAEL UF of 10 for the multiple effects of decreased cell-mediated response to SRBC and decreased stem cell bone recolonization.

In summary, there is high qualitative confidence for TCE immunotoxicity and moderate confidence in the cRfCs and cRfDs that can be derived from the available studies. Decreased thymus weight reported at relatively low exposures in nonautoimmune-prone mice is a clear indicator of immunotoxicity (Keil et al., 2009), and is therefore considered a candidate critical effect. A number of studies have also reported changes in markers of immunotoxicity at relatively low exposures. Therefore, among markers for autoimmune effects, the more sensitive measures of autoimmune changes in liver and spleen (Kaneko et al., 2000) and increased anti-dsDNA and anti-ssDNA antibodies (Keil et al., 2009) are considered the candidate critical effects. Similarly, for markers of immunosuppression, the more sensitive measures of decreased PFC response (Woolhiser et al., 2006), decreased stem cell bone marrow recolonization, and decreased cell-mediated response to SRBC [both from Sanders et al. (1982b)] are considered the candidate critical effects.

5.1.2.6. Candidate Critical Respiratory Tract Effects on the Basis of Applied Dose

As summarized in Section 4.11.1.5, available data are suggestive of TCE causing respiratory tract toxicity, based primarily on short-term studies in mice and rats. However, these studies are generally at high inhalation exposures and over durations of <2 weeks. Thus, these were not considered critical effects because such data are not necessarily indicators of longer-term effects at lower exposure and are not likely to be the most sensitive noncancer endpoints for chronic exposures. Therefore, cRfCs and cRfDs were not developed for them.

5.1.2.7. Candidate Critical Reproductive Effects on the Basis of Applied Dose

As summarized in Section 4.11.1.6, both human and experimental animal studies have associated TCE exposure with adverse reproductive effects. The strongest evidence of hazard is for effects on sperm and male reproductive outcomes, with evidence from multiple human studies and several experimental animal studies. There is also substantial evidence for effects on the male reproductive tract and male serum hormone levels, as well as evidence for effects on male reproductive behavior. There are fewer data and more limited support for effects on female reproduction. Studies with numerical dose-response information are summarized in Table 5-7, with their corresponding cRfCs or cRfDs summarized in Table 5-8.

Table 5-7. Summary of studies of reproductive effects suitable for dose-response assessment

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Effects on sperm, male reproductive outcomes				**Sections 4.8.1.1–4.8.1.2**
Chia et al. (1996)	Human, 85 men (37 low exposure, 48 high exposure)	Inhalation: Mean personal air TCE: 29.6 ppm; Mean U-TCA: 22.4 mg/g creatinine	Decreased normal sperm morphology and hyperzoospermia.	Table 4-85
Land et al. (1981)	Mouse, C57BlxC3H (F1), M, 5 or 10/group	Inhalation: 0, 200, 2,000 ppm, 4 hrs/d, 5 d exposure, 23 d rest	Increased percent morphologically abnormal epididymal sperm.	Table 4-86
Kan et al. (2007)	Mouse, CD-1, male, 4/group	Inhalation: 0 or 1,000 ppm, 6 hrs/d,5 d/wk, 4 wks	Abnormalities of the head and tail in sperm located in the epididymal lumen.	Table 4-86
Xu et al. (2004)	Mouse, CD-1, male, 4–27/group	Inhalation: 0 or 1,000 ppm, 6 hrs/d, 5 d/wk, 6 wks	Decreased in vitro sperm-oocyte binding and in vivo fertilization.	Table 4-86
Kumar et al. (2000b)	Rat, Wistar, male, 12–13/group	Inhalation: 0 or 376 ppm, 4 hrs/d, 5 d/wk, 2–10 wks exposed, 2–8 wks unexposed.	Multiple sperm effects; pre- and postimplantation losses.	Table 4-86
Kumar et al. (2001b)	Rat, Wistar, male, 6/group	Inhalation: 0 or 376 ppm, 4 hrs/d, 5 d/wk, 12 and 24 wks	Multiple sperm effects, increasing severity from 12 to 24 wks exposure.	Table 4-86

Table 5-7. Summary of studies of reproductive effects suitable for dose-response assessment (continued)

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
George et al. (1985)	Mouse, CD-1, male and female, 20 pairs/treatment group; 40 controls/sex	Oral: 0, 173, 362, or 737 mg/kg/d, Breeders exposed 1 wk premating, then for 13 wks; pregnant females exposed throughout gestation (i.e., 18 wks total)	Decreased sperm motility in F0 and F1 males.	Table 4-87
DuTeaux et al. (2004a)	Rat, Sprague-Dawley, male, 3/group, or Simonson albino (UC Davis), male, 3/group	Oral: 0, 143, or 270 mg/kg/d, 14 d	Decreased ability of sperm to fertilize oocytes collected from untreated females. Oxidative damage to sperm membrane in head and mid-piece.	Table 4-87
Male reproductive tract effects				**Section 4.8.1.2**
Forkert et al. (2002)	Mouse, CD-1, male, 6/group	Inhalation: 0 or 1,000 ppm, 6 hrs/d, 5 d/wk, 19 d over 4 wks	Sloughing of epididymal epithelial cells.	Table 4-86
Kan et al. (2007)	Mouse, CD-1, male, 4/group	Inhalation: 0 or 1,000 ppm, 6 hrs/d, 5 d/wk, 1–4 wks	Degeneration and sloughing of epididymal epithelial cells (more severe by 4 wks). Vesiculation in cytoplasm, disintegration of basolateral cell membranes, sloughing of epithelial cells.	Table 4-86
Kumar et al. (2000b)	Rat, Wistar, male, 12–13/group	Inhalation: 0 or 376 ppm, 4 hrs/d, 5 d/wk, 2–10 wks exposed, 2–8 wks unexposed	Smaller, necrotic spermatogenic tubules.	Table 4-86
Kumar et al. (2001b)	Rat, Wistar, male, 6/group	Inhalation: 0 or 376 ppm, 4 hrs/d, 5 d/wk, 12 and 24 wks	Decreased testes weight, numbers of spermatogenic cells and spermatids, testes atrophy, smaller tubules devoid of spermatocytes and spermatids, hyperplastic Leydig cells, altered testicular enzyme markers. Increasing severity from 12 to 24 wks of exposure.	Table 4-86

Table 5-7. Summary of studies of reproductive effects suitable for dose-response assessment (continued)

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
George et al. (1985)	Mouse, CD-1, male and female, 20 pairs/treatment group; 40 controls/sex	Oral: 0, 173, 362, or 737 mg/kg/d, Breeders exposed 1 wk premating, then for 13 wks; pregnant females exposed throughout gestation (i.e., 18 wks total)	Decreased testes and seminal vesicle weights in F0.	Table 4-87
George et al. (1986)	Rat, F334, males and female, 20 pairs/treatment group, 40 controls/sex	Oral: 0, 72, 186, or 389 mg/kg/d (estimated), Breeders exposed 1 wk premating, then for 13 wks; pregnant females exposed throughout gestation (i.e., 18 wks total)	Increased testes and epididymis weights in F0.	Table 4-87
Female maternal weight gain				**Section 4.8.3.2**
Carney et al. (2006)	Rat, Sprague-Dawley, females, 27 dams/group	Inhalation: 0, 50, 150, or 600 ppm, 6 hrs/d; GDs 6–20	Decreased body weight gain on GDs 6–9.	Table 4-96
Schwetz et al. (1975)	Rat, Sprague-Dawley, female, 20–35/group	Inhalation: 0 or 300 ppm, 7 hrs/d; GDs 6–15	Decreased body weight gain on GDs 6–9.	Table 4-96
Narotsky et al. (1995)	Rat, F344, females, 8–12 dams/group	Oral: 0, 10.1, 32, 101, 320, 475, 633, 844, or 1,125 mg/kg/d, GDs 6–15	Decreased body weight gain on GDs 6–8 and 6–20.	Table 4-98
Manson et al. (1984)	Rat, Long-Evans, female, 23–25/group	Oral: 0, 10, 100, or 1,000 mg/kg/d, 6 wks: 2 wks premating, 1 wk mating period, GDs 1–21	Decreased gestation body weight gain.	Table 4-87

Table 5-7. Summary of studies of reproductive effects suitable for dose-response assessment (continued)

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
George et al. (1986)	Rat, F334, males and female, 20 pairs/treatment group, 40 controls/sex	Oral: 0, 72, 186, or 389 mg/kg/d (estimated), Breeders exposed 1 wk premating, then for 13 wks; pregnant females exposed throughout gestation (i.e., 18 wks total)	Decreased term and postpartum dam body weight in F0 and F1.	Table 4-87
Female reproductive outcomes				**Section 4.8.3.2**
Narotsky et al. (1995)	Rat, F344, females, 8–12 dams/group	Oral: 0, 10.1, 32, 101, 320, 475, 633, 844, or 1,125 mg/kg/d, GDs 6–15	Delayed parturition.	Table 4-98
Reproductive behavior				**Section 4.8.1.2**
Zenick et al. (1984)	Rat, Long-Evans, male, 10/group	Oral: 0, 10, 100, or 1,000 mg/kg/d, 5 d/wk, 6 wks exposure, 4 wks recovery	Impaired copulatory performance.	Table 4-87
George et al. (1986)	Rat, F334, males and female, 20 pairs/treatment group, 40 controls/sex	Oral: 0, 72, 186, or 389 mg/kg/d (estimated), Breeders exposed 1 wk premating, then for 13 wks; pregnant females exposed throughout gestation (i.e., 18 wks total)	Decreased F0 mating in cross-over mating trials.	Table 4-87

Table 5-7. Summary of studies of reproductive effects suitable for dose-response assessment (continued)

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Reproductive effects from exposure to both sexes				**Section 4.8.1.2**
George et al. (1986)	Rat, F334, males and female, 20 pairs/treatment group, 40 controls/sex	Oral: 0, 72, 186, or 389 mg/kg/d (estimated), Breeders exposed 1 wk premating, then for 13 wks; pregnant females exposed throughout gestation (i.e., 18 wks total)	Decreased F0 litters/pair and live F1 pups/litter.	Table 4-87

Table 5-8. Reproductive effects in studies suitable for dose-response assessment, and corresponding cRfCs and cRfDs

Effect type Supporting studies[a]	Species	POD type	POD[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC (ppm)	cRfD (mg/kg/d)	Effect; comments
Effects on sperm, male reproductive outcomes												
Chia et al. (1996)	Human	BMDL	1.43	10	1	10	1	1	100	0.014		Hyperzoospermia; exposure estimates based on U-TCA from Ikeda et al. (1972); BMR = 10% extra risk
Land et al. (1981)	Mouse	BMDL	46.9	10	3	10	1	1	300	0.16		↑ abnormal sperm; BMR = 0.5 SD
Kan et al. (2007)	Mouse	LOAEL	180	10	3	10	10	1	3,000	0.060		↑ abnormal sperm; Land et al. (1981) cRfC preferred due to BMD modeling
Xu et al. (2004)	Mouse	LOAEL	180	10	3	10	10	1	3,000	0.060		↓ fertilization
Kumar et al. (2001b; 2000b)	Rat	LOAEL	45	10	3	10	10	1	3,000	0.015		Multiple sperm effects, increasing severity from 12 to 24 wks
Kumar et al. (2000b)	Rat	LOAEL	45	1	3	10	10	1	300	0.15		Pre- and postimplantation losses; UF_S = 1 due to exposure covered time period for sperm development; higher response for preimplantation losses
George et al. (1985)	Mouse	NOAEL	362	1	10	10	1	1	100		3.6	↓ sperm motility
DuTeaux et al., (2004a)	Rat	LOAEL	141	10	10	10	10	1	10,000[d]		0.014	↓ ability of sperm to fertilize in vitro
Male reproductive tract effects												
Forkert et al. (2002), Kan et al. (2007)	Mouse	LOAEL	180	10	3	10	10	1	3,000	0.060		Effects on epididymis epithelium
Kumar et al. (2001b; 2000b)	Rat	LOAEL	45	10	3	10	10	1	3,000	0.015		Testes effects, altered testicular enzyme markers, increasing severity from 12 to 24 wks
George et al. (1985)	Mouse	NOAEL	362	1	10	10	1	1	100		3.6	↓ testis/seminal vesicle weights
George et al. (1986)	Rat	NOAEL	186	1	10	10	1	1	100		1.9	↑ testis/epididymis weights

Table 5-8. Reproductive effects in studies suitable for dose-response assessment, and corresponding cRfCs and cRfDs (continued)

Effect type Supporting studies[a]	Species	POD type	POD[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC (ppm)	cRfD (mg/kg/d)	Effect; comments
Female maternal weight gain												
Carney et al. (2006)	Rat	BMDL	10.5	1	3	10	1	1	30	0.35		↓ Body weight gain; BMR = 10% decrease
Schwetz et al. (1975)	Rat	LOAEL	88	1	3	10	10	1	300	0.29		↓ maternal body weight; Carney et al. (2006) cRfC preferred due to BMD modeling
Narotsky et al. (1995)	Rat	BMDL	108	1	10	10	1	1	100		1.1	↓ Body weight gain; BMR = 10% decrease
Manson et al. (1984)	Rat	NOAEL	100	1	10	10	1	1	100		1.0	↓ Body weight gain; Narotsky et al. (1995) preferred due to BMD modeling (different strain)
George et al. (1986)	Rat	NOAEL	186	1	10	10	1	1	100		1.9	↓ postpartum body weight; Narotsky et al. (1995) cRfD preferred due to BMD modeling
Female reproductive outcomes												
Narotsky et al. (1995)	Rat	LOAEL	475	1	10	10	10	1	1,000		0.48	Delayed parturition
Reproductive behavior												
Zenick et al. (1984)	Rat	NOAEL	100	1	10	10	1	1	100		1.0	↓ copulatory performance in males
George et al. (1986)	Rat	LOAEL	389	1	10	10	10	1	1,000		0.39	↓ mating (both sexes exposed)
Reproductive effects from exposure to both sexes												
George et al. (1986)	Rat	BMDL	179	1	10	10	1	1	100		1.8	↓ number of litters/pair; BMR = 0.5 SD
	Rat	BMDL	152	1	10	10	1	1	100		1.5	↓ live pups/litter; BMR = 0.5 SD

[a]Shaded studies/endpoints were selected as candidate critical effects/studies.
[b]Adjusted to continuous exposure unless otherwise noted. For inhalation studies, adjustments yield a POD that is a HEC as recommended for a Category 3 gas in U.S. EPA (1994a) in the absence of PBPK modeling. Same units as cRfC (ppm) or cRfD (mg/kg/day).
[c]Product of individual UFs.
[d]EPA's report on the RfC and RfD processes (U.S. EPA, 2002b) recommends not deriving reference values with a composite UF of >3,000; however, composite UFs exceeding 3,000 are considered here because the derivation of the cRfCs and cRfDs is part of a screening process and the subsequent application of the PBPK model for candidate critical effects will reduce the values of some of the individual UFs.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

5.1.2.7.1. Male reproductive effects (effects on sperm and reproductive tract)

A number of available studies have reported functional and structural changes in sperm and male reproductive organs and effects on male reproductive outcomes following TCE exposure (see Table 5-8). A cRfC of 0.014 ppm was derived based on hyperzoospermia reported in the available human study (Chia et al., 1996), but there is substantial uncertainty in this estimate due to multiple issues.[29] Among the rodent inhalation studies, the cRfC of 0.2 ppm based on increased abnormal sperm in the mouse reported by Land et al. (1981) is considered relatively reliable because it is based on BMD modeling rather than a LOAEL or NOAEL. However, increased sperm abnormalities do not appear to be the most sensitive effect, as Kumar et al. (2001b; 2000b) reported a similar POD to be a LOAEL for reported multiple effects on sperm and testes, as well as altered testicular enzyme markers, in the rat. Although there are greater uncertainties associated with the cRfC of 0.02 ppm for this effect and a composite UF of 3,000 was applied to the POD, the uncertainties are generally typical of those encountered in RfC derivations.

Standard values of 3, 10, and 10 were used for the interspecies UF, the human variability UF, and the LOAEL-to-NOAEL UF, respectively. In addition, although the study would have qualified as a chronic exposure study based on its duration of 24 weeks (i.e., >10% of lifetime), statistically significant decreases in testicular weight and in sperm count and motility were already observed from subchronic exposure (12 weeks) to the same TCE exposure concentration and these effects became more severe after 24 weeks of exposure. Moreover, several testicular enzyme markers associated with spermatogenesis and germ cell maturation had significantly altered activities after 12 weeks of exposure, with more severe alterations at 24 weeks, and histological changes were also observed in the testes at 12 weeks, with the testes being severely deteriorated by 24 weeks. Thus, since the single exposure level used was already a LOAEL from subchronic exposure, and the testes were even more seriously affected by longer exposures, a subchronic-to-chronic UF of 10 was applied.[30] Note that for the cRfC derived for pre- and postimplantation losses reported by Kumar et al. (2000b), the subchronic-to-chronic UF was not applied because the exposure covered the time period for sperm development. This cRfC was

[29]Mean exposure estimates for the exposure groups were limited because they were defined in terms of ranges and because they were based on mean urinary TCA (mg/g creatinine). There is substantial uncertainty in the conversion of urinary TCA to TCE exposure level (see discussion of Mhiri et al. (2004), for neurotoxicity, above). In addition, there was uncertainty about the adversity of the effect being measured. While rodent evidence supports effects of TCE on sperm, and hyperzoospermia has reportedly been associated with infertility, the adversity of the hyperzoospermia (i.e., high sperm density) outcome measured in the Chia et al. (1996) study is unclear. Furthermore, the cut-point used to define hyperzoospermia in this study (i.e., >120 million sperm per mL ejaculate) is lower than some other reported cut-points, such as 200 and 250 million sperm/mL. A BMR of 10% extra risk was used on the assumption that this is a minimally adverse effect, but biological significance of this effect level is unclear.

[30]Alternatively, the value of the LOAEL-to-NOAEL UF could have been increased above 10 to reflect the extreme severity of the effects at the LOAEL after 24 weeks; however, the comparison of the 12- and 24-week results gives such a clear depiction of the progression of the effects, it was more compelling to frame the issue as a subchronic-to-chronic extrapolation issue.

0.2 ppm, similar to that derived from Land et al. (1981) based on BMD modeling of increases in abnormal sperm.

At a higher inhalation POD, Xu et al. (2004) reported decreased fertilization following exposure in male mice, and Forkert et al. (2002) and Kan et al. (2007) reported effects on the epididymal epithelium in male mice. Kan et al. (2007) reported degenerative effects on the epididymis as early as 1 week into exposure that became more severe at 4 weeks of exposure when the study ended; increases in abnormal sperm were also observed. As with the cRfC developed from the Kumar et al. (2001b; 2000b) studies, a composite UF of 3,000 was applied to these data, but the uncertainties are again typical of those encountered in RfC derivations. Standard values of 3 for the interspecies UF, 10 for the human variability UF, 10 for the LOAEL-to-NOAEL UF, and 10 for the subchronic-to-chronic UF were applied to each of the study PODs.

Among the oral studies, cRfDs derived for decreased sperm motility and changes in reproductive organ weights in rodents reported by George et al. (1986; 1985) were relatively high (2–4 mg/kg/day), and these effects were not considered candidate critical effects. The remaining available oral study of male reproductive effects is DuTeaux et al. (2004a), which reported decreased ability of sperm from TCE-exposed rats to fertilize eggs in vitro. This effect occurred in the absence of changes in combined testes/epididymes weight, sperm concentration or motility, or histological changes in the testes or epididymes. DuTeaux et al. (2004a) hypothesized that the effect is due to oxidative damage to the sperm. A LOAEL was used as the POD, and the standard UF values of 10 were used for each of the UFs, i.e., the subchronic-to-chronic UF (14-day study; substantially less than the 70-day time period for sperm development), the interspecies UF for oral exposures, the human variability UF, and the LOAEL-to-NOAEL UF. The resulting composite UF was 10,000,[31] and this yielded a cRfD of 0.01 mg/kg/day. The excessive magnitude of the composite UF, however, highlights the uncertainty in this estimate.

In summary, there is high qualitative confidence for TCE male reproductive tract toxicity and lower confidence in the cRfCs and cRfDs that can be derived from the available studies. Relatively high PODs are derived from several studies reporting less sensitive endpoints (George et al., 1986; George et al., 1985; 1981), and correspondingly higher cRfCs and cRfDs suggest that they are not likely to be critical effects. The studies reporting more sensitive endpoints also tend to have greater uncertainty. For the human study by Chia et al. (1996), as discussed above, there are uncertainties in the characterization of exposure and the adversity of the effect measured in the study. For the Kumar et al. (2001b; 2000a; 2000b), Forkert et al. (2002), and Kan et al. (2007) studies, the severity of the sperm and testes effects appears to be continuing to

[31]U.S. EPA's report on the RfC and RfD processes (U.S. EPA, 2002b) recommends not deriving reference values with a composite UF of >3,000; however, composite UFs exceeding 3,000 are considered here because the derivation of the cRfCs and cRfDs is part of a screening process and the subsequent application of the PBPK model for candidate critical effects will reduce the values of some of the individual UFs.

increase with duration even at the end of the study, so it is plausible that a lower exposure for a longer duration may elicit similar effects. For the DuTeaux et al. (2004a) study, there is also duration- and low-dose extrapolation uncertainty due to the short duration of the study in comparison to the time period for sperm development as well as the lack of a NOAEL at the tested doses. Overall, even though there are limitations in the quantitative assessment, there remains sufficient evidence to consider these to be candidate critical effects.

5.1.2.7.2. Other reproductive effects

With respect to female reproductive effects, several studies reporting decreased maternal weight gain were suitable for deriving candidate reference values (see Table 5-8). The cRfCs from the two inhalation studies (Carney et al., 2006; Schwetz et al., 1975) yielded virtually the same estimate (0.3–0.4 ppm), although the Carney et al. (2006) result is preferred due to the use of BMD modeling, which obviates the need for the 10-fold LOAEL-to-NOAEL UF used for Schwetz et al. (1975) (the other UFs, with a product of 30, were the same). The cRfDs for this endpoint from the three oral studies were within twofold of each other (1.1–1.9 mg/kg/day), with the same composite UFs of 100. The most sensitive estimate of Narotsky et al. (1995) is preferred due to the use of BMD modeling and the apparent greater sensitivity of the rat strain used.

With respect to other reproductive effects, the most reliable cRfD estimates of about 2 mg/kg/day, derived from BMD modeling with composite UFs of 100, are based on decreased litters/pair and decreased live pups/litter in rats reported in the continuous breeding study of George et al. (1986). Both of these effects were considered severe adverse effects, so a BMR of a 0.5 control SD shift from the control mean was used. Somewhat lower cRfDs of 0.4–1 mg/kg/day were derived based on delayed parturition in females (Narotsky et al., 1995), decreased copulatory performance in males (Zenick et al., 1984), and decreased mating for both exposed males and females in cross-over mating trials (George et al., 1986), all with composite UFs of 100 or 1,000, depending on whether a LOAEL or NOAEL was used.

In summary, there is moderate confidence both in the hazard and the cRfCs and cRfDs for reproductive effects other than the male reproductive effects discussed previously. While there are multiple studies suggesting decreased maternal body weight with TCE exposure, this systemic change may not be indicative of more sensitive reproductive effects. None of the estimates developed from other reproductive effects is particularly uncertain or unreliable. Therefore, delayed parturition (Narotsky et al., 1995) and decreased mating (George et al., 1986), which yielded the lowest cRfDs, were considered candidate critical effects. These effects were also included so that candidate critical reproductive effects from oral studies would not include only that reported by DuTeaux et al. (2004a), from which deriving the cRfD entailed a higher degree of uncertainty.

5.1.2.8. Candidate Critical Developmental Effects on the Basis of Applied Dose

As summarized in Section 4.11.1.7, both human and experimental animal studies have associated TCE exposure with adverse developmental effects. Weakly suggestive epidemiologic data and fairly consistent experimental animal data support TCE exposure posing a hazard for increased prenatal or postnatal mortality and decreased pre- or postnatal growth. In addition, congenital malformations following maternal TCE exposure have been reported in a number of epidemiologic and experimental animal studies. There is also some support for TCE effects on neurological and immunological development. Available human studies, while indicative of hazard, did not have adequate exposure information for quantitative estimates of PODs, so only experimental animal studies are considered here. Studies with numerical dose-response information are summarized in Table 5-9, with their corresponding cRfCs or cRfDs summarized in Table 5-10.

For pre- and postnatal mortality and growth, a cRfC of 0.06 ppm for resorptions, decreased fetal weight, and variations in skeletal development indicative of delays in ossification was developed based on the single available (rat) inhalation study considered (Healy et al., 1982) and utilizing the composite UF of 300 for an inhalation POD that is a LOAEL. The cRfDs for pre- and postnatal mortality derived from oral studies were within about a 10-fold range of 0.4–5 mg/kg/day, depending on the study and specific endpoint assessed. Of these, the estimate based on Narotsky et al. (1995) rat data was both the most sensitive and most reliable cRfD. The dose response for increased full-litter resorptions from this study is based on BMD modeling. Because of the severe nature of this effect, a BMR of 1% extra risk was used. The ratio of the resulting BMD to the BMDL was 5.7, which is on the high side, but given the severity of the effect and the low background response, a judgment was made to use 1% extra risk. Alternatively, a 10% extra risk could have been used, in which case the POD would have been considered more analogous to a LOAEL than a NOAEL, and a LOAEL-to-NOAEL UF of 10 would have been applied, ultimately resulting in the same cRfD estimate. The cRfDs for altered pre- and postnatal growth developed from the oral studies ranged about 10-fold from 0.8 to 8 mg/kg/day, all utilizing the composite UFs for the corresponding type of POD. The cRfDs for decreased fetal weight, both of which were based on NOAELs, were consistent, being about twofold apart (Narotsky et al., 1995; George et al., 1985). The cRfD based on postnatal growth at 21 days, reported in George et al. (1986), was lower and is preferred because it was based on BMD modeling. A BMR of 5% decrease in weight was used for postnatal growth at 21 days because decreases in weight gain so early in life were considered similar to effects on fetal weight.

Table 5-9. Summary of studies of developmental effects suitable for dose-response assessment

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Pre- and postnatal mortality				**Section 4.8.1.2 and 4.8.3.2**
George et al. (1985)	Mouse, CD-1, male and female, 20 pairs/treatment group; 40 controls/sex	Oral: 0, 173, 362, or 737 mg/kg/d, Breeders exposed 1 wk premating, then for 13 wks; pregnant females exposed throughout gestation (i.e., 18 wks total)	Increase perinatal mortality (PNDs 0–21)	Table 4-87
Narotsky et al. (1995)	Rat, F344, females, 8–12 dams/group	Oral: 0, 10.1, 32, 101, 320, 475, 633, 844, or 1,125 mg/kg/d, GDs 6–15	Increased resorptions, prenatal loss, and postnatal mortality	Table 4-98
Manson et al. (1984)	Rat, Long-Evans, female, 23–25/group	Oral: 0, 10, 100, or 1,000 mg/kg/d, 6 wks: 2 wks premating, 1 wk mating period, GDs 1–21	Increased neonatal deaths on PNDs 1, 10, and 14.	Table 4-87
Healy et al. (1982)	Rat, Wistar, females, 31–32 dams/group	Inhalation: 0 or 100 ppm, 4 hrs/d; GDs 8–21	Increased resorptions.	Table 4-96
Pre- and postnatal growth				**Section 4.8.3.2**
Healy et al. (1982)	Rat, Wistar, females, 31–32 dams/group	Inhalation: 0 or 100 ppm, 4 hrs/d; GDs 8–21	Decreased fetal weight, increased bipartite, or absent skeletal ossification centers	Table 4-96

Table 5-9. Summary of studies of developmental effects suitable for dose-response assessment (continued)

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Narotsky et al. (1995)	Rat, F344, females, 8–12 dams/group	Oral: 0, 10.1, 32, 101, 320, 475, 633, 844, or 1,125 mg/kg/d, GDs 6–15	Decreased pup body weight on PNDs 1 and 6.	Table 4-98
George et al. (1985)	Mouse, CD-1, male and female, 20 pairs/treatment group; 40 controls/sex	Oral: 0, 173, 362, or 737 mg/kg/d, Breeders exposed 1 wk premating, then for 13 wks; pregnant females exposed throughout gestation (i.e., 18 wks total)	Decreased live birth weights, PND 4 pup body weights.	Table 4-87
George et al. (1986)	Rat, F334, males and female, 20 pairs/treatment group, 40 controls/sex	Oral: 0, 72, 186, or 389 mg/kg/d (estimated), Breeders exposed 1 wk premating, then for 13 wks; pregnant females exposed throughout gestation (i.e., 18 wks total)	Decreased F1 body weight on PNDs 4–80.	Table 4-87
Congenital defects				**Section 4.8.3.2**
Narotsky et al. (1995)	Rat, F344, females, 8–12 dams/group	Oral: 0, 10.1, 32, 101, 320, 475, 633, 844, or 1,125 mg/kg/d, GDs 6–15	Increased incidence of eye defects.	Table 4-98
Johnson et al. (2003)	Rat, Sprague-Dawley, female, 9–13/group, 55 in control group	Oral: 0, 0.00045, 0.048, 0.218, or 129 mg/kg/d), GDs 0–22	Increased percentage of abnormal hearts; increased percentage of litters with abnormal hearts.	Table 4-98

Table 5-9. Summary of studies of developmental effects suitable for dose-response assessment (continued)

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Developmental neurotoxicity				**Sections 4.3.8.2 and 4.8.3.2**
George et al. (1986)	Rat, F334, males and female, 20 pairs/treatment group, 40 controls/sex	Oral: 0, 72, 186, or 389 mg/kg/d (estimated), Breeders exposed 1 wk premating, then for 13 wks; pregnant females exposed throughout gestation (i.e., 18 wks total)	Decreased locomotor, as assessed by increased time required for pups to cross the first grid in open-field testing.	Tables 4-34 and 4-98
Fredriksson et al. (1993)	Mouse, NMRI, male pups, 12 pups from 3 to 4 different litters/group	Oral: 0, 50, or 290 mg/kg/d, PNDs 10–16	Decreased rearing activity on PND 60.	Tables 4-34 and 4-98
Taylor et al. (1985)	Rat, Sprague-Dawley, females, no. dams/group not reported	Oral: 0, 312, 625, or 1,250 mg/L (0, 45, 80, or 140 mg/kg/d estimated), dams (and pups) exposed from 14 d prior to mating until end of lactation	Increased exploratory behavior in 60- and 90-d-old male rats (offspring).	Tables 4-34 and 4-98
Isaacson and Taylor (1989)	Rat, Sprague-Dawley, females, 6 dams/group	Oral: 0, 4.0, or 8.1 mg/d (0, 15, or 32 mg/kg/d estimated)[a], dams (and pups) exposed from 14 d prior to mating until end of lactation.	Decreased myelinated fibers in the stratum lacunosum-moleculare of pups; decreased myelin in the hippocampus.	Tables 4-34 and 4-98

Table 5-9. Summary of studies of developmental effects suitable for dose-response assessment (continued)

Effect type Study reference	**Species, strain (if applicable), sex, number used for dose-response assessment**	**Exposure(s) used for dose-response assessment**	**Endpoint(s) used for dose-response assessment**	**Chapter 4 Section/Table**
Developmental immunotoxicity				**Section 4.8.3.2**
Peden-Adams et al. (2006)	Mouse, B6C3F$_1$, dams and both sexes offspring, 5 dams/group; 5–7 pups/group at 3 wks; 4–5 pups/sex/group at 8 wks	Oral: 0, 1,400, or 14,000 ppb in water (0, 0.37, or 3.7 mg/kg/d estimated), parental mice and/or offspring exposed during mating, and from GDs 0 through 3 or 8 wks of age	Suppressed PFC responses in males and in females. Delayed hypersensitivity response increased at 8 wks of age in females. Splenic cell population decreased in 3-wk-old pups. Increased thymic T-cells at 8 wks of age. Delayed hypersensitivity response increased at 8 wks of age in males and females	Table 4-98

[a]The Isaacson and Taylor (1989) and Taylor et al. (1985) studies report different doses despite identical study designs and administered concentrations, both studies taking TCE degradation into account. Taylor et al. (1985) report total consumption of 646, 1,102, and 1,991 mg TCE for rats exposed to 312, 625, and 1,250 mg TCE/L drinking water, respectively. Dividing by the 56 days of exposure and the average 250 g per rat for female Sprague-Dawley rats of those ages yields estimated doses of roughly 45, 80, and 140 mg/kg/day, respectively. Isaacson and Taylor (1989) report average doses of TCE of 4.0 and 8.1 mg/day corresponding to exposures of 312 and 625 mg TCE/L drinking water, respectively. Dividing by the average 250 g per rat yields estimated doses of 16 and 32 mg/kg/day, respectively. Thus, the estimated doses for Taylor et al. (1985) are nearly 3 times higher than those for Isaacson and Taylor (1989), for reasons unknown.

Table 5-10. Developmental effects in studies suitable for dose-response assessment, and corresponding cRfCs and cRfDs

Effect type Supporting studies[a]	Species	POD type	POD[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC (ppm)	cRfD (mg/kg/d)	Effect; comments
Pre- and postnatal mortality												
George et al. (1985)	Mouse	NOAEL	362	1	10	10	1	1	100		3.6	↑ perinatal mortality
Narotsky et al. (1995)	Rat	LOAEL	475	1	10	10	10	1	1,000		0.48	Postnatal mortality; Manson et al. (1984) cRfD preferred for same endpoint due to NOAEL vs. LOAEL
Manson et al. (1984)	Rat	NOAEL	100	1	10	10	1	1	100		1.0	↑ neonatal death
Healy et al. (1982)	Rat	LOAEL	17	1	3	10	10	1	300	0.057		Resorptions
Narotsky et al. (1995)	Rat	BMDL	469	1	10	10	1	1	100		4.7	Prenatal loss; BMR = 1% extra risk
Narotsky et al. (1995)	Rat	BMDL	32.2	1	10	10	1	1	100		0.32	Resorptions; BMR = 1% extra risk
Pre- and postnatal growth												
Healy et al. (1982)	Rat	LOAEL	17	1	3	10	10	1	300	0.057		↓ fetal weight; skeletal effects
Narotsky et al. (1995)	Rat	NOAEL	844	1	10	10	1	1	100		8.4	↓ fetal weight
George et al. (1985)	Mouse	NOAEL	362	1	10	10	1	1	100		3.6	↓ fetal weight
George et al. (1986)	Rat	BMDL	79.7	1	10	10	1	1	100		0.80	↓ Body weight at d21; BMR = 5% decrease
Congenital defects												
Narotsky et al. (1995)	Rat	BMDL	60	1	10	10	1	1	100		0.60	Eye defects; low BMR (1%), but severe effect and low background. rate (<1%)
Johnson et al. (2003)	Rat	BMDL	0.0146	1	10	10	1	1	100		0.00015	Heart malformations (litters); BMR = 10% extra risk (only ~1/10 from each litter affected); highest-dose group (1,000-fold higher than next highest) dropped for model fit.
Johnson et al. (2003)	Rat	BMDL	0.0207	1	10	10	1	1	100		0.00021	Heart malformations (pups); BMR = 1% extra risk; preferred due to accounting for intralitter effects via nested model and pups being the unit of measure; highest-dose group (1,000-fold higher than next highest) dropped for model fit

Table 5-10. Developmental effects in studies suitable for dose-response assessment, and corresponding cRfCs and cRfDs (continued)

Effect type Supporting studies[a]	Species	POD type	POD[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC (ppm)	cRfD (mg/kg/d)	Effect; comments
Developmental neurotoxicity												
George et al. (1986)	Rat	BMDL	72.6	1	10	10	1	1	100		0.73	↓ locomotor activity; BMR = doubling of traverse time; results from females (males similar with BMDL = 92)
Fredriksson et al. (1993)	Mouse	LOAEL	50	3	10	10	10	1	3,000		0.017	↓ rearing postexposure; pup gavage dose; no effect at tested doses on locomotion behavior; UF_S = 3 because exposure only during PNDs 10–16
Taylor et al. (1985)	Rat	LOAEL	45	1	10	10	10	1	1,000		0.045	↑ exploration postexposure; estimated dam dose; less sensitive than Isaacson and Taylor (1989), but included because exposure is preweaning, so can utilize PBPK model
Isaacson and Taylor (1989)	Rat	LOAEL	16	1	10	10	10	1	1,000		0.016	↓ myelination in hippocampus; estimated dam dose
Developmental immunotoxicity												
Peden-Adams et al. (2006)	Mouse	LOAEL	0.37	1	10	10	10	1	1,000		0.00037	↓ PFC, ↑ DTH; POD is estimated dam dose (exposure throughout gestation and lactation + to 3 or 8 wks of age); UF LOAEL = 10 since multiple immunotoxicity effects

[a]Shaded studies/endpoints were selected as candidate critical effects/studies.
[b]Adjusted to continuous exposure unless otherwise noted. For inhalation studies, adjustments yield a POD that is a HEC as recommended for a Category 3 gas in U.S. EPA (1994a) in the absence of PBPK modeling. Same units as cRfC (ppm) or cRfD (mg/kg/day).
[c]Product of individual UFs.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

For congenital defects, there is relatively high confidence in the cRfD for eye defects in rats reported in Narotsky et al. (1995), derived using a composite UF of 100 for BMD modeling in a study of duration that encompasses the full window of eye development. However, the most sensitive developmental effect by far was heart malformations in the rat reported by Johnson et al. (2003), yielding a cRfD estimate of 0.0002 mg/kg/day, also with a composite UF of 100. As discussed in detail in Section 4.8 and summarized in Section 4.11.1.7, although this study has important limitations, the overall weight of evidence supports an effect of TCE on cardiac development, and this is the only study of heart malformations available for conducting dose-response analysis. Individual data were kindly provided by Dr. Johnson (personal communication from Paula Johnson, University of Arizona, to Susan Makris, EPA, 25 August 2008), and, for analyses for which the pup was the unit of measure, BMD modeling was done using nested models because accounting for the intralitter correlation improved model fit. For these latter analyses, a 1% extra risk of a pup having a heart malformation was used as the BMR because of the severity of the effect, since, for example, some of the types of malformations observed could have been fatal. The ratio of the resulting BMD to the BMDL was about three.

For developmental neurotoxicity, the cRfD estimates based on the four oral studies span a wide range from 0.02 to 0.8 mg/kg/day. The most reliable estimate, with a composite UF of 100, is based on BMD modeling of decreased locomotor activity in rats reported in George et al. (1986), although a nonstandard BMR of a twofold change was selected because the control SD appeared unusually small. The cRfDs developed for decreased rearing postexposure in mice (Fredriksson et al., 1993), increased exploration postexposure in rats (Taylor et al., 1985), and decreased myelination in the hippocampus of rats (Isaacson and Taylor, 1989), while being >10-fold lower, are all within a 3-fold range of 0.02–0.05 mg/kg/day. Importantly, there is some evidence from adult neurotoxicity studies of TCE causing demyelination, so there is additional biological support for the latter effect. There is greater uncertainty in the Fredriksson et al. (1993), the cRfD for which utilized a subchronic-to-chronic UF of 3 rather than 1, because exposure during PND 10–16 does not cover the full developmental window (Rice and Barone, 2000). The cRfDs derived from Taylor et al. (1985) and (Isaacson and Taylor, 1989) used the composite UF of 1,000 for a POD that is a LOAEL. While there is greater uncertainty in these endpoints, none of the uncertainties is particularly high, and they also appear to be more sensitive indicators of developmental neurotoxicity than that from George et al. (1986).

A cRfD of 0.0004 mg/kg/day was developed from the study (Peden-Adams et al., 2006) that reported developmental immunotoxicity. The main effects observed were significantly decreased PFC response and increased delayed-type hypersensitivity. The data on these effects were kindly provided by Dr. Peden-Adams (personal communication from Margie Peden-Adams, Medical University of South Carolina, to Jennifer Jinot, EPA, 26 August 2008); however, the dose-response relationships were sufficiently supralinear that attempts at BMD modeling did not result in adequate fits to these data. Thus, the LOAEL was used as the POD.

A LOAEL-to-NOAEL UF of 10 was used for the multiple effects of decreased PFC response and increased delayed-type hypersensitivity at the same dose. While there is uncertainty in this estimate, it is notable that decreased PFC response was also observed in an immunotoxicity study in adult animals (Woolhiser et al., 2006), lending biological plausibility to the effect.

In summary, there is moderate-to-high confidence both in the hazard and the cRfCs and cRfDs for developmental effects of TCE. It is also noteworthy that the PODs for the more sensitive developmental effects were similar to or, in most cases, lower than the PODs for the more sensitive reproductive effects, suggesting that developmental effects are not a result of paternal or maternal toxicity. Among inhalation studies, cRfCs were only developed for effects in rats reported in Healy et al. (1982), so the effects of resorptions, decreased fetal weight, and delayed skeletal ossification were considered candidate critical developmental effects. Because resorptions were also reported in oral studies, the most sensitive (rat) oral study (and most reliable for dose-response analysis) of Narotsky et al. (1995) was also selected as a candidate critical study for this effect. The confidence in the oral studies and candidate reference values developed for more sensitive endpoints is more moderate, but still sufficient for consideration as candidate critical effects. The most sensitive endpoints by far are the increased fetal heart malformations in rats reported by Johnson et al. (2003) and the developmental immunotoxicity in mice reported by Peden-Adams et al. (2006), and these are both considered candidate critical effects. Neurodevelopmental effects are a distinct type among developmental effects. Thus, the next most sensitive endpoints of decreased rearing postexposure in mice (Fredriksson et al., 1993), increased exploration postexposure in rats (Taylor et al., 1985), and decreased myelination in the hippocampus of rats (Isaacson and Taylor, 1989) are also considered candidate critical effects.

5.1.2.9. Summary of cRfCs, cRfDs, and Candidate Critical Effects

An overall summary of the cRfCs, cRfDs, and candidate critical effects across the health effect domains is shown in Tables 5-11 and 5-12. These tables present, for each type of noncancer effect, the relative ranges of the cRfC and cRfD developed for the different endpoints. The candidate critical effects selected above for each effect domain are shown in bold. As discussed above, these effects were generally selected to represent the most sensitive endpoints, across species where possible. From these candidate critical effects, candidate reference values based on internal dose-metrics from the PBPK model (p-cRfCs and p-cRfDs) were developed where possible. Effects within the same health effect domain were generally assumed to have the same relevant internal dose-metrics; thus, screening for the effects with the lowest cRfCs and cRfDs for each species within health effect domains on the basis of applied dose should capture the same endpoints which would have the lowest candidate reference values on the basis of an appropriate dose-metric. Application of the PBPK model is discussed in the next section.

Table 5-11. Ranges of cRfCs based on applied dose for various noncancer effects associated with inhalation TCE exposure[a]

cRfC range (ppm)	Neurological	Systemic/organ-specific	Immunological	Reproductive	Developmental
10–100	Impaired visual discrimination (rat)				
1–10		**Kidney meganucleocytosis (rat)** **↑ kidney weight (mouse)**			
0.1–1	Ototoxicity (rat) Hyperactivity (rat) Changes in locomotor activity (rat) **Trigeminal nerve effects (human)** Impaired visual function (rabbit) **↓ regeneration of sciatic nerve (rat)**	**↑ liver weight (rat)** **↑ liver weight (mouse)** **↑ kidney weight (rat)**	**↓ PFC response (rat)**	↓ maternal body weight gain (rat) ↑ abnormal sperm (mouse) pre/postimplantation losses (male rat exp)	
0.01–0.1	**↓ regeneration of sciatic nerve (mouse)** **Disturbed wakefulness (rat)**		**Autoimmune changes (MRL—lpr/lpr mouse)**	**Effects on epididymis epithelium (mouse)** **↓ fertilization (male mouse exp)** **Testes and sperm effects (rat)** **Hyperzoospermia (human)**	**Resorptions (female rat)** **↓ fetal weight (rat)** **Skeletal effects (rat)**

[a]Endpoints in **bold** were selected as candidate critical effects (see Sections 5.1.2.1–5.1.2.8).

Table 5-12. Ranges of cRfDs based on applied dose for various noncancer effects associated with oral TCE exposure[a]

cRfD range (mg/kg/d)	Neurological	Systemic/organ-specific	Immunological	Reproductive	Developmental
1–10	↑ neuromuscular changes (rat)	↓ Body weight (mouse)	↓ humoral response to SRBC (mouse)	↓ testis/seminal vesicle weight (mouse) ↓ sperm motility (mouse) ↑ testis/epididymis weight (rat) ↓ litters/pair (rat) ↓ live pups/litter (rat) ↓ Body weight gain (rat) ↓ copulatory performance (rat)	↓ fetal weight (rat) Prenatal loss (rat) ↓ fetal weight (mouse) ↑ neonatal mortality (mouse, rat)
0.1–1	↑ number rears (rat) ↑ foot splaying (rat) Trigeminal nerve effect (rat)	**↑ liver weight (mouse)** ↓ Body weight (mouse) ↓ Body weight (rat) **Toxic nephropathy** (other rat strains/sexes and **mouse**) **Meganucleocytosis (male Sprague-Dawley rat)**	Signs of autoimmune hepatitis (MRL +/+ mouse) Inflammation in various tissues (MRL +/+ mouse)	**Delayed parturition (rat)** **↓ mating (rat)**	↓ Body weight at PND 21 (rat) ↓ locomotor activity (rat) Eye defects (rat) **Resorptions (rat)**
0.01–0.1	**Degeneration of dopaminergic neurons (rat)**	**Toxic nephropathy (female Marshall rat)**	**↓ cell-mediated response to SRBC (mouse)** **↓ stem cell bone marrow recolonization (mouse)**	**↓ ability of sperm to fertilize (rat)**	**↑ exploration (postexposure) (rat)** **↓ rearing (postexposure) (mouse)** **↓ myelination in hippocampus (rat)**
0.001–0.01	**Demyelination in hippocampus (rat)**		**↑ anti-dsDNA and anti-ssDNA Abs (early marker for autoimmune disease) (mouse)**		
10^{-4}–0.001			**↓ thymus weight (mouse)**		**Immunotoxicity (↓ PFC, ↑ DTH) ($B6C3F_1$ mouse)** **Heart malformations (rat)**

[a]Endpoints in **bold** were selected as candidate critical effects (see Sections 5.1.2.1–5.1.2.8).

5.1.3. Application of PBPK Model to Inter- and Intraspecies Extrapolation for Candidate Critical Effects

For the candidate critical effects, the use of PBPK modeling of internal doses could justify, where appropriate, replacement of the UFs for pharmacokinetic inter- and intraspecies extrapolation. For more details on PBPK modeling used to estimate levels of dose-metrics corresponding to different exposure scenarios in rodents and humans, as well as a qualitative discussion of the uncertainties and limitations of the model, see Section 3.5.

Quantitative analyses of the PBPK modeling uncertainties and their implications for dose-response assessment, utilizing the results of the Bayesian analysis of the PBPK model, are discussed separately in Section 5.1.4.

5.1.3.1. Selection of Dose-metrics for Different Endpoints

One area of scientific uncertainty in noncancer dose-response assessment is the appropriate scaling between rodent and human doses for equivalent responses. As discussed above, the interspecies UF of 10 is usually thought of as a product of two factors of (approximately) three each for pharmacokinetics ($UF_{A\text{-}pk}$) and pharmacodynamics ($UF_{A\text{-}pd}$). In this assessment, EPA's cross-species scaling methodology, grounded in general principles of allometric variation of biologic processes, is used for describing pharmacokinetic equivalence (U.S. EPA, 1992, 2011a, 2005b; Allen and Fisher, 1993; Crump et al., 1989; Allen et al., 1987). Briefly, in the absence of adequate information to the contrary, the methodology determines pharmacokinetic equivalence across species through equal average lifetime concentrations or AUCs of the toxicant. Thus, in cases where the PBPK model can predict internal concentrations of the active moiety, equivalent daily AUCs are assumed to address cross-species pharmacokinetics, and the interspecies UF is reduced to 3 to account for the remaining pharmacodynamic factor.

In the absence of directly estimated AUCs, the cross-species scaling methodology assumes that, unless there is evidence to the contrary (U.S. EPA, 1992, 2011a, 2005b):

(1) The production of the active moiety(ies) is proportional to dose

(2) The clearance of the active moiety(ies) scales allometrically by body weight to the ¾ power; and

(3) The tissue distribution is equal across species.

Under these assumptions, for oral exposures, pharmacokinetic equivalence of AUCs between animals to humans is expressed on the basis of $mg/kg^{3/4}/day$, not mg/kg/day ("body weight scaling"). For inhalation exposures, pharmacokinetic equivalence would be on the basis of equivalent air concentrations, since the alveolar ventilation rate (which determines dose, for a

constant air concentration) scales approximately by body weight to the ¾ power, cancelling out the assumed scaling dependence of clearance.

However, when one or more metabolites are thought to be the toxicologically active compound(s), it is often the case that a PBPK model can predict the rate of production of the active moiety(ies) (i.e., the rate of metabolism) but cannot predict AUCs due to lack of data to inform clearance. In this case, assumption (1) above can be replaced by the PBPK model, while the other two cross-species scaling methodology assumptions are retained. The resulting pharmacokinetic equivalence can therefore be expressed on the basis of rate of metabolism/$kg^{3/4}$/day.[32] Thus, in cases where the PBPK model can predict the rate of production of the active metabolite(s), equivalent daily amounts metabolized through the appropriate pathway per unit body weight to the ¾ power are assumed to address cross-species pharmacokinetics, and the interspecies UF is reduced to 3 to account for the remaining pharmacodynamic factor.

In addition, in some cases when AUCs cannot be estimated, there are data to replace assumption (2), above, that the clearance of the active moiety(ies) scales allometrically by body weight to the ¾ power. Often, this is considered for toxicity associated with local (in situ) production of "reactive" metabolites whose concentrations cannot be directly measured in the target tissue. In such a case, an alternative approach of scaling the rate of local metabolism by target tissue mass, rather than body weight to the ¾ power, is appropriate if the metabolites are sufficiently reactive *and* are cleared by "spontaneous" deactivation (i.e., changes in chemical structure without the need of biological influences). In particular, use of this alternative scaling approach requires evidence that: (1) the active moiety or moieties do not leave the target tissue in appreciable quantities (i.e., are cleared primarily by in situ transformation to other chemical species and/or binding to/reactions with cellular components), and (2) the clearance of the active moieties from the target tissue is governed by biochemical reactions whose rates are independent of body weight (e.g., purely chemical reactions). If these conditions are met, equivalent daily amounts metabolized through the appropriate pathway per unit target tissue mass are assumed to address cross-species pharmacokinetics, and the interspecies UF is reduced to 3 to account for the remaining pharmacodynamic factor.

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[32]Consider a circulating stable metabolite X. Under a one-compartment model, at steady-state, the production of X will be equal to the clearance of X, so that

$$R_{met} = V_d \times BW \times C_X \times k_{cl},$$

where R_{met} = rate of production of X (mg/time), V_d = fractional volume of distribution, BW = body weight, C_X = concentration of X and k_{cl} = clearance of X in units of 1/time. Then, for the concentration C_X to be equivalent between experimental animals (A) and humans (H):

$$C_X = [R_{met}/BW \times k_{cl} \times V_d\ ]_H = [R_{met}/BW \times k_{cl} \times V_d]_A.$$

Under the cross-species scaling methodology, it is assumed that V_d is the same across species, so $[R_{met}/BW \times k_{cl}]_H = [R_{met}/BW \times k_{cl}]_A$. Next, under the cross-species scaling methodology, k_{cl} (with units of 1/time) is assumed to scale according to $BW^{1/4}$ (U.S. EPA, 2005b; U.S. EPA, 2011a), leading to:

$$R_{met\ (H)}/BW_H^{3/4} = R_{met\ (A)}/BW_A^{3/4}$$

Finally, there is the case where local metabolism, rather than systemically delivered metabolite(s), is thought to be involved in toxicity, but there are inadequate data to determine either the rate of local metabolism or its clearance. In this case, assumption (1) above can be replaced by the assumption that local metabolism will be proportional to blood concentration. Because tissue blood flow approximately scales allometrically by body weight to the ¾ power, combining this with assumptions (2) and (3) above will lead to the AUC of the parent compound in blood as an appropriate surrogate for local metabolism. Thus, in this case, equivalent daily AUCs of the parent compound are assumed to address cross-species pharmacokinetics, and the interspecies UF is reduced to 3 to account for the remaining pharmacodynamic factor.

To summarize, the internal dose-metric for addressing cross-species pharmacokinetics is based on the Agency's cross-species scaling methodology. The preferred dose-metric under this methodology is equivalent daily AUC of the active moiety (parent compound or metabolite). For metabolites, in cases where the rate of production, but not the rate of clearance, of the active moiety can be estimated, the preferred dose-metric is the rate of metabolism (through the appropriate pathway) scaled by body weight to the ¾ power. If there are sufficient data to consider the active metabolite moiety(ies) "reactive" and cleared through nonbiological processes, then the preferred dose-metric is the rate of metabolism (through the appropriate pathway) scaled by the tissue mass. Finally, if local metabolism is thought to be involved, but cannot be estimated with the available data, then the AUC of the parent compound in blood is considered an appropriate surrogate and thus the preferred dose-metric.

These dose-metrics were then also used in addressing the pharmacokinetic component, $UF_{H\text{-}pk}$, of the UF for human (intraspecies) variability. Because all of the dose-metrics used for TCE were for adults, and the dose-metrics are not very sensitive to the plausible range of adult body weight, for convenience the body weight ¾ scaling used for interspecies extrapolation was retained for characterization of human variability. However, it should be emphasized that this intraspecies characterization is of pharmacokinetics only, and not pharmacodynamics.

In general, an attempt was made to use tissue-specific dose-metrics representing particular pathways or metabolites identified from available data on the role of metabolism in toxicity for each endpoint (discussed in more detail below). The selection was limited to dose-metrics for which uncertainty and variability could be adequately characterized by the PBPK model (see Section 3.5). For most endpoints, sufficient information on the role of metabolites or mode of action was not available to identify likely relevant dose-metrics, and more "upstream" metrics representing either parent compound or total metabolism had to be used. The "primary" or "preferred" dose-metric referred to in subsequent tables has the greater biological support for its involvement in toxicity, whereas "alternative" dose-metrics are those that may also be plausibly involved (discussed further below). A discussion of the dose-metrics selected for particular noncancer endpoints follows.

5.1.3.1.1. Kidney toxicity (meganucleocytosis, increased kidney weight, toxic nephropathy)

As discussed in Sections 4.4.6–4.4.7, there is sufficient evidence to conclude that TCE-induced kidney toxicity is caused predominantly by GSH conjugation metabolites either produced in situ in or delivered systemically to the kidney. As discussed in Section 3.3.3.2, bioactivation of DCVG, DCVC, and *N*-acetyl-S-(1,2-dichlrovinyl)-L-cysteine (NAcDCVC) within the kidney, either by beta-lyase, flavin mono-oxygenase (FMO), or CYP, produces reactive species, any or all of which may cause nephrotoxicity. Therefore, multiple lines of evidence support the conclusion that renal bioactivation of DCVC is the preferred basis for internal dose extrapolations for TCE-induced kidney toxicity. However, uncertainties remain as to the relative contribution from each bioactivation pathway; and quantitative clearance data necessary to calculate the concentration of each species are lacking. Moreover, the estimates of the amount bioactivated are indirect, derived from the difference between overall GSH conjugation flux and NAcDCVC excretion (see Section 3.5.7.3.1).

Under the cross-species scaling methodology, the rate of renal bioactivation of DCVC would be scaled by body weight to the ¾ power. However, it is necessary to consider whether there are adequate data to support use of the alternative scaling by target tissue mass. For the beta-lyase pathway, Dekant et al. (1988) reported in trapping experiments that the postulated reactive metabolites decompose to stable (unreactive) metabolites in the presence of water. Moreover, the necessity of a chemical trapping mechanism to detect the reactive metabolites suggests a very rapid reaction such that it is unlikely that the reactive metabolites leave the site of production. Therefore, these data support the conclusion that, for this bioactivation pathway, clearance is chemical in nature and hence species-independent. If this were the only bioactivation pathway, then scaling by kidney weight would be supported. With respect to the FMO bioactivation pathway, Sausen and Elfarra (1991) reported that after direct dosing of the postulated reactive sulfoxide (DCVC sulfoxide), the sulfoxide was detected as an excretion product in bile. These data suggest that reactivity in the tissue to which the sulfoxide was delivered (the liver, in this case) is insufficient to rule out a significant role for enzymatic or other biologically mediated systemic clearance. Therefore, according to the criteria outlined above, for this bioactivation pathway, the data support scaling the rate of metabolism by body weight to the ¾ power. For P450-mediated bioactivation producing NAcDCVC sulfoxide, the only relevant data on clearance are from a study of the structural analogue to DCVC, fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (FDVE) (Sheffels et al., 2004), which reported that the postulated reactive sulfoxide was detected in urine. This suggests that the sulfoxide is sufficiently stable to be excreted by the kidney and supports the scaling of the rate of metabolism by body weight to the ¾ power.

Therefore, because the contributions to TCE-induced nephrotoxicity from each possible bioactivation pathway are not clear, and the scaling by body weight to the ¾ power is supported

for two of the identified three bioactivation pathways, it is decided here to scale the DCVC bioactivation rate by body weight to the ¾ power. The primary internal dose-metric for TCE-induced kidney toxicity is thus, the weekly rate of DCVC bioactivation per unit body weight to the ¾ power (**ABioactDCVCBW34 [mg/kg^{¾}/week]**). However, it should be noted that due to the larger relative kidney weight in rats as compared to humans, scaling by kidney weight instead of body weight to the ¾ power would only change the quantitative interspecies extrapolation by about twofold,[33] so the sensitivity of the results to the scaling choice is relatively small. In addition, quantitative estimates for this dose-metric are only available in rats and humans, and not in mice. Accordingly, this metric was only used for extrapolating results from rat toxicity studies.

An alternative dose-metric that also involves the GSH conjugation pathway is the amount of GSH conjugation scaled by the ¾ power of body weight **(AMetGSHBW34 [mg/kg^{¾}/week])**. This dose-metric uses the total flux of GSH conjugation as the toxicologically-relevant dose, and, thus, incorporates any direct contributions from DCVG and DCVC, which are not addressed in the DCVC bioactivation metric. The rationale for scaling by body weight to the ¾ power rather than target tissue mass is the same as above. Because of the lack of availability of the DCVC bioactivation dose-metric in mice, the GSH conjugation metric is used as the primary dose-metric for the nephrotoxicity endpoint in studies of mice.

Another alternative dose-metric is the total amount of TCE metabolism (oxidation and GSH conjugation together) scaled by the ¾ power of body weight **(TotMetabBW34 [mg/kg^{¾}/week])**. This dose-metric uses the total flux of TCE metabolism as the toxicologically relevant dose, and, thus, incorporates the possible involvement of oxidative metabolites, acting either additively or interactively, in addition to GSH conjugation metabolites in nephrotoxicity (see Section 4.4.6). However, this dose-metric is given less weight than those involving GSH conjugation because, as discussed in Sections 4.4.6, the weight of evidence supports the conclusion that GSH conjugation metabolites play a predominant role in nephrotoxicity. The rationale for scaling by body weight to the ¾ power rather than target tissue mass is the same as above.

5.1.3.1.2. Liver weight increases (hepatomegaly)

As discussed in Section 4.5.6, there is substantial evidence that oxidative metabolism is involved in TCE hepatotoxicity, based primarily on similarities in noncancer effects with a number of oxidative metabolites of TCE (e.g., CH, TCA, and DCA). While TCA is a stable, circulating metabolite, CH and DCA are relatively short-lived, although enzymatically cleared (see Section 3.3.3.1). As discussed in Section 4.5.6.2.1, there is substantial evidence that TCA

[33]The range of the difference is 2.1–2.4-fold using the posterior medians for the relative kidney weight in rats and humans from the PBPK model described in Section 3.5 (see Table 3-38), and body weights of 0.3–0.4 kg for rats and 60–70 kg for humans.

alone does not adequately account for the hepatomegaly induced by TCE; therefore, unlike in previous dose-response analyses (Clewell and Andersen, 2004; Barton and Clewell, 2000), the AUC of TCA in plasma or in liver were not considered as dose-metrics. However, there are inadequate data across species to quantify the dosimetry of CH and DCA, and other intermediates of oxidative metabolism (such as TCE-oxide or dichloroacetylchloride) may be involved in hepatomegaly. Thus, due to uncertainties as to the active moiety(ies), but given the strong evidence associating TCE liver effects with oxidative metabolism in the liver, hepatic oxidative metabolism is the preferred basis for internal dose extrapolations of TCE-induced liver weight increases.

Under the cross-species scaling methodology, the rate of hepatic oxidative metabolism would be scaled by body weight to the ¾ power. However, it is necessary to consider whether there are adequate data to support use of the alternative scaling by target tissue mass. Several of the oxidative metabolites are stable and systemically available, and several of those that are cleared rapidly are metabolized enzymatically, so, according to the criteria discussed above, there are insufficient data to support the conclusions that the active moiety or moieties do not leave the target tissue in appreciable quantities and are cleared by mechanisms whose rates are independent of body weight.

Therefore, the primary internal dose-metric for TCE-induced liver weight changes is selected to be the weekly rate of hepatic oxidation per unit body weight to the ¾ power (AMetLiv1BW34 [mg/kg^{¾}/week]). The use of this dose-metric is also supported by the analysis in Section 4.5.6.2.1 showing much more consistency in the dose-response relationships for TCE-induced hepatomegaly across studies and routes of exposure using this metric and the total oxidative metabolism dose-metric (discussed below) as compared to the AUC of TCE in blood. It should be noted that due to the larger relative liver weight in mice as compared to humans, scaling by liver weight instead of body weight to the ¾ power would only change the quantitative interspecies extrapolation by about fourfold,[34] so the sensitivity of the results to the scaling choice is relatively modest.

It is also known that the lung has substantial capacity for oxidative metabolism, with some proportion of the oxidative metabolites produced there entering systemic circulation. Thus, it is possible that extrahepatic oxidative metabolism can contribute to TCE-induced hepatomegaly. Therefore, the total amount of oxidative metabolism of TCE scaled by the ¾ power of body weight (**TotOxMetabBW34 [mg/kg^{¾}/week]**) was selected as an alternative dose-metric (the rationale for the body weight to the ¾ power scaling is analogous to that for hepatic oxidative metabolism, above).

[34]The range of the difference is 3.5–3.9-fold using the posterior medians for the relative liver weight in mice and humans from the PBPK model described in Section 3.5 (see Table 3-37), and body weights of 0.03–0.04 kg for mice and 60–70 kg for humans.

5.1.3.1.3. Developmental toxicity—heart malformations

As discussed in Section 4.8.3.2.1, several studies have reported that the prenatal exposure to TCE oxidative metabolites TCA or DCA also induces heart malformations, suggesting that oxidative metabolism is involved in TCE-induced heart malformations. However, there are inadequate data across species to quantify the dosimetry of DCA, and it is unclear if other products of TCE oxidative metabolism are involved. Therefore, the total amount of oxidative metabolism of TCE scaled by the ¾ power of body weight (TotOxMetabBW34 [mg/kg^{¾}/week]) was selected as the primary dose-metric. The rationale for the scaling by body weight to the ¾ power is analogous to that for hepatic oxidative metabolism, above.

An alternative dose-metric that is considered here is the AUC of TCE in (maternal) blood (AUCCBld [mg-hour/L/day]). The placenta is a highly perfused tissue, and TCE is known to cross the placenta to the fetus, with rats showing similar (within twofold) maternal and fetal blood TCE concentrations (see Section 3.2). This dose-metric accounts for the possible roles either of local metabolism or of TCE itself.

5.1.3.1.4. Reproductive toxicity—decreased ability of sperm to fertilize oocytes

The decreased ability of sperm to fertilize oocytes observed by DuTeaux et al. (2004a) occurred in the absence of changes in combined testes/epididymes weight, sperm concentration or motility, or histological changes in the testes or epididymes. However, there was evidence of oxidative damage to the sperm, and DuTeaux et al. (2003) previously reported the ability of the rat epididymis and efferent ducts to metabolize TCE oxidatively. Based on this evidence, DuTeaux et al. (2004a) hypothesized that the decreased ability to fertilize is due to oxidative damage to the sperm from local metabolism. Thus, the primary dose-metric for this endpoint is selected to be the AUC of TCE in blood (AUCCBld [mg-hour/L/day]), based on the assumption that in situ oxidation of systemically-delivered TCE (the flow rate of which scales as body weight to the ¾ power) is the determinant of toxicity.

Because metabolites causing oxidative damage may be delivered systemically to the target tissue, an alternative dose-metric that is considered here is total oxidative metabolism of TCE scaled by the ¾ power of body weight (TotOxMetabBW34 [mg/kg^{¾}/day]). The rationale for the scaling by body weight to the ¾ power is analogous to that for hepatic oxidative metabolism, above. Because oxidative metabolites make up the majority of TCE metabolism, total metabolism gives very similar results (within 1.2-fold) to total oxidative metabolism and is therefore not included as a dose-metric.

5.1.3.1.5. Other reproductive and developmental effects and neurological effects and immunologic effects

For all other candidate critical endpoints listed in Tables 5-11 and 5-12, including developmental effects other than heart malformations and reproductive effects other than

decreased ability of sperm to fertilize, there is insufficient information for site-specific determinations of an appropriate dose-metric. While TCE metabolites and/or metabolizing enzymes have been reported in some of these tissues (e.g., male reproductive tract), their general roles in toxicity in the respective tissues have not been established. The choice of total metabolism as the primary dose-metric is based on the observation that, in general, TCE toxicity is associated with metabolism rather than the parent compound. It is acknowledged that there is no compelling evidence that definitively establishes one metric as more plausible than the other in any particular case. Nonetheless, as a general inference in the absence of specific data, total metabolism is viewed as more likely to be involved in toxicity than the concentration of TCE itself.

Therefore, given that the majority of the toxic and carcinogenic responses in many tissues to TCE appears to be associated with metabolism, the primary dose-metric is selected to be total metabolism of TCE scaled by the ¾ power of body weight (TotMetabBW34 [mg/kg^{¾}/day]). The rationale for the scaling by body weight to the ¾ power is analogous to that for the other metabolism dose-metrics, above. Because oxidative metabolites make up the majority of TCE metabolism, total oxidative metabolism gives very similar results (within 1.2-fold) to total metabolism and is therefore not included as a dose-metric.

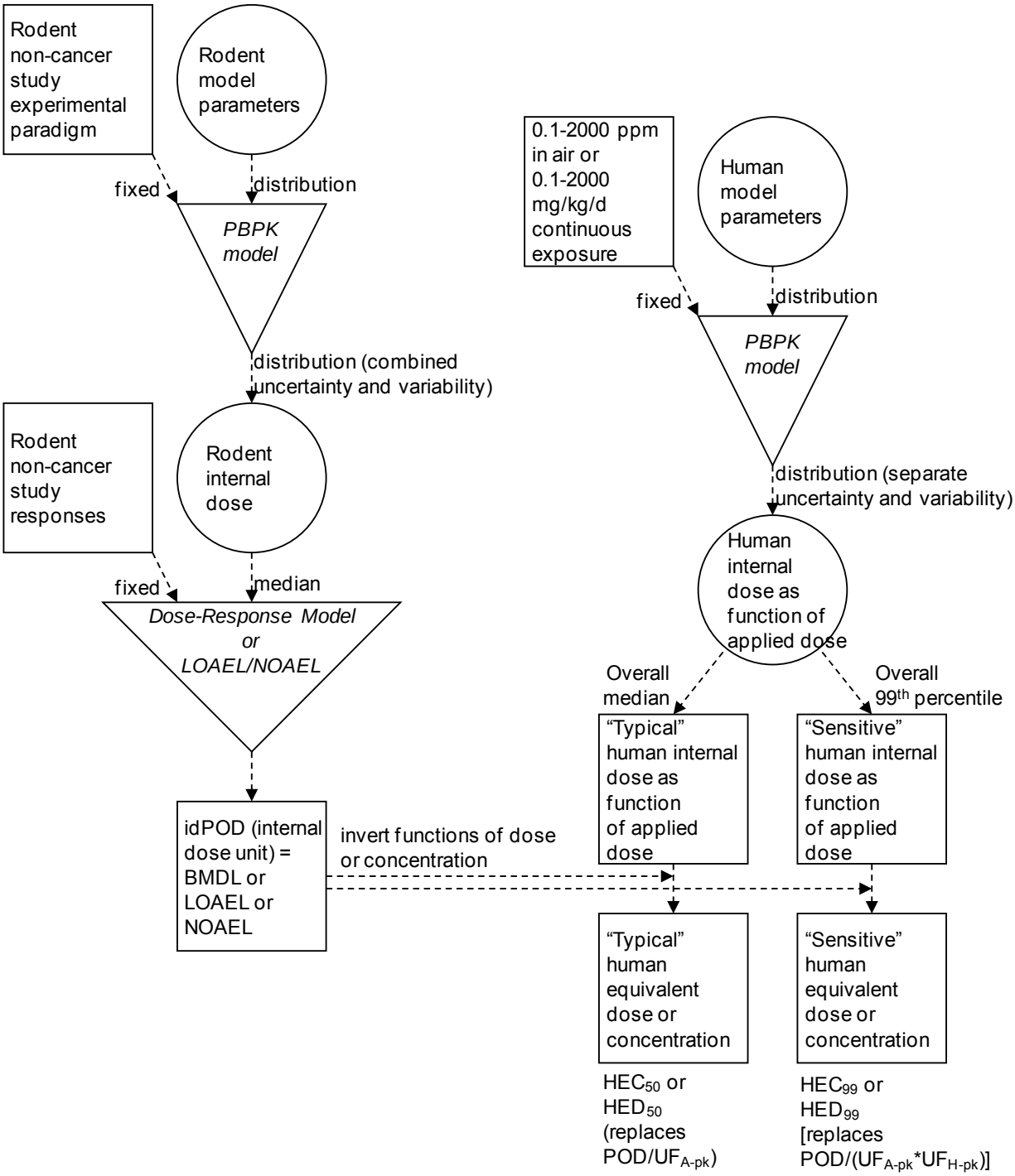
An alternative dose-metric that is considered here is the AUC of TCE in blood (AUCCBld [mg-hour/L/day]). This dose-metric would account for the possible role of local metabolism, which is determined by TCE delivered in blood via systemic circulation to the target tissue (the flow rate of which scales as body weight to the ¾ power), and the possible role of TCE itself. This dose-metric would also be most applicable to tissues that have similar tissue:blood partition coefficients across and within species.

Because the PBPK model described in Section 3.5 did not include a fetal compartment, the maternal internal dose-metric is taken as a surrogate for developmental effects in which exposure was before or during pregnancy (Johnson et al., 2003; Narotsky et al., 1995; Fredriksson et al., 1993; Taylor et al., 1985). This was considered reasonable because TCE and the major circulating metabolites (TCA and TCOH) appear to cross the placenta (see Sections 3.2, 3.3, and 4.10 (Fisher et al., 1989; Ghantous et al., 1986)), and maternal metabolizing capacity is generally greater than that of the fetus (see Section 4.10). In the cases where exposure continues after birth (Peden-Adams et al., 2006; Isaacson and Taylor, 1989), no PBPK model-based internal dose was used. Because of the complicated fetus/neonate dosing that includes transplacental, lactational, and direct (if dosing continues postweaning) exposure, the maternal internal dose is no more accurate a surrogate than applied dose in this case.

5.1.3.2. Methods for Inter- and Intraspecies Extrapolation Using Internal Doses[35]

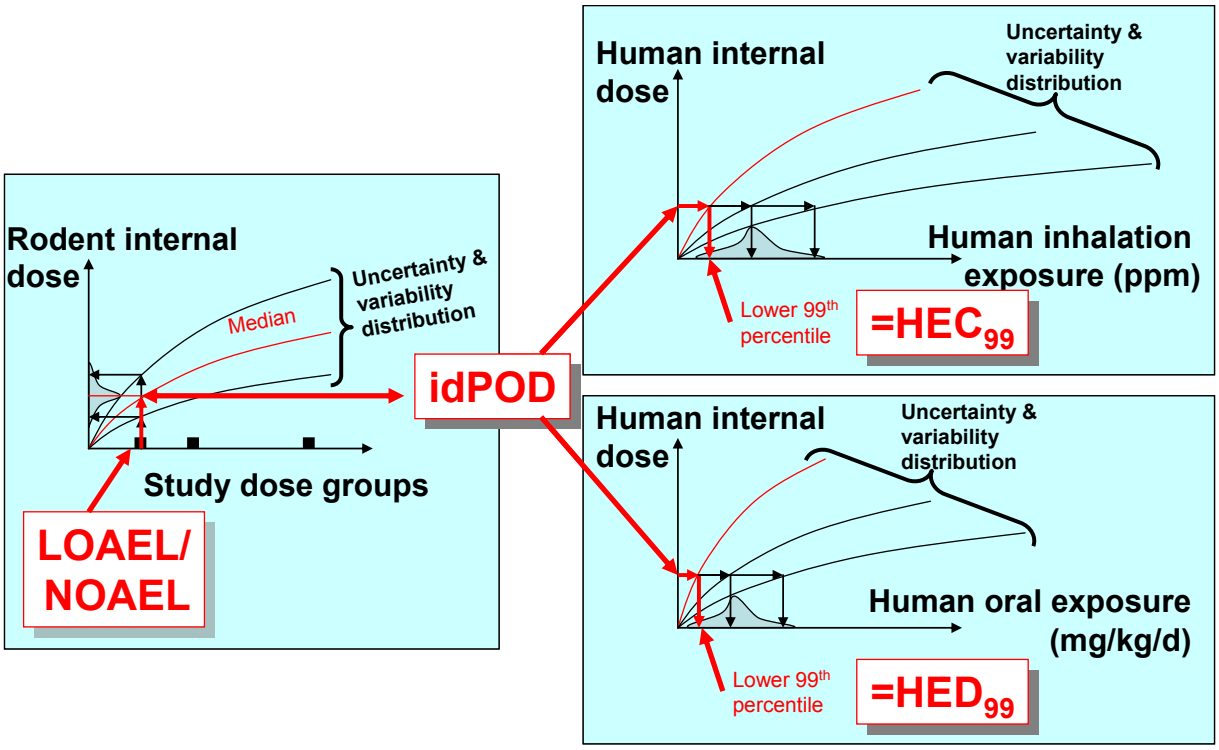
As shown in Figures 5-2 and 5-3, the general approach taken to use the internal dose-metrics in deriving HECs and HEDs was to first apply the rodent PBPK model to get rodent values for the dose-metrics corresponding to the applied doses in a study reporting noncancer effects. The idPOD is then obtained either directly from the internal dose corresponding to the applied dose LOAEL or NOAEL, or by dose-response modeling of responses with respect to the internal doses to derive a BMDL in terms of internal dose. Separately, the human PBPK model is run for a range of continuous exposures from 10^{-1} to 2×10^{3} ppm or mg/kg/day to obtain the relationship between human exposure and internal dose for the same dose-metric used for the rodent. The human equivalent exposure (HEC or HED) corresponding to the idPOD is derived by interpolation. It should be noted that median values of dose-metrics were used for rodents, whereas both median and 99th percentile values were used for humans. As discussed in Section 3.5, the rodent population model characterizes study-to-study variation, while, within a study, animals with the same sex/species/strain combination were assumed to be identical pharmacokinetically and represented by the group average (typically the only data reported). Therefore, use of median dose-metric values can be interpreted as assuming that the animals in the noncancer toxicity study were all "typical" animals and the idPOD is for a rodent that is pharmacokinetically "typical." In practice, the use of median or mean internal doses for rodents did not make much difference except when the uncertainty in the rodent dose-metric was high. The impact of the uncertainty in the rodent PBPK dose-metrics is analyzed quantitatively in Section 5.1.4.2.

[35]An alternative approach (e.g., Clewell et al., 1995) applies the UFs to the internal dose prior to using the human PBPK model to derive a human exposure level. As noted by Barton and Clewell (2000) for previous TCE PBPK models, because the human PBPK model for TCE is linear for all the dose metrics over very broad dose and concentration ranges, essentially identical results would be obtained using this alternative approach. Specifically, for all the primary dose metrics, the difference in the two approaches is less than two-fold, with the results from the critical studies differing by <0.1%. For some studies using AUCBld as an alternative dose metric, the difference ranged from three- to -sevenfold. Overall, use of the alternative approach would not significantly change the noncancer dose-response assessment of TCE, and the derived RfC and RfD would be identical.



Square nodes indicate point values, circle nodes indicate distributions, and the inverted triangle indicates a (deterministic) functional relationship.

Figure 5-2. Flow-chart for dose-response analyses of rodent noncancer effects using PBPK model-based dose-metrics.



In the case where BMD modeling is performed, the applied dose values are replaced by the corresponding median internal dose estimate, and the idPOD is the modeled BMDL in internal dose units.

Figure 5-3. Schematic of combined interspecies, intraspecies, and route-to-route extrapolation from a rodent study LOAEL or NOAEL.

The human population model characterizes individual-to-individual variation, in addition to its uncertainty. The "median" value for the HEC or HED was calculated as a point of comparison but was not actually used for derivation of candidate reference values. Because the RfC and RfD are intended to characterize the dose below which a sensitive individual would likely not experience adverse effects, the overall 99th percentile of the combined uncertainty and variability distribution was used for deriving the HEC and HED (denoted HEC_{99} and HED_{99}) from each idPOD.[36] As shown in Figures 5-2 and 5-3, the HEC_{99} or HED_{99} replaces the quantity $POD/(UF_{A\text{-}pk} \times UF_{H\text{-}pk})$ in the calculation of the RfC or RfD (i.e., the pharmacokinetic components of the UFs representing interspecies extrapolation and human interindividual variability).

As calculated, the extrapolated HEC_{99} and HED_{99} can be interpreted as being the dose or exposure for which there is 99% likelihood that a randomly selected individual will have an internal dose less than or equal to the idPOD derived from the rodent study. By contrast, the HEC_{50} and HED_{50} can be interpreted as being the dose or exposure for which there is 50% likelihood that a randomly selected individual will have an internal dose less than or equal to the idPOD derived from the rodent study. Values of HEC_{99} or HED_{99} are shown for each study and dose-metric considered in Tables 5-13 through 5-18. In addition, values of HEC_{50} or HED_{50} are shown for comparison, to give a sense of the difference between the median and the 99% confidence bound for combined uncertainty and variability. The separate contributions of uncertainty and variability in the human PBPK model are analyzed quantitatively, along with the uncertainty in the rodent PBPK dose-metrics as mentioned above, in Section 5.1.4.2.

[36]While for uncertainty, a 95th percentile is often selected by convention, there is no explicit guidance on the selection of the percentile for human toxicokinetic variability. Ideally, all sources of uncertainty and variability would be included, and percentile selected that is more in line with the levels of risk at which cancer dose-response is typically characterized (e.g., 10^{-6} to 10^{-4}) along with a level of confidence. However, only toxicokinetic uncertainty and variability is assessed quantitatively. Because the distribution here incorporates both uncertainty and variability simultaneously, a percentile higher than the 95th (a conventional choice for uncertainty *only*) was selected. However, percentiles greater than the 99th percentile are likely to be progressively less reliable due to the unknown shape of the tail of the input uncertainty and variability distributions for the PBPK model parameters (which were largely assumed to be normal or lognormal), and the fact that only 42 individuals were incorporated in the PBPK model for characterization of uncertainty and inter-individual variability (see Section 3.5). This concern is somewhat ameliorated because the candidate reference values also incorporate use of UFs to account for inter- and intraspecies toxicodynamic sensitivity.

Table 5-13. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical neurological effects

Effect type Candidate critical studies[a]	Species	POD type	HEC_{50} or HED_{50}	POD, HEC_{99}, or HED_{99}[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC or p-cRfC (ppm)	cRfD or p-cRfD (mg/kg/d)	Candidate critical effect; comments [dose-metric]
Trigeminal nerve effects													
Ruijten et al. (1991)	Human	LOAEL		14	1	1	10	3	1	30	0.47		Trigeminal nerve effects
		HEC	14	5.3	1	1	3	3	1	10	0.53		[TotMetabBW34]
		HEC	14	8.3	1	1	3	3	1	10	0.83		[AUCCBld]
		HED	7.4	7.3	1	1	3	3	1	10		0.73	[TotMetabBW34] (route-to-route)
		HED	59	14	1	1	3	3	1	10		1.4	[AUCCBld] (route-to-route)
Cognitive effects													
Isaacson et al. (1990)	Rat	LOAEL		47	10	10	10	10	1	10,000[d]		0.0047	demyelination in hippocampus
		HED	9.4	9.2	10	3	3	10	1	1,000		0.0092	[TotMetabBW34]
		HED	31	4.3	10	3	3	10	1	1,000		0.0043	[AUCCBld]
		HEC	18	7.1	10	3	3	10	1	1,000	0.0071		[TotMetabBW34] (route-to-route)
		HEC	3.8	2.3	10	3	3	10	1	1,000	0.0023		[AUCCBld] (route-to-route)
Mood and sleep disorders													
Arito et al. (1994)	Rat	LOAEL		12	3	3	10	10	1	1,000	0.012		Changes in wakefulness
		HEC	13	4.8	3	3	3	10	1	300	0.016		[TotMetabBW34]
		HEC	15	9.0	3	3	3	10	1	300	0.030		[AUCCBld]
		HED	6.6	6.5	3	3	3	10	1	300		0.022	[TotMetabBW34] (route-to-route)
		HED	65	15	3	3	3	10	1	300		0.051	[AUCCBld] (route-to-route)

Table 5-13. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical neurological effects (continued)

Effect type Candidate critical studies[a]	Species	POD type	HEC_{50} or HED_{50}	POD, HEC_{99}, or HED_{99}[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC or p-cRfC (ppm)	cRfD or p-cRfD (mg/kg/d)	Candidate critical effect; comments [dose-metric]
Other neurological effects													
Kjellstrand et al. (1987)	Rat	LOAEL		300	10	3	10	10	1	3,000	0.10		↓ regeneration of sciatic nerve
		HEC	274	93	10	3	3	10	1	1,000	0.093		[TotMetabBW34]
		HEC	487	257	10	3	3	10	1	1,000	0.26		[AUCCBld]
		HED	110	97	10	3	3	10	1	1,000		0.097	[TotMetabBW34] (route-to-route)
		HED	436	142	10	3	3	10	1	1,000		0.14	[AUCCBld] (route-to-route)
	Mouse	LOAEL		150	10	3	10	10	1	3,000	0.050		↓ regeneration of sciatic nerve
		HEC	378	120	10	3	3	10	1	1,000	0.12		[TotMetabBW34]
		HEC	198	108	10	3	3	10	1	1,000	0.11		[AUCCBld]
		HED	145	120	10	3	3	10	1	1,000		0.12	[TotMetabBW34] (route-to-route)
		HED	237	76	10	3	3	10	1	1,000		0.076	[AUCCBld] (route-to-route)
Gash et al. (2008)	Rat	LOAEL		710	10	10	10	10	1	10,000[d]		0.071	degeneration of dopaminergic neurons
		HED	56	53	10	3	3	10	1	1,000		0.053	[TotMetabBW34]
		HED	571	192	10	3	3	10	1	1,000		0.19	[AUCCBld]
		HEC	126	47	10	3	3	10	1	1,000	0.047		[TotMetabBW34] (route-to-route)
		HEC	679	363	10	3	3	10	1	1,000	0.36		[AUCCBld] (route-to-route)

[a]Shaded rows represent the p-cRfC or p-cRfD using the preferred PBPK model dose-metric.
[b]Applied dose POD adjusted to continuous exposure unless otherwise noted. POD, HEC_{99}, and HED_{99} have same units as cRfC (ppm) or cRfD (mg/kg/day).
[c]Product of individual UFs, rounded to 3, 10, 30, 100, 300, 1,000, 3,000, or 10,000 [see Footnote d below].
[d]EPA's report on the RfC and RfD processes (U.S. EPA, 2002b) recommends not deriving reference values with a composite UF of >3,000; however, composite UFs exceeding 3,000 are considered here because the derivation of the cRfCs and cRfDs is part of a screening process and the application of the PBPK model for candidate critical effects reduces the values of some of the individual UFs for the p-cRfCs and p-cRfDs.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

Table 5-14. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical kidney effects

Effect type Candidate critical studies[a]	**Species**	**POD type**	**HEC_{50} or HED_{50}**	**POD, HEC_{99}, or HED_{99}[b]**	**UF_S**	**UF_A**	**UF_H**	**UF_L**	**UF_D**	**UF^c**	**cRfC or p-cRfC (ppm)**	**cRfD or p-cRfD (mg/kg/d)**	**Candidate critical effect; comments [dose-metric]**
Histological changes in kidney													
Maltoni (1986) (inhalation)	Rat	BMDL		40.2	1	3	10	1	1	30	1.3		meganucleocytosis; BMR = 10%
		HEC	0.28	0.038	1	3	3	1	1	10	0.0038		[ABioactDCVCBW34]
		HEC	0.45	0.058	1	3	3	1	1	10	0.0058		[AMetGSHBW34]
		HEC	39	15.3	1	3	3	1	1	10	1.5		[TotMetabBW34]
		HED	0.22	0.023	1	3	3	1	1	10		0.0023	[ABioactDCVCBW34] (route-to-route)
		HED	0.35	0.036	1	3	3	1	1	10		0.0036	[AMetGSHBW34] (route-to-route)
		HED	19	19	1	3	3	1	1	10		1.9	[TotMetabBW34] (route-to-route)
NCI (1976)	Mouse	LOAEL		620	1	10	10	30	1	3,000		0.21	toxic nephrosis
		HED	2.9	0.30	1	3	3	30	1	300		0.00101	[AMetGSHBW34]
		HED	51	48	1	3	3	30	1	300		0.160	[TotMetabBW34]
		HEC	3.9	0.50	1	3	3	30	1	300	0.00165		[AMetGSHBW34] (route-to-route)
		HEC	113	42	1	3	3	30	1	300	0.140		[TotMetabBW34] (route-to-route)

Table 5-14. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical kidney effects (continued)

Effect type Candidate critical studies[a]	**Species**	**POD type**	**HEC_{50} or HED_{50}**	**POD, HEC_{99}, or HED_{99}[b]**	**UF_S**	**UF_A**	**UF_H**	**UF_L**	**UF_D**	**UF^c**	**cRfC or p-cRfC (ppm)**	**cRfD or p-cRfD (mg/kg/d)**	**Candidate critical effect; comments [dose-metric]**
Histological changes in kidney													
NTP (1988)	Rat	BMDL		9.45	1	10	10	1	1	100		0.0945	Toxic nephropathy; BMR = 5%; female Marshall (most sensitive sex/strain)
		HED	0.033	0.0034	1	3	3	1	1	10		0.00034	[ABioactDCVCBW34]
		HED	0.053	0.0053	1	3	3	1	1	10		0.00053	[AMetGSHBW34]
		HED	0.75	0.74	1	3	3	1	1	10		0.074	[TotMetabBW34]
		HEC	0.042	0.0056	1	3	3	1	1	10	0.00056		[ABioactDCVCBW34] (route-to-route)
		HEC	0.067	0.0087	1	3	3	1	1	10	0.00087		[AMetGSHBW34] (route-to-route)
		HEC	1.4	0.51	1	3	3	1	1	10	0.051		[TotMetabBW34] (route-to-route)
Maltoni (1986) (oral)	Rat	BMDL		34	1	10	10	1	1	100		0.34	meganucleocytosis; BMR = 10%
		HED	0.15	0.015	1	3	3	1	1	10		0.0015	[ABioactDCVCBW34]
		HED	0.25	0.025	1	3	3	1	1	10		0.0025	[AMetGSHBW34]
		HED	11	11	1	3	3	1	1	10		0.11	[TotMetabBW34]
		HEC	0.19	0.025	1	3	3	1	1	10	0.0025		[ABioactDCVCBW34] (route-to-route)
		HEC	0.31	0.041	1	3	3	1	1	10	0.0041		[AMetGSHBW34] (route-to-route)
		HEC	22	8.5	1	3	3	1	1	10	0.85		[TotMetabBW34] (route-to-route)

Table 5-14. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical kidney effects (continued)

Effect type Candidate critical studies[a]	Species	POD type	HEC_{50} or HED_{50}	POD, HEC_{99}, or HED_{99}[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC or p-cRfC (ppm)	cRfD or p-cRfD (mg/kg/d)	Candidate critical effect; comments [dose-metric]
↑ Kidney/body weight ratio													
Kjellstrand et al. (1983b)	Mouse	BMDL		34.7	1	3	10	1	1	30	1.2		BMR = 10%
		HEC	0.88	0.12	1	3	3	1	1	10	0.012		[AMetGSHBW34]
		HEC	52	21	1	3	3	1	1	10	2.1		[TotMetabBW34]
		HED	0.69	0.070	1	3	3	1	1	10		0.0070	[AMetGSHBW34] (route-to-route)
		HED	25	25	1	3	3	1	1	10		2.5	[TotMetabBW34] (route-to-route)
Woolhiser et al. (2006)	Rat	BMDL		15.7	1	3	10	1	1	30	0.52		BMR = 10%
		HEC	0.099	0.013	1	3	3	1	1	10	0.0013		[ABioactDCVCBW34]
		HEC	0.17	0.022	1	3	3	1	1	10	0.0022		[AMetGSHBW34]
		HEC	29	11	1	3	3	1	1	10	1.1		[TotMetabBW34]
		HED	0.078	0.0079	1	3	3	1	1	10		0.00079	[ABioactDCVCBW34] (route-to-route)
		HED	0.13	0.013	1	3	3	1	1	10		0.0013	[AMetGSHBW34] (route-to-route)
		HED	14	14	1	3	3	1	1	10		1.4	[TotMetabBW34] (route-to-route)

[a]Shaded rows represent the p-cRfC or p-cRfD using the preferred PBPK model dose-metric.
[b]Applied dose POD adjusted to continuous exposure unless otherwise noted. POD, HEC_{99}, and HED_{99} have same units as cRfC or cRfD.
[c]Product of individual UFs, rounded to 3, 10, 30, 100, 300, 1,000, or 3,000.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

Table 5-15. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical liver effects

Effect type Candidate critical studies[a]	**Species**	**POD type**	**HEC_{50} or HED_{50}**	**POD, HEC_{99}, or HED_{99}[b]**	**UF_S**	**UF_A**	**UF_H**	**UF_L**	**UF_D**	**UF[c]**	**cRfC or p-cRfC (ppm)**	**cRfD or p-cRfD (mg/kg/d)**	**Candidate critical effect; comments [dose-metric]**
↑ Liver/body weight ratio													
Kjellstrand et al. (1983b)	Mouse	BMDL		21.6	1	3	10	1	1	30	0.72		BMR = 10% increase
		HEC	25	9.1	1	3	3	1	1	10	0.91		[AMetLiv1BW34]
		HEC	75	24.9	1	3	3	1	1	10	2.5		[TotOxMetabBW34]
		HED	9.0	7.9	1	3	3	1	1	10		0.79	[AMetLiv1BW34] (route-to-route)
		HED	32	25.7	1	3	3	13	1	10		2.6	[TotOxMetabBW34] (route-to-route)
Woolhiser et al. (2006)	Rat	BMDL		25	1	3	10	1	1	30	0.83		BMR = 10% increase
		HEC	53	19	1	3	3	1	1	10	1.9		[AMetLiv1BW34]
		HEC	46	16	1	3	3	1	1	10	1.6		[TotOxMetabBW34]
		HED	19	16	1	3	3	1	1	10		1.6	[AMetLiv1BW34] (route-to-route)
		HED	20	17	1	3	3	1	1	10		1.7	[TotOxMetabBW34] (route-to-route)
Buben and O'Flaherty (1985)	Mouse	BMDL		82	1	10	10	1	1	100		0.82	BMR = 10% increase
		HED	12	10	1	3	3	1	1	10		1.0	[AMetLiv1BW34]
		HED	15	13	1	3	3	1	1	10		1.3	[TotOxMetabBW34]
		HEC	32	11	1	3	3	1	1	10	1.1		[AMetLiv1BW34] (route-to-route)
		HEC	34	11	1	3	3	1	1	10	1.1		[TotOxMetabBW34] (route-to-route)

[a]Shaded rows represent the p-cRfC or p-cRfD using the preferred PBPK model dose-metric.
[b]Applied dose POD adjusted to continuous exposure unless otherwise noted. POD, HEC_{99}, and HED_{99} have same units as cRfC (ppm) or cRfD (mg/kg/day).
[c]Product of individual UFs, rounded to 3, 10, 30, 100, 300, 1,000, or 3,000.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

Table 5-16. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical immunological effects

Effect type Candidate critical studies[a]	Species	POD type	HEC_{50} or HED_{50}	POD, HEC_{99}, or HED_{99}[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC or p-cRfC (ppm)	cRfD or p-cRfD (mg/kg/d)	Candidate critical effect; comments [dose-metric]
↓ Thymus weight													
Keil et al. (2009)	Mouse	LOAEL		0.35	1	10	10	10	1	1,000		0.00035	↓ thymus weight
		HED	0.049	0.048	1	3	3	10	1	100		0.00048	[TotMetabBW34]
		HED	0.20	0.016	1	3	3	10	1	100		0.00016	[AUCCBld]
		HEC	0.092	0.033	1	3	3	10	1	100	0.00033		[TotMetabBW34] (route-to-route)
		HEC	0.014	0.0082	1	3	3	10	1	100	0.000082		[AUCCBld] (route-to-route)
Autoimmunity													
Kaneko et al. (2000)	Mouse	LOAEL		70	10	3	3	10	1	1,000	0.070		Changes in immunoreactive organs - liver (including sporadic necrosis in hepatic lobules), spleen; UF_H = 3 due to autoimmune-prone mouse
		HEC	97	37	10	3	1	10	1	300	0.12		[TotMetabBW34]
		HEC	121	69	10	3	1	10	1	300	0.23		[AUCCBld]
		HED	44	42	10	3	1	10	1	300		0.14	[TotMetabBW34] (route-to-route)
		HED	181	57	10	3	1	10	1	300		0.19	[AUCCBld] (route-to-route)
Keil et al. (2009)	Mouse	LOAEL		0.35	1	10	10	3	1	300		0.0012	↑ anti-dsDNA and anti-ssDNA Abs (early markers for autoimmune disease)
		HED	0.049	0.048	1	3	3	3	1	30		0.0016	[TotMetabBW34]
		HED	0.20	0.016	1	3	3	3	1	30		0.00053	[AUCCBld]
		HEC	0.092	0.033	1	3	3	3	1	30	0.0011		[TotMetabBW34] (route-to-route)
		HEC	0.014	0.0082	1	3	3	3	1	30	0.00027		[AUCCBld] (route-to-route)

Table 5-16. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical immunological effects (continued)

Effect type Candidate critical studies[a]	Species	POD type	HEC_{50} or HED_{50}	POD, HEC_{99}, or HED_{99}[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC or p-cRfC (ppm)	cRfD or p-cRfD (mg/kg/d)	Candidate critical effect; comments [dose-metric]
Immunosuppression													
Woolhiser et al. (2006)	Rat	BMDL		24.9	10	3	10	1	1	300	0.083		↓ PFC response; BMR = 1 SD change; dropped highest dose
		HEC	29	11	10	3	3	1	1	100	0.11		[TotMetabBW34]; all does groups
		HEC	263	140	10	3	3	1	1	100	1.4		[AUCCBld]; all does groups
		HED	14	14	10	3	3	1	1	100		0.14	[TotMetabBW34] (route-to-route); all does groups
		HED	282	91	10	3	3	1	1	100		0.91	[AUCCBld] (route-to-route); all does groups
Sanders et al. (1982b)	Mouse	LOAEL		18	1	10	10	10	1	1000		0.018	↓ stem cell bone marrow recolonization (sustained); ↓ cell-mediated response to SRBC (largely transient during exposure); females more sensitive
		HED	2.5	2.5	1	3	3	10	1	100		0.025	[TotMetabBW34]
		HED	8.8	0.84	1	3	3	10	1	100		0.0084	[AUCCBld]
		HEC	4.8	1.7	1	3	3	10	1	100	0.017		[TotMetabBW34] (route-to-route)
		HEC	0.73	0.43	1	3	3	10	1	100	0.0043		[AUCCBld] (route-to-route)

[a]Shaded rows represent the p-cRfC or p-cRfD using the preferred PBPK model dose-metric.
[b]Applied ose POD adjusted to continuous exposure unless otherwise noted. POD, HEC_{99}, and HED_{99} have same units as cRfC (ppm) or cRfD (mg/kg/day).
[c]Product of individual UFs, rounded to 3, 10, 30, 100, 300, 1,000, or 3,000.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

Table 5-17. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical reproductive effects

Effect type Candidate critical studies[a]	**Species**	**POD type**	**HEC_{50} or HED_{50}**	**POD, HEC_{99}, or HED_{99}[b]**	**UF_S**	**UF_A**	**UF_H**	**UF_L**	**UF_D**	**UF[c]**	**cRfC or p-cRfC (ppm)**	**cRfD or p-cRfD (mg/kg/d)**	**Candidate critical effect; comments [dose-metric]**
Effects on sperm, male reproductive outcomes													
Chia et al. (1996)	Human	BMDL		1.4	10	1	10	1	1	100	0.014		Hyperzoospermia; BMR = 10% extra risk
		HEC	1.4	0.50	10	1	3	1	1	30	0.0017		[TotMetabBW34]
		HEC	1.4	0.83	10	1	3	1	1	30	0.0028		[AUCCBld]
		HED	0.74	0.73	10	1	3	1	1	30		0.024	[TotMetabBW34] (route-to-route)
		HED	15	1.6	10	1	3	1	1	30		0.053	[AUCCBld] (route-to-route)
Xu et al. (2004)	Mouse	LOAEL		180	10	3	10	10	1	3,000	0.060		↓ fertilization
		HEC	190	67	10	3	3	10	1	1,000	0.067		[TotMetabBW34]
		HEC	321	170	10	3	3	10	1	1,000	0.17		[AUCCBld]
		HED	80	73	10	3	3	10	1	1,000		0.073	[TotMetabBW34] (route-to-route)
		HED	324	104	10	3	3	10	1	1,000		0.10	[AUCCBld] (route-to-route)
Kumar et al. (2000b); (2001b)	Rat	LOAEL		45	10	3	10	10	1	3,000	0.015		Multiple sperm effects, increasing severity from 12 to 24 wks
		HEC	32	13	10	3	3	10	1	1,000	0.013		[TotMetabBW34]
		HEC	91	53	10	3	3	10	1	1,000	0.053		[AUCCBld]
		HED	16	16	10	3	3	10	1	1,000		0.016	[TotMetabBW34] (route-to-route)
		HED	157	49	10	3	3	10	1	1,000		0.049	[AUCCBld] (route-to-route)

Table 5-17. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical reproductive effects (continued)

Effect type Candidate critical studies[a]	**Species**	**POD type**	**HEC_{50} or HED_{50}**	**POD, HEC_{99}, or HED_{99}[b]**	**UF_S**	**UF_A**	**UF_H**	**UF_L**	**UF_D**	**UF[c]**	**cRfC or p-cRfC (ppm)**	**cRfD or p-cRfD (mg/kg/d)**	**Candidate critical effect; comments [dose-metric]**
DuTeaux et al. (2004a)	Rat	LOAEL		141	10	10	10	10	1	10,000[d]		0.014	↓ ability of sperm to fertilize in vitro
		HED	66	16	10	3	3	10	1	1,000		0.016	[AUCCBld]
		HED	65	42	10	3	3	10	1	1,000		0.042	[TotOxMetabBW34]
		HEC	16	9.3	10	3	3	10	1	1,000	0.0093		[AUCCBld] (route-to-route)
		HEC	160	43	10	3	3	10	1	1,000	0.043		[TotOxMetabBW34] (route-to-route)
Male reproductive tract effects													
Forkert et al. (2002); Kan et al. (2007)	Mouse	LOAEL		180	10	3	10	10	1	3,000	0.060		Effects on epididymis epithelium
		HEC	190	67	10	3	3	10	1	1,000	0.067		[TotMetabBW34]
		HEC	321	170	10	3	3	10	1	1,000	0.17		[AUCCBld]
		HED	80	73	10	3	3	10	1	1,000		0.073	[TotMetabBW34] (route-to-route)
		HED	324	104	10	3	3	10	1	1,000		0.10	[AUCCBld] (route-to-route)
Kumar et al. (2000b, 2001b)	Rat	LOAEL		45	10	3	10	10	1	3,000	0.015		Testes effects, testicular enzyme markers, increasing severity from 12 to 24 wks
		HEC	32	13	10	3	3	10	1	1,000	0.013		[TotMetabBW34]
		HEC	91	53	10	3	3	10	1	1,000	0.053		[AUCCBld]
		HED	16	16	10	3	3	10	1	1,000		0.016	[TotMetabBW34] (route-to-route)
		HED	157	49	10	3	3	10	1	1,000		0.049	[AUCCBld] (route-to-route)
Female reproductive outcomes													
Narotsky et al. (1995)	Rat	LOAEL		475	1	10	10	10	1	1,000		0.48	Delayed parturition
		HED	47	44	1	3	3	10	1	100		0.44	[TotMetabBW34]
		HED	350	114	1	3	3	10	1	100		1.1	[AUCCBld]
		HEC	98	37	1	3	3	10	1	100	0.37		[TotMetabBW34] (route-to-route)
		HEC	363	190	1	3	3	10	1	100	1.9		[AUCCBld] (route-to-route)

Table 5-17. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical reproductive effects (continued)

Effect type Candidate critical studies[a]	Species	POD type	HEC_{50} or HED_{50}	POD, HEC_{99}, or HED_{99}[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC or p-cRfC (ppm)	cRfD or p-cRfD (mg/kg/d)	Candidate critical effect; comments [dose-metric]
Reproductive behavior													
George et al. (1986)	Rat	LOAEL		389	1	10	10	10	1	1,000		0.39	↓ mating (both sexes exposed)
		HED	85	77	1	3	3	10	1	100		0.77	[TotMetabBW34]
		HED	167	52	1	3	3	10	1	100		0.52	[AUCCBld]
		HEC	204	71	1	3	3	10	1	100	0.71		[TotMetabBW34] (route-to-route)
		HEC	103	60	1	3	3	10	1	100	0.60		[AUCCBld] (route-to-route)

[a]Shaded rows represent the p-cRfC or p-cRfD using the preferred PBPK model dose-metric.
[b]Applied dose POD adjusted to continuous exposure unless otherwise noted. POD, HEC_{99}, and HED_{99} have same units as cRfC (ppm) or cRfD (mg/kg/day).
[c]Product of individual UFs, rounded to 3, 10, 30, 100, 300, 1,000, 3,000, or 10,000 (see footnote [d] below).
[d]EPA's report on the RfC and RfD processes (U.S. EPA, 2002b) recommends not deriving reference values with a composite UF of >3,000; however, composite UFs exceeding 3,000 are considered here because the derivation of the cRfCs and cRfDs is part of a screening process and the application of the PBPK model for candidate critical effects reduces the values of some of the individual UFs for the p-cRfCs and p-cRfDs.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

Table 5-18. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical developmental effects

Effect type Candidate critical studies[a]	Species	POD type	HEC_{50} or HED_{50}	POD, HEC_{99}, or HED_{99}[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC or p-cRfC (ppm)	cRfD or p-cRfD (mg/kg/d)	Candidate critical effect; comments [dose-metric]
Pre- and postnatal mortality													
Healy et al. (1982)	Rat	LOAEL		17	1	3	10	10	1	300	0.057		Resorptions
		HEC	16	6.2	1	3	3	10	1	100	0.062		[TotMetabBW34]
		HEC	23	14	1	3	3	10	1	100	0.14		[AUCCBld]
		HED	8.7	8.5	1	3	3	10	1	100		0.085	[TotMetabBW34] (route-to-route)
		HED	73	20	1	3	3	10	1	100		0.20	[AUCCBld] (route-to-route)
Narotsky et al. (1995)	Rat	BMDL		32.2	1	10	10	1	1	100		0.32	Resorptions; BMR = 1% extra risk
		HED	29	28	1	3	3	1	1	10		2.8	[TotMetabBW34]
		HED	95	29	1	3	3	1	1	10		2.9	[AUCCBld]
		HEC	57	23	1	3	3	1	1	10	2.3		[TotMetabBW34] (route-to-route)
		HEC	40	24	1	3	3	1	1	10	2.4		[AUCCBld] (route-to-route)
Pre- and postnatal growth													
Healy et al. (1982)	Rat	LOAEL		17	1	3	10	10	1	300	0.057		↓ fetal weight; skeletal effects
		HEC	16	6.2	1	3	3	10	1	100	0.062		[TotMetabBW34]
		HEC	23	14	1	3	3	10	1	100	0.14		[AUCCBld]
		HED	8.7	8.5	1	3	3	10	1	100		0.085	[TotMetabBW34] (route-to-route)
		HED	73	20	1	3	3	10	1	100		0.20	[AUCCBld] (route-to-route)

Table 5-18. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical developmental effects (continued)

Effect type Candidate critical studies[a]	Species	POD type	HEC_{50} or HED_{50}	POD, HEC_{99}, or HED_{99}[b]	UF_S	UF_A	UF_H	UF_L	UF_D	UF[c]	cRfC or p-cRfC (ppm)	cRfD or p-cRfD (mg/kg/d)	Candidate critical effect; comments [dose-metric]
Congenital defects													
Johnson et al. (2003)	Rat	BMDL		0.0207	1	10	10	1	1	100		0.00021	Heart malformations (pups); BMR = 1% extra risk; highest-dose group (1,000-fold higher than next highest) dropped to improve model fit
		HED	0.0058	0.0052	1	3	3	1	1	10		0.00052	[TotOxMetabBW34]
		HED	0.019	0.0017	1	3	3	1	1	10		0.00017	[AUCCBld]
		HEC	0.012	0.0037	1	3	3	1	1	10	0.00037		[TotOxMetabBW34] (route-to-route)
		HEC	0.0016	0.00093	1	3	3	1	1	10	0.000093		[AUCCBld] (route-to-route)
Developmental neurotoxicity													
Fredriksson et al. (1993)	Mouse	LOAEL		50	3	10	10	10	1	3,000		0.017	↓ rearing postexposure; pup gavage dose
		HED	4.2	4.1	3	3	3	10	1	300		0.014	[TotMetabBW34]
		HED	27	3.5	3	3	3	10	1	300		0.012	[AUCCBld]
		HEC	8.0	3.0	3	3	3	10	1	300	0.010		[TotMetabBW34] (route-to-route)
		HEC	3.1	1.8	3	3	3	10	1	300	0.0061		[AUCCBld] (route-to-route)
Taylor et al. (1985)	Rat	LOAEL		45	1	10	10	10	1	1,000		0.045	↑ exploration postexposure; estimated dam dose
		HED	11	11	1	3	3	10	1	100		0.11	[TotMetabBW34]
		HED	30	4.1	1	3	3	10	1	100		0.041	[AUCCBld]
		HEC	22	8.4	1	3	3	10	1	100	0.084		[TotMetabBW34] (route-to-route)
		HEC	3.7	2.2	1	3	3	10	1	100	0.022		[AUCCBld] (route-to-route)
Isaacson and Taylor (1989)	Rat	LOAEL		16	1	10	10	10	1	1,000		0.016	↓ myelination in hippocampus; estimated dam dose

Table 5-18. cRfCs and cRfDs (based on applied dose) and p-cRfCs and p-cRfDs (based on PBPK modeled internal dose-metrics) for candidate critical developmental effects (continued)

Effect type Candidate critical studies[a]	**Species**	**POD type**	**HEC_{50} or HED_{50}**	**POD, HEC_{99}, or HED_{99}[b]**	**UF_S**	**UF_A**	**UF_H**	**UF_L**	**UF_D**	**UF[c]**	**cRfC or p-cRfC (ppm)**	**cRfD or p-cRfD (mg/kg/d)**	**Candidate critical effect; comments [dose-metric]**
Developmental immunotoxicity													
Peden-Adams et al. (2006)	Mouse	LOAEL		0.37	1	10	10	10	1	1,000		0.00037	↓ PFC, ↑DTH; POD is estimated dam dose (exposure throughout gestation and lactation + to 3 or 8 wks of age)

[a]Shaded rows represent the p-cRfC or p-cRfD using the preferred PBPK model dose-metric or, in the cases where the PBPK model was not used, the cRfD or cRfC based on applied dose.
[b]Applied dose POD adjusted to continuous exposure unless otherwise noted. POD, HEC_{99}, and HED_{99} have same units as cRfC (ppm) or cRfD (mg/kg/day).
[c]Product of individual UFs, rounded to 3, 10, 30, 100, 300, 1,000, or 3,000.

UF_S = subchronic-to-chronic UF; UF_A = interspecies UF; UF_H = human variability UF; UF_L = LOAEL-to-NOAEL UF; UF_D = database UF

Because they are derived from rodent internal dose estimates, the HEC and HED are derived in the same manner independent of the route of administration of the original rodent study. Therefore, a route-to-route extrapolation from an oral (inhalation) study in rodents to a HEC (HED) in humans is straight-forward. As shown in Tables 5-13–5-18, route-to-route extrapolation was performed for a number of endpoints with low cRfCs and cRfDs to derive p-cRfDs and p-cRfCs.

5.1.3.3. Results and Discussion of p-RfCs and p-RfDs for Candidate Critical Effects

Tables 5-13–5-18 present the p-cRfCs and p-cRfDs developed using the PBPK internal dose-metrics, along with the cRfCs and cRfDs based on applied dose for comparison, for each health effect domain.

The greatest impact of using the PBPK model was, as expected, for kidney effects, since as discussed in Sections 3.3 and 3.5, some toxicokinetic data indicate substantially more GSH conjugation of TCE and subsequent bioactivation of GSH-conjugates in humans relative to rats or mice. In addition, as discussed in Sections 3.3 and 3.5, the available in vivo data indicate high interindividual variability in the amount of TCE conjugated with GSH. The overall impact is that the p-cRfCs and p-cRfDs based on the preferred dose-metric of bioactivated DCVC are 300–400-fold lower than the corresponding cRfCs and cRfDs based on applied dose. As shown in Figure 3-20 in Section 3.5, for this dose-metric there is about a 30–100-fold difference (depending on exposure route and level) between rats and humans in the "central estimates" of interspecies differences for the fraction of TCE that is bioactivated as DCVC. The uncertainty in the human central estimate is only on the order of 2-fold (in either direction), while that in the rat central estimate is substantially greater, about 10-fold (in either direction). In addition, the interindividual variability about the human median estimate is on the order of 10-fold (in either direction). However, as noted in Section 3.3.3.2, there are a number of discrepancies in estimates for the extent of GSH conjugation that may be related to different analytical methods, and it is possible that GSH conjugation data to which the PBPK model was calibrated overestimated the extent of DCVG formation by a substantial amount. Thus, there remain significant uncertainties in the human estimates of GSH conjugation derived from the PBPK model. Moreover, the estimates of the amount bioactivated are indirect, derived from the difference between overall GSH conjugation flux and NAcDCVC excretion (see Section 3.5.7.3.1). Therefore, while there is a high degree of confidence in the nephrotoxic hazard posed by TCE, there is less confidence in the p-cRfCs and p-RfDs derived using GSH conjugation dose-metrics for these effects.

In addition, in two cases in which BMD modeling was employed, using internal dose-metrics led to a sufficiently different dose-response shape so as to change the resulting reference value by greater than fivefold. For the Woolhiser et al. (2006) decreased PFC response, this occurred with the AUC of TCE in blood dose-metric, leading to a p-cRfC 17-fold higher than the

cRfC based on applied dose. However, the model fit for this effect using this metric was substantially worse than the fit using the preferred metric of Total oxidative metabolism. Moreover, whereas an adequate fit was obtained with applied dose only with the highest-dose group dropped, all of the dose groups were included when the total oxidative metabolism dose-metric was used while still resulting in a good model fit. Therefore, it appears that using this metric resolves some of the low-dose supralinearity in the dose-response curve. Nonetheless, the overall impact of the preferred metric was minimal, as the p-cRfC based on the Total oxidative metabolism metric was less than 1.4-fold larger than the cRfC based on applied dose. The second case in which BMD modeling based on internal doses changed the candidate reference value by more than fivefold was for resorptions reported by Narotsky et al. (1995). Here, the p-cRfDs were seven- to eightfold larger than the corresponding cRfD based on applied dose. However, for applied dose, there is substantial uncertainty in the low-dose curvature of the dose-response curve. This uncertainty persisted with the use of internal dose-metrics, so the BMD remains somewhat uncertain (see figures in Appendix F).In the remaining cases, which generally involved the "generic" dose-metrics of total metabolism and AUC of TCE in blood, the p-cRfCs and p-cRfDs were within fivefold of the corresponding cRfC or cRfD based on applied dose, with the vast majority within threefold. This suggests that the standard UFs for inter- and intraspecies pharmacokinetic variability are fairly accurate in capturing these differences for these TCE studies.

5.1.4. Uncertainties in cRfCs and cRfDs

5.1.4.1. Qualitative Uncertainties

An underlying assumption in deriving a reference value for a noncancer effect is that the dose-response relationship has a threshold. Thus, a fundamental uncertainty is the validity of that assumption. For some effects, in particular effects on very sensitive processes (e.g., developmental processes) or effects for which there is a nontrivial background level and even small exposures may contribute to background disease processes in more susceptible people, a practical threshold (i.e., a threshold within the range of environmental exposure levels of regulatory concern) may not exist.

Nonetheless, under the assumption of a threshold, the desired exposure level to have as a reference value is the maximum level at which there is no appreciable risk for an adverse effect in (nonnegligible) sensitive subgroups (of humans). However, because it is not possible to know what this level is, "uncertainty factors" are used to attempt to address quantitatively various aspects, depending on the data set, of qualitative uncertainty.

First there is uncertainty about the POD for the application of UFs. Conceptually, the POD should represent the maximum exposure level at which there is no appreciable risk for an adverse effect in the study population under study conditions (i.e., the threshold in the dose-response relationship). Then, the application of the relevant UFs is intended to convey that

exposure level to the corresponding exposure level for sensitive human subgroups exposed continuously for a lifetime. In fact, it is again not possible to know that exposure level even for a laboratory study because of experimental limitations (e.g., the power to detect an effect, dose spacing, measurement errors, etc.), and crude approximations like the NOAEL or a BMDL are used. If a LOAEL is used as the POD, then the LOAEL-to-NOAEL UF is applied as an adjustment factor to get a better approximation of the desired exposure level (threshold), but the necessary extent of adjustment is unknown.

If a BMDL is used as the POD, there are uncertainties regarding the appropriate dose-response model to apply to the data, but these should be minimal if the modeling is in the observable range of the data. There are also uncertainties about what BMR to use to best approximate the desired exposure level (threshold, see above). For continuous endpoints, in particular, it is often difficult to identify the level of change that constitutes the "cut-point" for an adverse effect. Sometimes, to better approximate the desired exposure level, a BMR somewhat below the observable range of the data is selected. In such cases, the model uncertainty is increased, but this is a trade-off to reduce the uncertainty about the POD not being a good approximation for the desired exposure level.

For each of these types of PODs, there are additional uncertainties pertaining to adjustments to the administered exposures (doses). Typically, administered exposures (doses) are converted to equivalent continuous exposures (daily doses) over the study exposure period under the assumption that the effects are related to concentration × time, independent of the daily (or weekly) exposure regimen (i.e., a daily exposure of 6 hours to 4 ppm is considered equivalent to 24 hours of exposure to 1 ppm). However, the validity of this assumption is generally unknown, and, if there are dose-rate effects, the assumption of $C \times t$ equivalence would tend to bias the POD downwards. Where there is evidence that administered exposure better correlates to the effect than equivalent continuous exposure averaged over the study exposure period (e.g., visual effects), administered exposure was not adjusted. For the PBPK analyses in this assessment, the actual administered exposures are taken into account in the PBPK modeling, and equivalent daily values (averaged over the study exposure period) for the dose-metrics are obtained (see above, Section 5.1.3.2). Additional uncertainties about the PBPK-based estimates include uncertainties about the appropriate dose-metric for each effect, although for some effects there was better information about relevant dose-metrics than for others (see Section 5.1.3.1). Furthermore, as discussed in Section 3.3.3.2, there remains substantial uncertainty in the extrapolation of GSH conjugation from rodents to humans due to limitations in the available data.

Second, there is uncertainty about the UFs. The human variability UF is to some extent an adjustment factor because, for more sensitive people, the dose-response relationship shifts to lower exposures. However, there is uncertainty about the extent of the adjustment required (i.e., about the distribution of human susceptibility). Therefore, in the absence of data on a more

sensitive population(s) or on the distribution of susceptibility in the general population, an UF of 10 is generally used, in part for pharmacokinetic variability and in part for pharmacodynamic variability. The PBPK analyses in this assessment attempt to account for the pharmacokinetic portion of human variability using human data on pharmacokinetic variability. A quantitative uncertainty analysis of the PBPK-derived dose-metrics used in the assessment is presented in Section 5.1.4.2. There is still uncertainty regarding the susceptible subgroups for TCE exposure and the extent of pharmacodynamic variability.

If the data used to determine a particular POD are from laboratory animals, an interspecies extrapolation UF is used. This UF is also to some extent an adjustment factor for the expected scaling for toxicologically-equivalent doses across species (i.e., according to body weight to the ¾ power for oral exposure). However, there is also uncertainty about the true extent of interspecies differences for specific noncancer effects from specific chemical exposures. Often, the "adjustment" component of this UF has been attributed to pharmacokinetics, while the "uncertainty" component has been attributed to pharmacodynamics, but as discussed above in Section 5.1.3.1, this is not the only interpretation supported. For oral exposures, the standard value for the interspecies UF is 10, which can be viewed as breaking down (approximately) to a factor of three for the "adjustment" (nominally pharmacokinetics) and a factor of three for the "uncertainty" (nominally pharmacodynamics). For inhalation exposures, no adjustment across species is generally assumed for fixed air concentrations (ppm equivalence), and the standard value for the interspecies UF is 3, reflecting only "uncertainty" (nominally pharmacodynamics). The PBPK analyses in this assessment attempt to account for the "adjustment" portion of interspecies extrapolation using rodent pharmacokinetic data to estimate internal doses for various dose-metrics. With respect to the "uncertainty" component, quantitative uncertainty analyses of the PBPK-derived dose-metrics used in the assessment are presented in Section 5.1.4.2. However, these only address the pharmacokinetic uncertainties in a particular dose-metric, and there is still uncertainty regarding the true dose-metrics. Nor do the PBPK analyses address the uncertainty in either cross-species pharmacodynamic differences (i.e., about the assumption that equal doses of the appropriate dose-metric convey equivalent risk across species for a particular endpoint from a specific chemical exposure) or in cross-species pharmacokinetic differences not accounted for by the PBPK model dose-metrics (e.g., departures from the assumed interspecies scaling of clearance of the active moiety, in the cases where only its production is estimated). A value of 3 is typically used for the "uncertainty" about cross-species differences, and this generally represents true uncertainty because it is usually unknown, even after adjustments have been made to account for the expected interspecies differences, whether humans have more or less susceptibility, and to what degree, than the laboratory species in question.

If only subchronic data are available, the subchronic-to-chronic UF is to some extent an adjustment factor because, if the effect becomes more severe with increasing exposure, then

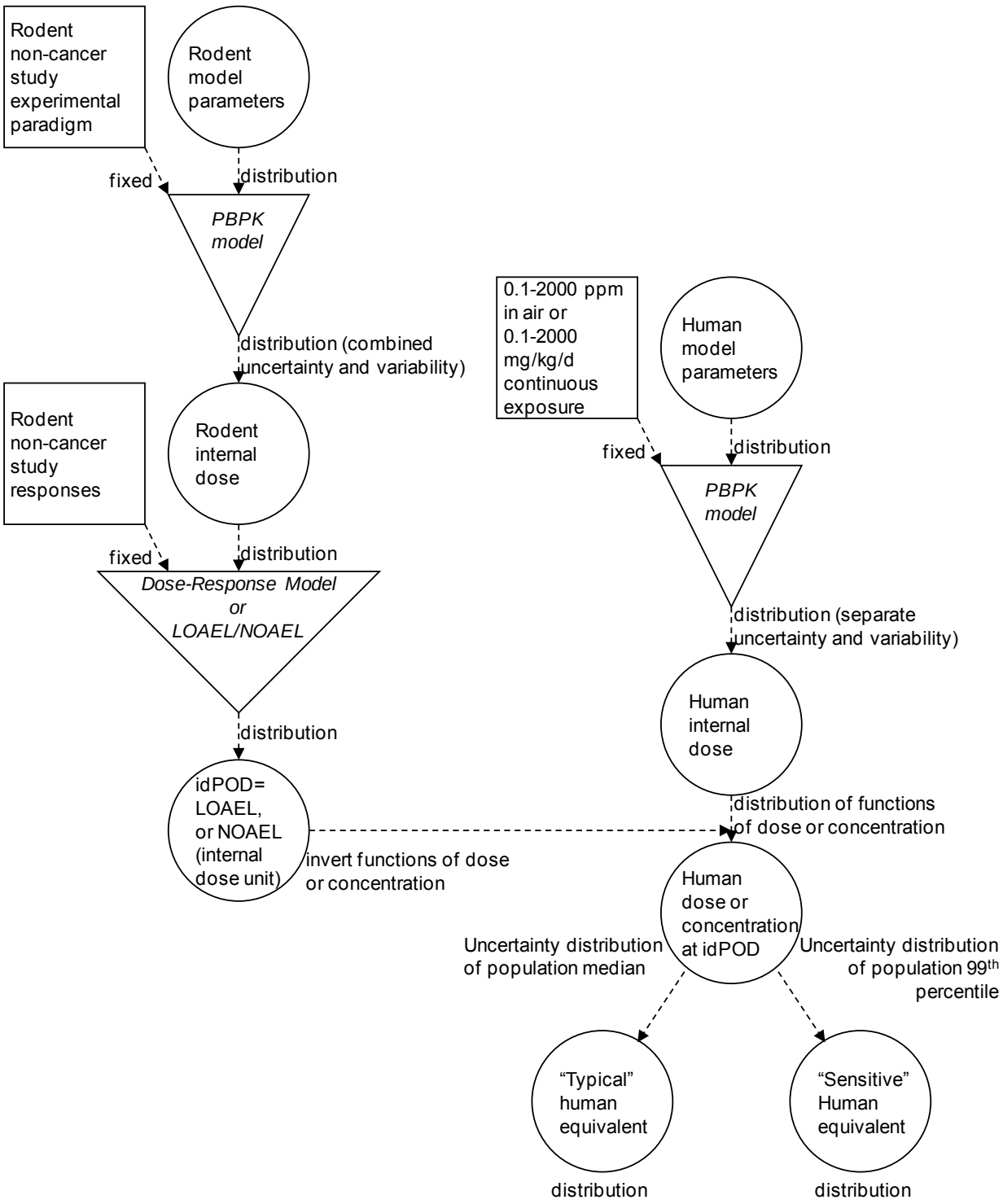
chronic exposure would shift the dose-response relationship to lower exposures. However, the true extent of the shift is unknown.

Sometimes a database UF is also applied to address limitations or uncertainties in the database. The overall database for TCE is quite extensive, with studies for many different types of effects, including two-generation reproductive studies, as well as neurological, immunological, and developmental immunological studies. In addition, there were sufficient data to develop a reliable PBPK model to estimate route-to-route extrapolated doses for some candidate critical effects for which data were only available for one route of exposure. Thus, there is a high degree of confidence that the TCE database was sufficient to identify sensitive endpoints.

5.1.4.2. Quantitative Uncertainty Analysis of PBPK Model-Based Dose-metrics for LOAEL- or NOAEL-Based PODs

The Bayesian analysis of the PBPK model for TCE generates distributions of uncertainty and variability in the internal dose-metrics that can be readily used for characterizing the uncertainty and variability in the PBPK model-based derivations of the HEC and HED. However, in the primary analysis, a number of simplifications are made including: (1) use of median estimates for rodent internal doses and (2) expressing the "sensitive human" HEC and HED in terms of combined uncertainty and variability. Therefore, a 2-dimensional quantitative uncertainty and variability analysis is performed, the objective of which is to characterize the impact of these assumptions.

As shown in Figure 5-4, the overall approach taken for the uncertainty analysis is similar to that used for the point estimates except for the carrying through of separate uncertainty and variability distributions throughout the analysis. In particular, to address simplification (1), above, the distribution of rodent internal dose estimates is carried through; and to address simplification (2), above, uncertainty and variability distributions in human internal dose estimates are kept distinct.



Square nodes indicate point values, circle nodes indicate distributions, and the inverted triangle indicates a (deterministic) functional relationship.

Figure 5-4. Flow-chart for uncertainty analysis of HECs and HEDs derived using PBPK model-based dose-metrics.

Because of a lack of tested software and limitations of time and resources, this analysis was not performed for idPODs based on BMD modeling, and was only performed for idPODs derived from a LOAEL or NOAEL. However, for those endpoints for which BMD modeling was performed, for the purposes of this uncertainty analysis, an alternative idPOD was used based on the study LOAEL or NOAEL.

In brief, the methodology involves an iterative process of sampling from three separate distributions—the uncertainty distribution of rodent PBPK model parameters, the uncertainty distribution of human population PBPK parameters, and the variability distribution of human individual PBPK model parameters—the latter two of which are related hierarchically. For a sample from the rodent parameter distribution, the corresponding idPOD is calculated. Then, an individual is sampled from a human population distribution, which itself is sampled from the uncertainty distribution of population parameters. For this individual, a human equivalent exposure (HEC or HED) corresponding to the idPOD is derived by interpolation. Taking multiple individuals from this population, a HEC or HED corresponding to the median and 99th percentile individuals is then derived. Repeating this process (starting again with a sample from the rodent distribution) results in two distributions (both reflecting uncertainty): one of "typical" individuals represented by the distribution of population medians, and one of "sensitive" individuals represented by the distribution of an upper percentile of the population (e.g., 99th percentile). This uncertainty reflects both uncertainty in the rodent internal dose and uncertainty in the human population parameters. Thus, for selected quantiles of the population and level of confidence (e.g., Xth percentile individual at Yth% confidence), the interpretation is that at the resulting HEC or HED, there is Y% confidence that X% of the population has an internal dose less than that of the rodent in the toxicity study.

As shown in Tables 5-19–5-23, the HEC_{99} and HED_{99} derived using the rodent median dose-metrics and the combined uncertainty and variability in human dose-metrics is generally near (within 1.3-fold of) the median confidence level estimate of the HEC and HED for the 99th percentile individual. Therefore, the interpretation is that there is about 50% confidence that human exposure at the HEC_{99} or HED_{99} will, in 99% of the human population, lead to an internal dose less than or equal to that in the subjects (rodent or human) exposed at the POD in the corresponding study.

Table 5-19. Comparison of —sensitive individual" HECs or HEDs for neurological effects based on PBPK modeled internal dose-metrics at different levels of confidence and sensitivity, at the NOAEL or LOAEL

Candidate critical effect Candidate critical study[a] (species)	POD type	Ratio HEC/D_{50}: HEC/D_{99}	HEC_X or HED_X[b]			[Dose-metric]
			$X = 99$	$X = 99$, median	$X = 99$, 95lcb	
Neurological						
Trigeminal nerve effects Ruijten et al. (1991) (human)	HEC	2.62	5.4	5.4	2.6	[TotMetabBW34]
	HEC	1.68	8.3	8.3	4.9	[AUCCBld]
	HED	1.02	7.3	7.2	3.8	[TotMetabBW34] (rtr)
	HED	4.31	14	16	8.0	[AUCCBld] (rtr)
Demyelination in hippocampus Isaacson et al. (1990) (rat)	HED	1.02	9.21	9.20	7.39	[TotMetabBW34]
	HED	7.20	4.29	5.28	2.52	[AUCCBld]
	HEC	2.59	7.09	6.77	4.94	[TotMetabBW34] (rtr)
	HEC	1.68	2.29	2.42	0.606	[AUCCBld] (rtr)
Changes in wakefulness Arito et al. (1994) (rat)	HEC	2.65	4.79	4.86	2.37	[TotMetabBW34]
	HEC	1.67	9	9.10	4.63	[AUCCBld]
	HED	1.02	6.46	6.50	3.39	[TotMetabBW34] (rtr)
	HED	4.25	15.2	18.0	8.33	[AUCCBld] (rtr)
↓ Regeneration of sciatic nerve Kjellstrand et al. (1987) (rat)	HEC	2.94	93.1	93.6	38.6	[TotMetabBW34]
	HEC	1.90	257	266	114	[AUCCBld]
	HED	1.13	97.1	96.8	43.4	[TotMetabBW34] (rtr)
	HED	3.08	142	147	78.0	[AUCCBld] (rtr)
↓ Regeneration of sciatic nerve Kjellstrand et al. (1987) (mouse)	HEC	3.16	120	125	48.8	[TotMetabBW34]
	HEC	1.84	108	111	59.7	[AUCCBld]
	HED	1.21	120	121	57.0	[TotMetabBW34] (rtr)
	HED	2.13	75.8	79.1	53.4	[AUCCBld] (rtr)
Degeneration of dopaminergic neurons Gash et al. (2008) (rat)	HED	1.06	53	53.8	17.1	[TotMetabBW34]
	HED	2.98	192	199	94.7	[AUCCBld]
	HEC	2.70	46.8	47.9	14.2	[TotMetabBW34] (rtr)

[a]Shaded rows denote results for the primary dose-metric.
[b]HEC_{99} = the 99th percentile of the combined human uncertainty and variability distribution of continuous exposure concentrations that lead to the (fixed) median estimate of the rodent internal dose at the POD; $HEC_{99,median}$ (or $HEC_{99,95lcb}$) = the median (or 95th percentile lower confidence bound) estimate of the uncertainty distribution of continuous exposure concentrations for which the 99th percentile individual has an internal dose less than the (uncertain) rodent internal dose at the POD.

rtr = route-to-route extrapolation using PBPK model and the specified dose-metric

Table 5-20. Comparison of —sensitive individual" HECs or HEDs for kidney and liver effects based on PBPK modeled internal dose-metrics at different levels of confidence and sensitivity, at the NOAEL or LOAEL

Candidate critical effect Candidate critical study[a] (species)	POD type	Ratio HEC/D_{50}: HEC/D_{99}	HEC_X or HED_X[b]			[Dose-metric]
			$X = 99$	$X = 99$, median	$X = 99$, 95lcb	
Kidney						
Meganucleocytosis [NOAEL][c] Maltoni et al. (1986) (rat inhalation)	HEC	7.53	0.0233	0.0260	0.00366	[ABioactDCVCBW34]
	HEC	7.70	0.0364	0.0411	0.00992	[AMetGSHBW34]
	HEC	2.57	8.31	7.97	4.03	[TotMetabBW34]
	HED	9.86	0.0140	0.0156	0.00216	[ABioactDCVCBW34] (rtr)
	HED	9.83	0.0223	0.0242	0.00597	[AMetGSHBW34] (rtr)
	HED	1.02	10.6	10.7	5.75	[TotMetabBW34] (rtr)
Toxic nephrosis NCI (1976) (mouse)	HED	9.51	0.30	0.32	0.044	[AMetGSHBW34]
	HED	1.05	48	48.9	16.2	[TotMetabBW34]
	HEC	7.78	0.50	0.514	0.0703	[AMetGSHBW34] (rtr)
	HEC	2.67	42	43.5	13.7	[TotMetabBW34] (rtr)
Toxic nephropathy [LOAEL][c] NTP (1988) (rat)	HED	9.75	0.121	0.126	0.0177	[ABioactDCVCBW34]
	HED	9.64	0.193	0.210	0.0379	[AMetGSHBW34]
	HED	1.03	33.1	33.1	11.1	[TotMetabBW34]
	HEC	7.55	0.201	0.204	0.0269	[ABioactDCVCBW34] (rtr)
	HEC	7.75	0.314	0.353	0.0676	[AMetGSHBW34] (rtr)
	HEC	2.59	28.2	27.2	8.77	[TotMetabBW34] (rtr)
Meganucleocytosis [NOAEL][c] Maltoni et al. (1986) (rat oral)	HED	9.85	0.0133	0.0145	0.00158	[ABioactDCVCBW34]
	HED	9.86	0.0214	0.0249	0.00366	[AMetGSHBW34]
	HED	1.02	8.7	8.57	4.95	[TotMetabBW34]
	HEC	7.55	0.022	0.0249	0.00256	[ABioactDCVCBW34] (rtr)
	HEC	7.71	0.0349	0.0424	0.00615	[AMetGSHBW34] (rtr)
	HEC	2.60	6.66	6.31	3.70	[TotMetabBW34] (rtr)
↑ Kidney/body weight ratio [NOAEL][c] Kjellstrand et al. (1983a) (mouse)	HEC	7.69	0.111	0.103	0.00809	[AMetGSHBW34]
	HEC	2.63	34.5	33.7	13.5	[TotMetabBW34]
	HED	9.78	0.068	0.00641	0.00497	[AMetGSHBW34] (rtr)
	HED	1.03	39.9	39.2	17.9	[TotMetabBW34] (rtr)

Table 5-20. Comparison of —sensitive individual" HECs or HEDs for kidney and liver effects based on PBPK modeled internal dose-metrics at different levels of confidence and sensitivity, at the NOAEL or LOAEL (continued)

Candidate critical effect Candidate critical study (species)	POD type	Ratio HEC/D_{50}: HEC/D_{99}	HEC_X or HED_X			[Dose-metric]
			$X = 99$	$X = 99$, median	$X = 99$, 95lcb	
↑ Kidney/body weight ratio [NOAEL][c] Woolhiser et al. (2006) (rat)	HEC	7.53	0.0438	0.0481	0.00737	[ABioactDCVCBW34]
	HEC	7.70	0.0724	0.0827	0.0179	[AMetGSHBW34]
	HEC	2.54	16.1	15.2	7.56	[TotMetabBW34]
	HED	9.84	0.0264	0.0282	0.00447	[ABioactDCVCBW34] (rtr)
	HED	9.81	0.0444	0.0488	0.0111	[AMetGSHBW34] (rtr)
	HED	1.02	19.5	19.2	10.5	[TotMetabBW34] (rtr)
Liver						
↑ Liver/body weight ratio [LOAEL][c] Kjellstrand et al. (1983a) (mouse)	HEC	2.85	16.2	16.3	6.92	[AMetLiv1BW34]
	HEC	3.63	40.9	38.1	15.0	[TotOxMetabBW34]
	HED	1.16	14.1	14.1	5.85	[AMetLiv1BW34] (rtr)
	HED	1.53	40.1	39.4	17.9	[TotOxMetabBW34] (rtr)
↑ Liver/body weight ratio [NOAEL][c] Woolhiser et al. (2006) (rat)	HEC	2.86	20.7	21.0	11.0	[AMetLiv1BW34]
	HEC	2.94	18.2	17.1	8.20	[TotOxMetabBW34]
	HED	1.20	17.8	17.7	9.94	[AMetLiv1BW34] (rtr)
	HED	1.21	19.6	19.3	10.5	[TotOxMetabBW34] (rtr)
↑ Liver/body weight ratio [LOAEL][c] Buben and O'Flaherty (1985) (mouse)	HED	1.14	8.82	8.95	4.17	[AMetLiv1BW34]
	HED	1.14	9.64	9.78	5.28	[TotOxMetabBW34]
	HEC	2.80	10.1	9.97	4.83	[AMetLiv1BW34] (rtr)
	HEC	3.13	7.83	7.65	4.23	[TotOxMetabBW34] (rtr)

[a]Shaded rows denote results for the primary dose-metric.
[b]HEC_{99} = the 99th percentile of the combined human uncertainty and variability distribution of continuous exposure concentrations that lead to the (fixed) median estimate of the rodent internal dose at the POD; $HEC_{99,median}$ (or $HEC_{99,95lcb}$) = the median (or 95th percentile lower confidence bound) estimate of the uncertainty distribution of continuous exposure concentrations for which the 99th percentile individual has an internal dose less than the (uncertain) rodent internal dose at the POD.
[c]BMDL used for p-cRfC or p-cRfD, but LOAEL or NOAEL (as noted) used for uncertainty analysis.

rtr = route-to-route extrapolation using PBPK model and the specified dose-metric

Table 5-21. Comparison of —sensitive individual" HECs or HEDs for immunological effects based on PBPK modeled internal dose-metrics at different levels of confidence and sensitivity, at the NOAEL or LOAEL

Candidate critical effect Candidate critical study[a] (species)	POD type	Ratio HEC/D_{50}: HEC/D_{99}	HEC_X or HED_X[b]			[Dose-metric]
			$X = 99$	$X = 99$, median	$X = 99$, 95lcb	
Immunological						
Changes in immunoreactive organs—liver (including sporadic necrosis in hepatic lobules), spleen Kaneko et al. (2000) (mouse)	HEC	2.65	36.7	38.3	16.0	[TotMetabBW34]
	HEC	1.75	68.9	70.0	37.1	[AUCCBld]
	HED	1.04	42.3	43.3	21.3	[TotMetabBW34] (rtr)
	HED	3.21	56.5	59.0	39.8	[AUCCBld] (rtr)
↑ Anti-dsDNA and anti-ssDNA Abs (early markers for auto-immune disease); ↓ thymus weight Keil et al. (2009) (mouse)	HED	1.02	0.0482	0.0483	0.0380	[TotMetabBW34]
	HED	12.1	0.0161	0.0189	0.00363	[AUCCBld]
	HEC	2.77	0.0332	0.0337	0.0246	[TotMetabBW34] (rtr)
	HEC	1.69	0.00821	0.00787	0.00199	[AUCCBld] (rtr)
↓ PFC response [NOAEL][c] Woolhiser et al. (2006) (rat)	HEC	2.54	16.1	15.2	7.56	[TotMetabBW34]
	HEC	1.73	59.6	60.1	26.2	[AUCCBld]
	HED	1.02	19.5	19.2	10.5	[TotMetabBW34] (rtr)
	HED	3.21	52	55.9	33.0	[AUCCBld] (rtr)
↓ Stem cell bone marrow recolonization; ↓ cell-mediated response to SRBC Sanders et al. (1982b) (mouse)	HED	1.02	2.48	2.48	1.94	[TotMetabBW34]
	HED	10.5	0.838	0.967	0.187	[AUCCBld]
	HEC	2.77	1.72	1.75	1.28	[TotMetabBW34] (rtr)
	HEC	1.68	0.43	0.412	0.103	[AUCCBld] (rtr)

[a]Shaded rows denote results for the primary dose-metric.
[b]HEC_{99} = the 99th percentile of the combined human uncertainty and variability distribution of continuous exposure concentrations that lead to the (fixed) median estimate of the rodent internal dose at the POD; $HEC_{99,median}$ (or $HEC_{99,95lcb}$) = the median (or 95th percentile lower confidence bound) estimate of the uncertainty distribution of continuous exposure concentrations for which the 99th percentile individual has an internal dose less than the (uncertain) rodent internal dose at the POD.
[c]BMDL used for p-cRfC or p-cRfD, but LOAEL or NOAEL (as noted) used for uncertainty analysis.

rtr = route-to-route extrapolation using PBPK model and the specified dose-metric

Table 5-22. Comparison of —sensitive individual" HECs or HEDs for reproductive effects based on PBPK modeled internal dose-metrics at different levels of confidence and sensitivity, at the NOAEL or LOAEL

Candidate critical effect Candidate critical study[a] (species)	POD type	Ratio HEC/D_{50}: HEC/D_{99}	HEC_X or HED_X[b]			[Dose-metric]
			$X = 99$	$X = 99$, median	$X = 99$, 95lcb	
Reproductive						
Hyperzoospermia Chia et al. (1996) (human)	HEC	2.78	0.50	0.53	0.25	[TotMetabBW34]
	HEC	1.68	0.83	0.83	0.49	[AUCCBld]
	HED	1.02	0.73	0.71	0.37	[TotMetabBW34] (rtr)
	HED	9.69	1.6	2.0	0.92	[AUCCBld] (rtr)
↓ Fertilization Xu et al. (2004) (mouse)	HEC	2.85	66.6	72.3	26.6	[TotMetabBW34]
	HEC	1.89	170	171	97.1	[AUCCBld]
	HED	1.09	73.3	76.9	32.9	[TotMetabBW34] (rtr)
	HED	3.11	104	109	67.9	[AUCCBld] (rtr)
Multiple sperm effects, testicular enzyme markers Kumar et al. (2001b; 2000b) (rat)	HEC	2.53	12.8	12.2	6.20	[TotMetabBW34]
	HEC	1.72	53.2	54.4	23.2	[AUCCBld]
	HED	1.02	15.8	15.7	8.60	[TotMetabBW34] (rtr)
	HED	3.21	48.8	52.6	30.6	[AUCCBld] (rtr)
↓ Ability of sperm to fertilize in vitro DuTeaux et al. (2004a) (rat)	HED	4.20	15.6	18.1	4.07	[AUCCBld]
	HED	1.57	41.7	41.9	32.0	[TotOxMetabBW34]
	HEC	1.67	9.3	10.1	2.09	[AUCCBld] (rtr)
	HEC	3.75	42.5	55.6	39.1	[TotOxMetabBW34] (rtr)
Effects on epididymis epithelium Forkert et al. (2002); Kan et al. (2007) (mouse)	HEC	2.85	66.6	72.3	26.6	[TotMetabBW34]
	HEC	1.89	170	171	97.1	[AUCCBld]
	HED	1.09	73.3	76.9	32.9	[TotMetabBW34] (rtr)
	HED	3.11	104	109	67.9	[AUCCBld] (rtr)
Testes effects Kumar et al. (2001b; 2000b) (rat)	HEC	2.53	12.8	12.2	6.20	[TotMetabBW34]
	HEC	1.72	53.2	54.4	23.2	[AUCCBld]
	HED	1.02	15.8	15.7	8.60	[TotMetabBW34] (rtr)
	HED	3.21	48.8	52.6	30.6	[AUCCBld] (rtr)
Delayed parturition Narotsky et al. (1995) (rat)	HED	1.06	44.3	43.9	15.1	[TotMetabBW34]
	HED	3.07	114	119	47.7	[AUCCBld]
	HEC	2.66	36.9	35.3	11.6	[TotMetabBW34] (rtr)
	HEC	1.91	190	197	48.1	[AUCCBld] (rtr)

Table 5-22. Comparison of —sensitive individual" HECs or HEDs for reproductive effects based on PBPK modeled internal dose-metrics at different levels of confidence and sensitivity, at the NOAEL or LOAEL (continued)

Candidate critical effect Candidate critical study (species)	POD type	Ratio HEC/D_{50}: HEC/D_{99}	HEC_X or HED_X			[Dose-metric]
			$X = 99$	$X = 99$, median	$X = 99$, 95lcb	
↓ Mating (both sexes exposed) George et al. (1986) (rat)	HED	1.10	77.4	77.1	34.2	[TotMetabBW34]
	HED	3.21	51.9	55.8	14.7	[AUCCBld]
	HEC	2.86	71.1	70.0	29.5	[TotMetabBW34] (rtr)
	HEC	1.73	59.5	63.3	8.14	[AUCCBld] (rtr)

[a]Shaded rows denote results for the primary dose-metric.
[b]HEC_{99} = the 99th percentile of the combined human uncertainty and variability distribution of continuous exposure concentrations that lead to the (fixed) median estimate of the rodent internal dose at the POD; $HEC_{99,median}$ (or $HEC_{99,95lcb}$) = the median (or 95th percentile lower confidence bound) estimate of the uncertainty distribution of continuous exposure concentrations for which the 99th percentile individual has an internal dose less than the (uncertain) rodent internal dose at the POD.

rtr = route-to-route extrapolation using PBPK model and the specified dose-metric

Table 5-23. Comparison of —sensitive individual" HECs or HEDs for developmental effects based on PBPK modeled internal dose-metrics at different levels of confidence and sensitivity, at the NOAEL or LOAEL

Candidate critical effect Candidate critical study[a] (species)	POD type	Ratio HEC/D_{50}: HEC/D_{99}	HEC$_X$ or HED$_X$[b]			[Dose-metric]
			X = 99	X = 95, median	X = 95, 95lcb	
Developmental						
Resorptions Healy et al. (1982) (rat)	HEC	2.58	6.19	6.02	3.13	[TotMetabBW34]
	HEC	1.69	13.7	13.9	7.27	[AUCCBld]
	HED	1.02	8.5	8.50	4.61	[TotMetabBW34] (rtr)
	HED	3.68	19.7	22.4	11.5	[AUCCBld] (rtr)
Resorptions [LOAEL][c] Narotsky et al. (1995) (rat)	HED	1.06	44.3	43.9	15.1	[TotMetabBW34]
	HED	3.07	114	119	47.7	[AUCCBld]
	HEC	2.66	36.9	35.3	11.6	[TotMetabBW34] (rtr)
	HEC	1.91	190	197	48.1	[AUCCBld] (rtr)
↓ Fetal weight; skeletal effects Healy et al. (1982) (rat)	HEC	2.58	6.19	6.02	3.13	[TotMetabBW34]
	HEC	1.69	13.7	13.9	7.27	[AUCCBld]
	HED	1.02	8.5	8.50	4.61	[TotMetabBW34] (rtr)
	HED	3.68	19.7	22.4	11.5	[AUCCBld] (rtr)
Heart malformations (pups) [LOAEL][c] Johnson et al. (2003) (rat)	HED	1.02	0.012	0.012	0.0102	[TotOxMetabBW34]
	HED	11.6	0.00382	0.00476	0.00112	[AUCCBld]
	HEC	2.75	0.00848	0.00866	0.00632	[TotOxMetabBW34] (rtr)
	HEC	1.70	0.00216	0.00221	0.000578	[AUCCBld] (rtr)
↓ Rearing postexposure Fredriksson et al. (1993) (mouse)	HED	1.02	4.13	4.19	2.22	[TotMetabBW34]
	HED	7.69	3.46	4.21	0.592	[AUCCBld]
	HEC	2.71	2.96	2.96	1.48	[TotMetabBW34] (rtr)
	HEC	1.68	1.84	1.81	0.302	[AUCCBld] (rtr)
↑ Exploration postexposure Taylor et al. (1985) (rat)	HED	1.02	10.7	10.7	8.86	[TotMetabBW34]
	HED	7.29	4.11	5.08	1.16	[AUCCBld]
	HEC	2.57	8.36	7.94	5.95	[TotMetabBW34] (rtr)
	HEC	1.68	2.19	2.31	0.580	[AUCCBld] (rtr)

[a]Shaded rows denote results for the primary dose-metric.
[b]HEC$_{99}$ = the 99th percentile of the combined human uncertainty and variability distribution of continuous exposure concentrations that lead to the (fixed) median estimate of the rodent internal dose at the POD; HEC$_{99,median}$ (or HEC$_{99,95lcb}$) = the median (or 95th percentile lower confidence bound) estimate of the uncertainty distribution of continuous exposure concentrations for which the 99th percentile individual has an internal dose less than the (uncertain) rodent internal dose at the POD.
[c]BMDL used for p-cRfC or p-cRfD, but LOAEL or NOAEL (as noted) used for uncertainty analysis.

rtr = route-to-route extrapolation using PBPK model and the specified dose-metric

In several cases, the uncertainty, as reflected in the ratio between the 95 and 50% confidence bounds on the 99th percentile individual, was rather high (e.g., ≥5-fold), and reflected primarily uncertainty in the rodent internal dose estimates, discussed previously in Section 3.5.7. The largest uncertainties (ratios between 95 to 50% confidence bounds of 8–10-fold) were for kidney effects in mice using the AMetGSHBW34 dose-metric (Kjellstrand et al., 1983a; NCI, 1976). More moderate uncertainties (ratios between 95 to 50% confidence bounds of five- to eightfold) were evident in some oral studies using the AUCCBld dose-metric (Keil et al., 2009; Fredriksson et al., 1993; George et al., 1986; Sanders et al., 1982b), as well as in studies reporting kidney effects in rats in which the ABioactDCVCBW34 or AMetGSHBW34 dose-metrics were used (Woolhiser et al., 2006; NTP, 1988; Maltoni et al., 1986). Therefore, in these cases, a POD that is protective of the 99th percentile individual at a confidence level higher than 50% could be as much as an order of magnitude lower.

For comparison, Tables 5-19 and 5-23 also show the ratios of the overall 50th percentile to the overall 99th percentile HECs and HEDs, reflecting combined human uncertainty and variability at the median study/endpoint idPOD. The smallest ratios (up to 1.2-fold) are for total, oxidative, and hepatic oxidative metabolism dose-metrics from oral exposures, due to the large hepatic first-pass effect resulting in virtually all of the oral intake being metabolized before systemic circulation. Conversely, the large hepatic first-pass results in high variability in the blood concentration of TCE following oral exposures, with ratios up to 12-fold at low exposures (e.g., 90 vs. 99% first-pass would result in amounts metabolized differing by about 10% but TCE blood concentrations differing by about 10-fold). From inhalation exposures, there is moderate variability in these metrics, about two- to threefold. For GSH conjugation and bioactivated DCVC, however, variability is high (8–10-fold) for both exposure routes, which follows from the incorporation in the PBPK model analysis of the data from Lash et al. (1999b) showing substantial interindividual variability in GSH conjugation in humans.

Finally, it is important to emphasize that this analysis only addresses pharmacokinetic uncertainty and variability, so other aspects of extrapolation addressed in the UFs (e.g., LOAEL to NOAEL, subchronic to chronic, and pharmacodynamic differences), discussed above, are not included in the level of confidence.

5.1.5. Summary of Noncancer Reference Values

5.1.5.1. Preferred Candidate Reference Values (cRfCs, cRfD, p-cRfCs, and p-cRfDs) for Candidate Critical Effects

The candidate critical effects that yielded the lowest p-cRfC or p-cRfD for each type of effect, based on the primary dose-metric, are summarized in Tables 5-24 (p-cRfCs) and 5-25 (p-cRfDs). These results are extracted from Tables 5-13 to 5-18. In cases where a route-to-route extrapolated p-cRfC (p-cRfD) is lower than the lowest p-cRfC (p-cRfD) from an inhalation

(oral) study, both values are presented in the table. In addition, if there is greater than usual uncertainty associated with the lowest p-cRfC or p-cRfD for a type of effect, then the endpoint with the next lowest value is also presented. Furthermore, given those selections, the same sets of critical effects and studies are displayed across both tables, with the exception of two oral studies for which route-to-route extrapolation was not performed. Tables 5-24 and 5-25 are further summarized in Tables 5-26 and 5-27 to present the overall preferred p-cRfC and p-cRfD for each type of noncancer effect. The purpose of these summary tables is to show the most sensitive endpoints for each type of effect and the apparent relative sensitivities (based on reference value estimates) of the different types of effects.

Table 5-24. Lowest p-cRfCs or cRfCs for different effect domains

Effect domain Effect type	**Candidate critical effect (species/critical study)**	**p-cRfC or cRfC in ppm (composite UF)**		
		Preferred dose-metric[a]	**Default methodology**	**Alternative dose-metrics/studies (Tables 5-13–5-18)**
Neurologic				
Trigeminal nerve effects	Trigeminal nerve effects (human/Ruijten et al., 1991)	0.54 (10)	0.47 (30)	0.83 (10)
Cognitive effects	Demyelination in hippocampus (rat/Isaacson et al., 1990)	0.0071 (1,000)	– [rtr]	0.0023 (1,000)
Mood/sleep changes	Changes in wakefulness (rat/Arito et al., 1994)	0.016 (300)	0.012 (1,000)	0.030 (300)
Kidney				
Histological changes	*Toxic nephropathy (rat/NTP, 1988)*	*0.00056* (10)	– [rtr]	0.00087–1.3 (10–300)
	Toxic nephrosis (mouse/NCI, 1976)	0.0017 (300)	– [rtr]	
	Meganucleocytosis (rat/Maltoni et al., 1986)	0.0025 (10)	– [rtr]	
↑ Kidney weight	↑ kidney weight (rat/Woolhiser et al., 2006)	0.0013 (10)	0.52 (30)	0.0022–2.1 (10–30)
Liver				
↑ Liver weight	↑ liver weight (mouse/Kjellstrand et al., 1983a)	0.91 (10)	0.72 (30)	0.83–2.5 (10–30)
Immunologic				
↓ Thymus weight	**↓ thymus weight (mouse/Keil et al., 2009)**	**0.00033** (100)	– [rtr]	0.000082 (100)
Immuno-suppression	↓ cell-mediated response to SRBC ↓ stem cell recolonization (mouse/Sanders et al., 1982b)	0.017 (100)	– [rtr]	0.0043–1.4 (100)
	Decreased PFC response (rat/Woolhiser et al., 2006)	0.11 (100)	0.083 (300)	
Autoimmunity	↑ anti-dsDNA and anti-ssDNA Abs (mouse/Keil et al., 2009)	0.0011 (30)	– [rtr]	0.00027–0.23 (30–300)
	Autoimmune organ changes (mouse/Kaneko et al., 2000)	0.12 (300)	0.070 (1,000)	

Table 5-24. Lowest p-cRfCs or cRfCs for different effect domains (continued)

Effect domain Effect type	Candidate critical effect (species/critical study)	p-cRfC or cRfC in ppm (composite UF)		
		Preferred dose-metric[a]	**Default methodology**	**Alternative dose-metrics/studies (Tables 5-13–5-18)**
Reproductive				
Effects on sperm and testes	↓ ability of sperm to fertilize (rat/DuTeaux et al., 2004a)	0.0093 (1,000)	– [rtr]	0.028–0.17 (30–1,000)
	Multiple effects (rat/Kumar et al., 2001b, 2000b)	0.013 (1,000)	0.015 (3,000)	
	Hyperzoospermia (human/Chia et al., 1996)[b]	0.017 (30)	0.014 (100)	
Developmental				
Congenital defects	**Heart malformations (rat/Johnson et al., 2003)**	**0.00037** (10)	– [rtr]	0.000093 (10)
Developmental neurotoxicity	↓ rearing postexposure (rat/Fredriksson et al., 1993)	0.028 (300)	– [rtr]	0.0077–0.084 (100–300)
Pre/postnatal mortality/growth	Resorptions/↓ fetal weight/ skeletal effects (rat/Healy et al., 1982)	0.062 (100)	0.057 (300)	0.14–2.4 (10–100)

[a]The critical effects/studies and p-cRfCs used to derive the RfC are in **bold**; supporting effects/studies and p-cRfCs in *italics*.
[b]Greater than usual degree of uncertainty (see Section 5.1.2).

rtr = route-to-route extrapolated result

Table 5-25. Lowest p-cRfDs or cRfDs for different effect domains

Effect domain Effect type	Candidate critical effect (species/critical study)	p-cRfD or cRfD in mg/kg/d (composite UF)		
		Preferred dose-metric[a]	**Default methodology**	**Alternative dose-metrics/studies (Tables 5-13–5-18)**
Neurologic				
Trigeminal nerve effects	Trigeminal nerve effects (human/Ruijten et al., 1991)	0.73 (10)	– [rtr]	1.4 (10)
Cognitive effects	Demyelination in hippocampus (rat/Isaacson et al., 1990)	0.0092 (1,000)	0.0047 (10,000[b])	0.0043 (1,000)
Mood/sleep changes	Changes in wakefulness (rat/Arito et al., 1994)	0.022 (300)	– [rtr]	0.051 (300)
Kidney				
Histological changes	*Toxic nephropathy* *(rat/NTP, 1988)*	*0.00034* (10)	0.0945 (100)	0.00053–1.9 (10–300)
	Toxic nephrosis (mouse/NCI, 1976)	0.0010 (300)		
	Meganucleocytosis (rat/Maltoni et al., 1986)	0.0015 (10)	0.34 (100)	
↑ Kidney weight	↑ *kidney weight* *(rat/Woolhiser et al., 2006)*	*0.00079* (10)	– [rtr]	0.0013–2.5 (10)
Liver				
↑ Liver weight	↑ liver weight (mouse/Kjellstrand et al., 1983a)	0.79 (10)	– [rtr]	0.82–2.6 (10–100)
Immunologic				
↓ Thymus weight	↓ **thymus weight** **(mouse/Keil et al., 2009)**	**0.00048** (100)	0.00035 (1,000)	0.00016 (100)
Immuno-suppression	↓ cell-mediated response to SRBC ↓ stem cell recolonization (mouse/Sanders et al., 1982b)	0.025 (100)	0.018 (1000)	0.0084–0.91 (100)
	Decreased PFC response (rat/Woolhiser et al., 2006)	0.14 (100)	– [rtr]	
Autoimmunity	↑ anti-dsDNA and anti-ssDNA Abs (mouse/Keil et al., 2009)	0.0016 (30)	0.0012 (300)	0.00053–0.19 (30–300)
	Autoimmune organ changes (mouse/Kaneko et al., 2000)	0.14 (300)	– [rtr]	

Table 5-25. Lowest p-cRfDs or cRfDs for different effect domains (continued)

Effect domain Effect type	Candidate critical effect (species/critical study)	p-cRfD or cRfD in mg/kg/d (composite UF)		
		Preferred dose-metric[a]	**Default methodology**	**Alternative dose-metrics/studies (Tables 5-13–5-18)**
Reproductive				
Effects on sperm and testes	↓ Ability of sperm to fertilize (rat/DuTeaux et al., 2004a)	0.016 (1,000)	0.014 (10,000[b])	0.042–0.10 (30–1,000)
	Multiple effects (rat/Kumar et al., 2001b, 2000b)	0.016 (1,000)	– [rtr]	
	Hyperzoospermia (human/Chia et al., 1996)[c]	0.024 (30)	– [rtr]	
Developmental				
Develop. immunotox.	↓ **PFC, ↑ DTH (rat/Peden-Adams et al., 2006)**[d]	**0.00037** (1,000)	Same as preferred	–
Congenital defects	**Heart malformations (rat/Johnson et al., 2003)**	**0.00052** (10)	0.00021 (100)	0.00017 (10)
Develop. neurotox.	↓ Rearing postexposure (rat/Fredriksson et al., 1993)[d]	0.016 (1,000)	Same as preferred	0.017–0.11 (100–3,000)
Pre/postnatal mortality/growth	Resorptions/↓ fetal weight/ skeletal effects (rat/Healy et al., 1982)	0.085 (100)	[rtr]	0.70–2.9 (10–100)

[a]The critical effects/studies and p-cRfDs or cRfDs used to derive the RfD are in **bold**; supporting effects/studies and p-cRfDs in *italics*.
[b]EPA's report on the RfC and RfD processes (U.S. EPA, 2002b) recommends not deriving reference values with a composite UF of >3,000; however, composite UFs exceeding 3,000 are considered here because the derivation of the cRfCs and cRfDs is part of a screening process and the application of the PBPK model for candidate critical effects reduces the values of some of the individual UFs for the p-cRfCs and p-cRfDs.
[c]Greater than usual degree of uncertainty (see Section 5.1.2).
[d]No PBPK model based analyses were done, so cRfD on the basis of applied dose only.

rtr = route-to-route extrapolated result (no value for default methodology)

Table 5-26. Lowest p-cRfCs for candidate critical effects for different types of effect based on primary dose-metric

Type of effect	Effect (primary dose-metric)	p-cRfC (ppm)
Neurological	Demyelination in hippocampus in rats (TotMetabBW34)	0.007 (rtr)
Kidney	Toxic nephropathy in rats (ABioactDCVCBW34)	0.0006 (rtr)
Liver	Increased liver weight in mice (AMetLiv1BW34)	0.9
Immunological	Decreased thymus weight in mice (TotMetabBW34)	0.0003 (rtr)
Reproductive	Decreased ability of rat sperm to fertilize (AUCCBld)	0.009 (rtr)[a]
Developmental	Heart malformations in rats (TotOxMetabBW34)	0.0004 (rtr)

[a]This value is supported by the p-cRfC value of 0.01 ppm for multiple testes and sperm effects from an inhalation study in rats.

rtr = route-to-route extrapolated result

Table 5-27. Lowest p-cRfDs for candidate critical effects for different types of effect based on primary dose-metric

Type of effect	Effect (primary dose-metric)	p-cRfD (mg/kg/d)
Neurological	Demyelination in hippocampus in rats (TotMetabBW34)	0.009
Kidney	Toxic nephropathy in rats (ABioactDCVCBW34)	0.0003
Liver	Increased liver weight in mice (AMetLiv1BW34)	0.8 (rtr)
Immunological	Decreased thymus weight in mice (TotMetabBW34)	0.0005
Reproductive	Decreased ability of rat sperm to fertilize (AUCCBld) and multiple testes and sperm effects (TotMetabBW34)[a]	0.02
Developmental	Heart malformations in rats (TotOxMetabBW34)	0.0005[b]

[a]Endpoints from two different studies yielded the same p-cRfD value.
[b]This value is supported by the cRfD value of 0.0004 mg/kg/day derived for developmental immunotoxicity effects in mice (Peden-Adams et al., 2006); however, no PBPK analyses were done for this latter effect, so the value of 0.0004 mg/kg/day is based on applied dose.

rtr = route-to-route extrapolated result

For neurological, kidney, immunological, and developmental effects, the lowest p-cRfCs were derived from oral studies by route-to-route extrapolation. This appears to be a function of the lack of comparable inhalation studies for many effects studied via the oral exposure route, for

which there is a larger database of studies. For the liver and reproductive effects, inhalation studies yielded a p-cRfC lower than the lowest route-to-route extrapolated p-cRfC for that type of effect. Conversely, the lowest p-cRfDs were derived from oral studies with the exception of reproductive effects, for which route-to-route extrapolation from an inhalation study in humans also yielded among the lowest p-cRfDs. The only effect for which there were comparable studies for comparing a p-cRfC from an inhalation study with a p-cRfC estimated by route-to-route extrapolation from an oral study was increased liver weight in the mouse. The primary dose-metric of amount of TCE oxidized in the liver yielded similar p-cRfCs of 1.0 and 1.1 ppm for the inhalation result and the route-to-route extrapolated result, respectively (see Table 5-15).

As can be seen in these tables, the most sensitive types of effects (the types with the lowest p-cRfCs and p-cRfDs) appear to be developmental, kidney, and immunological (adult and developmental) effects, and then neurological and reproductive effects, in that order. Lastly, the liver effects have p-cRfC and p-cRfD values that are about 3.5 orders of magnitude higher than those for developmental, kidney, and immunological effects.

5.1.5.2. RfC

The goal is to select an overall RfC that is well supported by the available data (i.e., without excessive uncertainty given the extensive database) and protective for all of the candidate critical effects, recognizing that individual candidate RfC values are by nature somewhat imprecise. The lowest candidate RfC values within each health effect category span a 3,000-fold range from 0.0003 to 0.9 ppm (see Table 5-26). One approach to selecting an RfC would be to select the lowest calculated value of 0.0003 ppm for decreased thymus weight in mice. However, as can be seen in Table 5-24, three p-cRfCs are in the relatively narrow range of 0.0003–0.0006 ppm at the low end of the overall range. Given the somewhat imprecise nature of the individual candidate RfC values, and the fact that multiple effects/studies lead to similar candidate RfC values, the approach taken in this assessment is to select an RfC supported by multiple effects/studies. The advantages of this approach, which is only possible when there is a relatively large database of studies/effects and when multiple candidate values happen to fall within a narrow range at the low end of the overall range, are that it leads to a more robust RfC (less sensitive to limitations of individual studies) and that it provides the important characterization that the RfC exposure level is similar for multiple noncancer effects rather than being based on a sole explicit critical effect.

Tables 5-28 and 5-29 summarize the PODs and UFs for the two critical and one supporting studies/effects, respectively, corresponding to the p-cRfCs that have been chosen as the basis of the RfC for TCE noncancer effects. Each of these lowest candidate p-cRfCs, ranging from 0.0003 to 0.0006 ppm, for developmental, immunologic, and kidney effects, are values derived from route-to-route extrapolation using the PBPK model. The lowest p-cRfC estimate (for a primary dose-metric) from an inhalation study is 0.001 ppm for kidney effects, which is

higher than the route-to-route extrapolated p-cRfC from the most sensitive oral study. For each of the candidate RfCs, the PBPK model was used for inter- and intraspecies extrapolation, based on the preferred dose-metric for each endpoint.

Table 5-28. Summary of critical studies, effects, PODs, and UFs used to derive the RfC

For the database, $UF_D = 1$ because there is minimal potential for deriving an underprotective toxicity value as a result of an incomplete characterization of TCE toxicity.

Keil et al. (2009)—Decreased thymus weight in female $B6C3F_1$ mice exposed for 30 wks by drinking water. • idPOD = 0.139 mg TCE metabolized/$kg^{¾}$/d, which is the PBPK model-predicted internal dose at the applied dose LOAEL of 0.35 mg/kg/d (continuous) (no BMD modeling due to inadequate model fit caused by supralinear dose-response shape) (see Appendix F, Section F.6.3). • HEC_{99} = 0.033 ppm (lifetime continuous exposure) derived from combined interspecies, intraspecies, and route-to-route extrapolation using PBPK model. • $UF_L = 10$ because POD is a LOAEL for an adverse effect. • $UF_A = 3$ because the PBPK model was used for interspecies extrapolation. • $UF_H = 3$ because the PBPK model was used to characterize human toxicokinetic variability. • p-cRfC = 0.033/100 = 0.00033 ppm (2 μg/m^3).
Johnson et al. (2003)—Fetal heart malformations in Sprague-Dawley rats exposed on GDs 1–22 by drinking water. • idPOD = 0.0142 mg TCE metabolized by oxidation/$kg^{¾}$/d, which is the BMDL from BMD modeling using PBPK model-predicted internal doses, with highest dose group (1,000-fold higher than next highest dose group) dropped, pup as unit of analysis, BMR = 1% (due to severity of defects, some of which could have been fatal), and a nested Log-logistic model to account for intralitter correlation (see Appendix F, Section F.6.4). • HEC_{99} = 0.0037 ppm (lifetime continuous exposure) derived from combined interspecies, intraspecies, and route-to-route extrapolation using PBPK model. • $UF_A = 3$ because the PBPK model was used for interspecies extrapolation. • $UF_H = 3$ because the PBPK model was used to characterize human toxicokinetic variability. • p-cRfC = 0.0037/10 = 0.00037 ppm (2 μg/m^3).

Table 5-29. Summary of supporting studies, effects, PODs, and UFs for the RfC

For the database, $UF_D = 1$ because there is minimal potential for deriving an underprotective toxicity value as a result of an incomplete characterization of TCE toxicity.

NTP (1988)—Toxic nephropathy in female Marshall rats exposed for 104 wks by gavage (5 d/wk). • idPOD = 0.0132 mg DCVC bioactivated/$kg^{¾}$/d, which is the BMDL from BMD modeling using PBPK model-predicted internal doses, BMR = 5% (clearly toxic effect), and log-logistic model (see Appendix F, Section F.6.1). • HEC_{99} = 0.0056 ppm (lifetime continuous exposure) derived from combined interspecies, intraspecies, and route-to-route extrapolation using PBPK model. • $UF_A = 3$ because the PBPK model was used for interspecies extrapolation. • $UF_H = 3$ because the PBPK model was used to characterize human toxicokinetic variability. • p-cRfC = 0.0056/10 = 0.00056 ppm (3 μg/m^3).

There is moderate confidence in the lowest p-cRfC for developmental effects (heart malformations) (see Section 5.1.2.8) and the lowest p-cRfC estimate for immunological effects

(see Section 5.1.2.5), and these are considered the critical effects used for deriving the RfC. For developmental effects, although the available study has important limitations, the overall weight of evidence supports an effect of TCE on cardiac development. For immunological effects, there is high confidence in the evidence for an immunotoxic hazard from TCE, but the available dose-response data preclude application of BMD modeling.

For kidney effects (see Section 5.1.2.2), there is high confidence in the evidence for a nephrotoxic hazard from TCE. Moreover, the lowest p-cRfC for kidney effects (toxic nephropathy) is derived from a chronic study and is based on BMD modeling. However, as discussed in Section 3.3.3.2, there remains substantial uncertainty in the extrapolation of GSH conjugation from rodents to humans due to limitations in the available data. In addition, the p-cRfC for toxic nephropathy had greater dose-response uncertainty since the estimation of its POD involved extrapolation from high response rates (>60%). Therefore, toxic nephropathy is considered supportive but is not used as a primary basis for the RfC. The other sensitive p-cRfCs for kidney effects in Table 5-19 were all within a factor of 5 of that for toxic nephropathy; however, these values similarly relied on the uncertain interspecies extrapolation of GSH conjugation.

As a whole, the estimates support an RfC of 0.0004 ppm (0.4 ppb or 2 $\mu g/m^3$). This value essentially reflects the midpoint between the similar p-cRfC estimates for the two critical effects (0.00033 ppm for decreased thymus weight in mice and 0.00037 ppm for heart malformations in rats), rounded to one significant figure. This value is also within a factor of 2 of the p-cRfC estimate of 0.0006 ppm for the supporting effect of toxic nephropathy in rats. Thus, there is robust support for an RfC of 0.0004 ppm provided by estimates for multiple effects from multiple studies. The estimates are based on PBPK model-based estimates of internal dose for interspecies, intraspecies, and route-to-route extrapolation, and there is sufficient confidence in the PBPK model and support from mechanistic data for one of the dose-metrics (TotOxMetabBW34 for the heart malformations). There is high confidence that ABioactDCVCBW34 and AMetGSHBW34 would be appropriate dose-metrics for kidney effects, but there is substantial uncertainty in the PBPK model predictions for these dose-metrics in humans (see Section 5.1.3.1). Note that there is some human evidence of developmental heart defects from TCE exposure in community studies (see Section 4.8.3.1.1) and of kidney toxicity in TCE-exposed workers (see Section 4.4.1).

In summary, the RfC is **0.0004 ppm** (0.4 ppb or 2 $\mu g/m^3$) based on route-to-route extrapolated results from oral studies for the critical effects of heart malformations (rats) and immunotoxicity (mice). This RfC value is further supported by route-to-route extrapolated results from an oral study of toxic nephropathy (rats).

5.1.5.3. RfD

As with the RfC determination above, the goal is to select an overall RfD that is well supported by the available data (i.e., without excessive uncertainty given the extensive database) and protective for all of the candidate critical effects, recognizing that individual candidate RfD values are by nature somewhat imprecise. The lowest candidate RfD values within each health effect category span a nearly 3,000-fold range from 0.0003 to 0.8 mg/kg/day (see Table 5-26). One approach to selecting an RfC would be to select the lowest calculated value of 0.0003 ppm for toxic nephropathy in rats. However, as can be seen in Table 5-25, multiple p-cRfDs or cRfDs from oral studies are in the relatively narrow range of 0.0003–0.0008 mg/kg/day at the low end of the overall range. Given the somewhat imprecise nature of the individual candidate RfD values, and the fact that multiple effects/studies lead to similar candidate RfD values, the approach taken in this assessment is to select an RfD supported by multiple effects/studies. The advantages of this approach, which is only possible when there is a relatively large database of studies/effects and when multiple candidate values happen to fall within a narrow range at the low end of the overall range, are that it leads to a more robust RfD (less sensitive to limitations of individual studies) and that it provides the important characterization that the RfD exposure level is similar for multiple noncancer effects rather than being based on a sole explicit critical effect.

Tables 5-30 and 5-31 summarize the PODs and UFs for the three critical and two supporting studies/effects, respectively, corresponding to the p-cRfDs or cRfDs that have been chosen as the basis of the RfD for TCE noncancer effects. Two of the lowest p-cRfDs for the primary dose-metrics—0.0008 mg/kg/day for increased kidney weight in rats and 0.0005 mg/kg/day for both heart malformations in rats and decreased thymus weights in mice—are derived using the PBPK model for inter- and intraspecies extrapolation, and a third—0.0003 mg/kg/day for increased toxic nephropathy in rats—is derived using the PBPK model for inter- and intraspecies extrapolation as well as route-to-route extrapolation from an inhalation study. The other of these lowest values—0.0004 mg/kg/day for developmental immunotoxicity (decreased PFC response and increased delayed-type hypersensitivity) in mice—is based on applied dose.

Table 5-30. Summary of critical studies, effects, PODs, and UFs used to derive the RfD

For the database, $UF_D = 1$ because there is minimal potential for deriving an underprotective toxicity value as a result of an incomplete characterization of TCE toxicity.

Keil et al. (2009)—Decreased thymus weight in female $B6C3F_1$ mice exposed for 30 wks by drinking water. • idPOD = 0.139 mg TCE metabolized/$kg^{3/4}$/d, which is the PBPK model-predicted internal dose at the applied dose LOAEL of 0.35 mg/kg/d (continuous) (no BMD modeling due to inadequate model fit caused by supralinear dose-response shape) (see Appendix F, Section F.6.3). • HED_{99} = 0.048 mg/kg/d (lifetime continuous exposure) derived from combined interspecies and intraspecies extrapolation using PBPK model. • $UF_L = 10$ because POD is a LOAEL for an adverse effect. • $UF_A = 3$ because the PBPK model was used for interspecies extrapolation. • $UF_H = 3$ because the PBPK model was used to characterize human toxicokinetic variability. • p-cRfD = 0.048/100 = 0.00048 mg/kg/d.
Peden-Adams et al. (2006)—Decreased PFC response (3 and 8 wks), and increased delayed-type hypersensitivity (8 wks) in pups exposed from GDs 0–3- or 8 wks of age through drinking water (placental and lactational transfer, and pup ingestion). • POD = 0.37 mg/kg/d is the applied dose LOAEL (estimated daily dam dose) (no BMD modeling due to inadequate model fit caused by supralinear dose-response shape). No PBPK modeling was attempted due to lack of appropriate models/parameters to account for complicated fetal/pup exposure pattern (see Appendix F, Section F.6.5). • $UF_L = 10$ because POD is a LOAEL for multiple adverse effects. • $UF_A = 10$ for interspecies extrapolation because PBPK model was not used. • $UF_H = 10$ for human variability because PBPK model was not used. • cRfD = 0.37/1,000 = 0.00037 mg/kg/d.
Johnson et al. (2003)—Fetal heart malformations in Sprague-Dawley rats exposed on GDs 1–22 by drinking water. • idPOD = 0.0142 mg TCE metabolized by oxidation/$kg^{3/4}$/d, which is the BMDL from BMD modeling using PBPK model-predicted internal doses, with highest dose group (1,000-fold higher than next highest dose group) dropped, pup as unit of analysis, BMR = 1% (due to severity of defects, some of which could have been fatal), and a nested Log-logistic model to account for intralitter correlation (see Appendix F, Section F.6.4). • HED_{99} = 0.0051 mg/kg/d (lifetime continuous exposure) derived from combined interspecies and intraspecies extrapolation using PBPK model. • $UF_A = 3$ because the PBPK model was used for interspecies extrapolation. • $UF_H = 3$ because the PBPK model was used to characterize human toxicokinetic variability. • p-cRfD = 0.0051/10 = 0.00051 mg/kg/d.

Table 5-31. Summary of supporting studies, effects, PODs, and UFs for the RfD

For the database, $UF_D = 1$ because there is minimal potential for deriving an underprotective toxicity value as a result of an incomplete characterization of TCE toxicity.

NTP (1988)—Toxic nephropathy in female Marshall rats exposed for 104 wks by gavage (5 d/wk). • idPOD = 0.0132 mg DCVC bioactivated/kg$^{3/4}$/d, which is the BMDL from BMD modeling using PBPK model-predicted internal doses, BMR = 5% (clearly toxic effect), and Log-logistic model (see Appendix F, Section F.6.1). • HED_{99} = 0.0034 mg/kg/d (lifetime continuous exposure) derived from combined interspecies and intraspecies extrapolation using PBPK model. • $UF_A = 3$ because the PBPK model was used for interspecies extrapolation. • $UF_H = 3$ because the PBPK model was used to characterize human toxicokinetic variability. • p-cRfD = 0.0034/10 = 0.00034 mg/kg/d.
Woolhiser et al. (2006)—Increased kidney weight in female Sprague-Dawley rats exposed for 4 wks by inhalation (6 hrs/d, 5 d/wk). • idPOD = 0.0309 mg DCVC bioactivated/kg$^{3/4}$/d, which is the BMDL from BMD modeling using PBPK model-predicted internal doses, BMR = 10%, and Hill model with constant variance (see Appendix F, Section F.6.2). • HED99 = 0.0079 mg/kg/d (lifetime continuous exposure) derived from combined interspecies and intraspecies extrapolation using PBPK model. • $UF_S = 1$ because Kjellstrand et al. (1983a) reported that in mice, kidney effects after exposure for 120 d was no more severe than those after 30 d exposure. • $UF_A = 3$ because the PBPK model was used for interspecies extrapolation. • $UF_H = 3$ because the PBPK model was used to characterize human toxicokinetic variability. • p-cRfC = 0.0079/10 = 0.00079 mg/kg/d.

There is moderate confidence in the p-cRfDs for decreased thymus weights (see Section 5.1.2.5) and heart malformations (see Section 5.1.2.8) and the cRfD for developmental immunological effects (see Section 5.1.2.8), and these effects are considered the critical effects used for deriving the RfD. For heart malformations, although the available study has important limitations, the overall weight of evidence supports an effect of TCE on cardiac development. For adult and developmental immunological effects, there is high confidence in the evidence for an immunotoxic hazard from TCE. However, the available dose-response data for immunological effects preclude application of BMD modeling.

For kidney effects (see Section 5.1.2.2), there is high confidence in the evidence for a nephrotoxic hazard from TCE. Moreover, the two lowest p-cRfDs for kidney effects (toxic nephropathy and increased kidney weight) are both based on BMD modeling and one is derived from a chronic study. However, as discussed in Section 3.3.3.2, there remains substantial uncertainty in the extrapolation of GSH conjugation from rodents to humans due to limitations in the available data. In addition, the p-cRfD value for toxic nephropathy had greater dose-response uncertainty since the estimation of its POD involved extrapolation from high response rates (>60%). Therefore, kidney effects are considered supportive but are not used as a primary basis for the RfD.

As a whole, the estimates support an RfD of 0.0005 mg/kg/day. This value is within 20% of the estimates for the critical effects—0.0004 mg/kg/day for developmental immunotoxicity (decreased PFC and increased delayed-type hypersensitivity) in mice, and 0.0005 mg/kg/day for both heart malformations in rats and decreased thymus weights in mice. This value is also within approximately a factor of 2 of the supporting effect estimates of 0.0003 mg/kg/day for toxic nephropathy in rats and 0.0008 mg/kg/day for increased kidney weight in rats. Thus, there is strong, robust support for an RfD of 0.0005 mg/kg/day provided by the concordance of estimates derived from multiple effects from multiple studies. The estimates for kidney effects, thymus effects, and developmental heart malformations are based on PBPK model-based estimates of internal dose for interspecies and intraspecies extrapolation, and there is sufficient confidence in the PBPK model and support from mechanistic data for one of the dose-metrics (TotOxMetabBW34 for the heart malformations). There is high confidence that ABioactDCVCBW34 would be an appropriate dose-metric for kidney effects, but there is substantial uncertainty in the PBPK model predictions for this dose-metric in humans (see Section 5.1.3.1). Note that there is some human evidence of developmental heart defects from TCE exposure in community studies (see Section 4.8.3.1.1) and of kidney toxicity in TCE-exposed workers (see Section 4.4.1).

In summary, the RfD is **0.0005 mg/kg/day** based on the critical effects of heart malformations (rats), adult immunological effects (mice), and developmental immunotoxicity (mice), all from oral studies. This RfD value is further supported by results from an oral study for the effect of toxic nephropathy (rats) and route-to-route extrapolated results from an inhalation study for the effect of increased kidney weight (rats).

5.2. DOSE-RESPONSE ANALYSIS FOR CANCER ENDPOINTS

This section describes the dose-response analysis for cancer endpoints. Section 5.2.1 discusses the analyses of data from chronic rodent bioassays. Section 5.2.2 discusses the analyses of human epidemiologic data. Section 5.2.3 discusses the choice of the preferred inhalation unit risk and oral slope factor estimates, as well as the application of ADAFs to the slope factor and unit risk estimates.

5.2.1. Dose-Response Analyses: Rodent Bioassays

This section describes the calculation of cancer slope factor and unit risk estimates based on rodent bioassays. First, all of the available studies (i.e., chronic rodent bioassays) were considered, and those suitable for dose-response modeling were selected for analysis (see Section 5.2.1.1). Then dose-response modeling using the linearized multistage model was performed using applied doses (default dosimetry) as well as PBPK model-based internal doses (see Section 5.2.1.2). Bioassays for which time-to-tumor data were available were analyzed using poly-3 adjustment techniques and using a Multistage Weibull model. In addition, a cancer

potency estimate for different cancer types combined was derived from bioassays in which there was more than one type of tumor response in the same sex and species. Slope factor and unit risk estimates based on PBPK model-estimated internal doses were then extrapolated to human population slope factor and unit risk estimates using the human PBPK model. From these results (see Section 5.2.1.3), estimates from the most sensitive bioassay (i.e., that with the greatest slope factor or unit risk estimate) for each combination of administration route, sex, and species, based on the PBPK model-estimated internal doses, were considered as candidate slope factor or unit risk estimates for TCE. Uncertainties in the rodent-based dose-response analyses are described in Section 5.2.1.4.

5.2.1.1. Rodent Dose-Response Analyses: Studies and Modeling Approaches

The rodent cancer bioassays that were identified for consideration for dose-response analysis are listed in Tables 5-32 (inhalation bioassays) and 5-33 (oral bioassays) for each sex/species combination. The bioassays selected for dose-response analysis are marked with an asterisk; rationales for rejecting the bioassays that were not selected are provided in the "Comments" columns of the tables. For the selected bioassays, the tissues/organs that exhibited a TCE-associated carcinogenic response and for which dose-response modeling was performed are listed in the "Tissue/Organ" columns.

Table 5-32. Inhalation bioassays

Study	Strain	Tissue/organ	Comments
Female mice			
Fukuda et al. (1983)[a]	Crj:CD-1 (ICR)	Lung	
Henschler et al. (1980)[a]	Han:NMRI	Lymphoma	
Maltoni et al. (1986)[a]	$B6C3F_1$	Liver, Lung	
Maltoni et al. (1986)	Swiss	–	No dose-response
Male mice			
Henschler et al. (1980)	Han:NMRI	–	No dose-response
Maltoni et al. (1986)	$B6C3F_1$	Liver	Exp #BT306: excessive fighting
Maltoni et al. (1986)	$B6C3F_1$	Liver	Exp #BT306bis. Results similar to Swiss mice
Maltoni et al. (1986)[a]	Swiss	Liver	
Female rats			
Fukuda et al. (1983)	Sprague-Dawley	–	No dose-response
Henschler et al. (1980)	Wistar	–	No dose-response
Maltoni et al. (1986)	Sprague-Dawley	–	No dose-response
Male rats			
Henschler et al. (1980)	Wistar	–	No dose-response
Maltoni et al. (1986)[a]	Sprague-Dawley	Kidney, Leydig cell, Leukemia	

[a]Selected for dose-response analysis.

—No dose-response" = no tumor incidence data suitable for dose-response modeling

Table 5-33. Oral bioassays

Study	Strain	Tissue/organ	Comments
Female mice			
Henschler et al. (1984)	Han:NMRI	–	Toxicity, no dose-response
NCI (1976)[a]	$B6C3F_1$	Liver, lung, sarcomas and lymphomas	
NTP (1990)	$B6C3F_1$	Liver, lung, lymphomas	Single dose
Van Duuren et al. (1979)	Swiss	Liver	Single dose, no dose-response
Male mice			
Anna et al. (1994)	$B6C3F_1$	Liver	Single dose
Bull et al. (2002)	$B6C3F_1$	Liver	Single dose
Henschler et al. (1984)	Han:NMRI	–	Toxicity, no dose-response
NCI (1976)[a]	$B6C3F_1$	Liver	
NTP (1990)	$B6C3F_1$	Liver	Single dose
Van Duuren et al. (1979)	Swiss	–	Single dose, no dose-response
Female rats			
NCI (1976)	Osborne-Mendel	–	Toxicity, no dose-response
NTP (1988)	ACI	–	No dose-response
NTP (1988)[a]	August	Leukemia	
NTP (1988)	Marshall	–	No dose-response
NTP (1988)	Osborne-Mendel	Adrenal cortex	Adenomas only
NTP (1990)	F344/*N*	–	No dose-response
Male rats			
NCI (1976)	Osborne-Mendel	–	Toxicity, no dose-response
NTP (1988)	ACI	–	No dose-response
NTP (1988)[a]	August	Subcutaneous tissue sarcomas	
NTP (1988)[a]	Marshall	Testes	
NTP (1988)[a]	Osborne-Mendel	Kidney	
NTP (1990)[a]	F344/*N*	Kidney	

[a]Selected for dose-response analysis.

—No dose-response" = no tumor incidence data suitable for dose-response modeling

The general approach used was to model each sex/species/bioassay tumor response to determine the most sensitive bioassay response (in terms of HEC or HED) for each sex/species combination. The various modeling approaches, model selection, and slope factor and unit risk derivation are discussed below. Modeling was done using the applied dose or exposure (default dosimetry) and several internal dose-metrics. The dose-metrics used in the dose-response modeling are discussed in Section 5.2.1.2. Because of the large volume of analyses and results, detailed discussions about how the data were modeled using the various dosimetry and modeling approaches and results for individual data sets are provided in Appendix G. The overall results are summarized and discussed in Section 5.2.1.3.

Most tumor responses were modeled using the multistage model in EPA's BMDS (www.epa.gov/ncea/bmds). The multistage model is a flexible model, capable of fitting most cancer bioassay data, and it is EPA's long-standing model for the modeling of such cancer data. The multistage model has the general form

$$P(d) = 1 - \exp\left[-\left(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k\right)\right]$$

where *P(d)* represents the lifetime risk (probability) of cancer at dose *d*, and parameters $q_i \geq 0$, for $i = 0, 1, \ldots, k$. For each data set, the multistage model was evaluated for one stage and (n – 1) stages, where n is the number of dose groups in the bioassay. A detailed description of how the data were modeled, as well as tables of the dose-response input data and figures of the multistage modeling results, is provided in Appendix G.

Only models with acceptable fit ($p > 0.05$) were considered.[37] If 1-parameter and 2-parameter models were both acceptable (in no case was there a 3-parameter model), then the more parsimonious model (i.e., the 1-parameter model) was selected unless the inclusion of the 2nd parameter resulted in a statistically significant[38] improvement in fit. If two different 1-parameter models were available (e.g., a 1-stage model and a 3-stage model with β_1 and β_2 both equal to 0), then the one with the best fit, as indicated by the lowest AIC value, was selected. If the AIC values were the same (to three significant figures), then the lower-stage model was selected. Visual fit and scaled χ^2 residuals were also considered for confirmation in model selection. For two data sets, the highest-dose group was dropped to improve the fit in the lower dose range.

From the selected model for each data set, the maximum likelihood estimate (MLE) for the dose corresponding to a specified level of risk (i.e., the BMD) and its 95% lower confidence bound (BMDL) were estimated.[39] In most cases, the risk level, or BMR, was 10% extra risk;[40] however, in a few cases with low response rates, a BMR of 5%, or even 1%, extra risk was used to avoid extrapolation above the range of the data. As discussed in Section 4.4, there is sufficient evidence to conclude that a mutagenic mode of action is operative for TCE-induced kidney tumors, so linear extrapolation from the BMDL to the origin was used to derive slope factor and unit risk estimates for this site. The weight of evidence also supports involvement of processes of cytotoxicity and regenerative proliferation in the carcinogenicity of TCE, although not with the extent of support as for a mutagenic mode of action. In particular, data linking TCE-induced

[37]When considering multiple types of model for noncancer effects, $p > 0.10$ is used. For cancer, there is a prior preference for the multistage model, thus the $p > 0.05$ (which increases the probability of accepting the preferred model).
[38]Using a standard criterion for nested models, that the difference in -2 × log-likelihood exceeds 3.84 (the 95th percentile of $\chi 2$ [1]).
[39]BMDS estimates confidence intervals using the profile likelihood method.
[40]Extra risk over the background tumor rate is defined as $[P(d) - P(0)] / [1 - P(0)]$, where $P(d)$ represents the lifetime risk (probability) of cancer at dose *d*.

proliferation to increased mutation or clonal expansion are lacking, as are data informing the quantitative contribution of cytotoxicity. Moreover, it is unlikely that any contribution from cytotoxicity leads to a non-linear dose-response relationship near the POD for rodent kidney tumors, since maximal levels of toxicity are reached before the onset of tumors. Finally, because any possible involvement of a cytotoxicity mode of action would be additional to mutagenicity, the dose-response relationship would nonetheless be expected to be linear at low doses. Therefore, the additional involvement of a cytotoxicity mode of action does not provide evidence against the use of linear extrapolation from the POD.

For all other cancer types, the available evidence supports the conclusion that the mode(s) of action for TCE-induced rodent tumors is unknown, as discussed in Sections 4.5–4.10 and summarized in Section 4.11.2.3. Therefore, linear extrapolation was also used based on the general principles outlined in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b) and reviewed below in Section 5.2.1.4.1. Thus, for all TCE-associated rodent tumors, slope factor and unit risk estimates are equal to BMR/BMDL (e.g., $0.10/BMDL_{10}$ for a BMR of 10%). See Section 5.2.1.3 for a summary of the slope factor and unit risk estimates for each sex/species/bioassay/tumor type.

Some of the bioassays exhibited differential early mortality across the dose groups, and, for three such male rat studies (identified with checkmarks in the "Time-to-tumor" column of Table 5-34), analyses that take individual animal survival times into account were performed. (For bioassays with differential early mortality occurring primarily before the time of the 1st tumor [or 52 weeks, whichever came first], the effects of early mortality were largely accounted for by adjusting the tumor incidence for animals at risk, as described in Appendix G, and the dose-response data were modeled using the regular multistage model, as discussed above, rather than approaches that account for individual animal survival times.)

Two approaches were used to take individual survival times into account. First, EPA's Multistage Weibull (MSW) software[41] was used for time-to-tumor modeling. The Multistage Weibull time-to-tumor model has the general form:

$$P(d,t) = 1 - \exp\left[-\left(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k\right) \times \left(t - t_0\right)^z\right]$$

[41]This software is available on U.S. EPA's BMDS Web site (www.epa.gov/ncea/bmds).

Table 5-34. Specific dose-response analyses performed and dose-metrics used

Bioassay	Strain	Endpoint	Applied dose	PBPK-based—primary dose-metric[a]	PBPK-based—alternative dose-metric(s)[a]	Time-to-tumor
INHALATION						
Female mice						
Fukuda et al. (1983)	Crj:CD-1 (ICR)	Lung adenomas and carcinomas	√	AMetLngBW34	TotOxMetabBW34 AUCCBld	
Henschler et al. (1980)	Han:NMRI	Lymphoma	√	TotMetabBW34	AUCCBld	
Maltoni et al. (1986)	$B6C3F_1$	Liver hepatomas	√	AMetLiv1BW34	TotOxMetabBW34	
		Lung adenomas and carcinomas	√	AMetLngBW34	TotOxMetabBW34 AUCCBld	
		Combined risk	√			
Male mice						
Maltoni et al. (1986)	Swiss	Liver hepatomas	√	AMetLiv1BW34	TotOxMetabBW34	
Female rats						
None selected						
Male rats						
Maltoni et al. (1986)	Sprague-Dawley	Kidney adenomas and carcinomas	√	ABioactDCVCBW34	AMetGSHBW34 TotMetabBW34	
		Leydig cell tumors	√	TotMetabBW34	AUCCBld	
		Leukemias	√	TotMetabBW34	AUCCBld	
		Combined risk	√			

Table 5-34. Specific dose-response analyses performed and dose-metrics used (continued)

Bioassay	Strain	Endpoint	Applied dose	PBPK-based—primary dose-metric	PBPK-based—alternative dose-metric(s)	Time-to-tumor
ORAL						
Female mice						
NCI (1976)	$B6C3F_1$	Liver carcinomas	√	AMetLiv1BW34	TotOxMetabBW34	
		Lung adenomas and carcinomas	√	AMetLngBW34	TotOxMetabBW34 AUCCBld	
		Multiple sarcomas/lymphomas	√	TotMetabBW34	AUCCBld	
		Combined risk	√			
Male mice						
NCI (1976)	$B6C3F_1$	Liver carcinomas	√	AMetLiv1BW34	TotOxMetabBW34	
Female rats						
NTP (1988)	August	Leukemia	√	TotMetabBW34	AUCCBld	
Male rats						
NTP (1988)	August	Subcutaneous tissue sarcomas	√	TotMetabBW34	AUCCBld	
NTP (1988)	Marshall	Testicular interstitial cell tumors	√	TotMetabBW34	AUCCBld	√
NTP (1988)	Osborne-Mendel	Kidney adenomas and carcinomas	√	ABioactDCVCBW34	AMetGSHBW34 TotMetabBW34	√
NTP (1990)	F344/*N*	Kidney adenomas and carcinomas	√	ABioactDCVCBW34	AMetGSHBW34 TotMetabBW34	√

[a]PBPK-based dose-metric abbreviations:
ABioactDCVCBW34 = Amount of DCVC bioactivated in the kidney per unit body weight$^{3/4}$ (mg DCVC/kg$^{3/4}$/week).
AMetGSHBW34 = Amount of TCE conjugated with GSH per unit body weight$^{3/4}$ (mg TCE/kg$^{3/4}$/week).
AMetLiv1BW34 = Amount of TCE oxidized per unit body weight$^{3/4}$ (mg TCE/kg$^{3/4}$/week).
AMetLngBW34 = Amount of TCE oxidized in the respiratory tract per unit body weight$^{3/4}$ (mg TCE/kg$^{3/4}$/week).
AUCCBld = Area under the curve of the venous blood concentration of TCE (mg-hr/L/week).
TotMetabBW34 = Total amount of TCE metabolized per unit body weight$^{3/4}$ (mg TCE/kg$^{3/4}$/week).
TotOxMetabBW34 = Total amount of TCE oxidized per unit body weight$^{3/4}$ (mg TCE/kg$^{3/4}$/week).

where $P(d,t)$ represents the probability of a tumor by age t for dose d, and parameters $z \geq 1$, $t_0 \geq 0$, and $q_i \geq 0$ for $i = 0,1,...,k$, where k = the number of dose groups; the parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death. (All of our analyses used the model for incidental tumors, which has no t_0 term.) Although the fit of the MSW model can be assessed visually using the plot feature of the MSW software, because there is no applicable goodness-of-fit statistic with a well-defined asymptotic distribution, an alternative survival-adjustment technique, "poly-3 adjustment," was also applied (Portier and Bailer, 1989). This technique was used to adjust the tumor incidence denominators based on the individual animal survival times.[42] The adjusted incidence data then served as inputs for EPA's BMDS multistage model, and model (i.e., stage) selection was conducted as already described above. Under both survival-adjustment approaches, BMDs and BMDLs were obtained and slope factor and unit risks were derived as discussed above for the standard multistage model approach. See Appendix G for a more detailed description of the MSW modeling and for the results of both the MSW and poly-3 approaches for the individual data sets. A comparison of the results for the three different data sets and the various dose-metrics used is presented in Section 5.2.1.3.

For bioassays that exhibited more than one type of tumor response in the same sex and species (these studies have a row for "combined risk" in the "Endpoint" column of Table 5-34), the cancer potency for the different cancer types combined was estimated, in accordance with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b). The combined tumor risk estimate describes the risk of developing tumors for *any* (not all together) of the cancer types that exhibited a TCE-associated tumor response; this estimate then represents the total excess cancer risk. The model for the combined tumor risk is also multistage, with the sum of the stage-specific multistage coefficients from the individual tumor models serving as the stage-specific coefficients for the combined risk model (i.e., for each q_i, $q_{i[combined]} = q_{i1} + q_{i2} + ... + q_{ik}$, where the q_is are the coefficients for the powers of dose and k is the number of cancer types being combined) (NRC, 1994; Bogen, 1990). This model assumes that the occurrences of two or more cancer types are independent. Although the resulting model equation can be readily solved for a given BMR to obtain an MLE (BMD) for the combined risk, the confidence bounds for the combined risk estimate were not calculated by modeling software available during the development of this assessment. Therefore, the confidence bounds on the combined BMD were estimated using a Bayesian approach, computed using Markov chain Monte Carlo techniques and implemented using the freely available WinBugs software (Spiegelhalter et al., 2003). Use of WinBugs for derivation of a distribution of BMDs for a single multistage model has been demonstrated by Kopylev et al. (2007), and this approach can be straightforwardly generalized to

[42]Each tumorless animal is weighted by its fractional survival time (number of days on study divided by 728 days, the typical number of days in a 2-year bioassay) raised to the power of 3 to reflect the fact that animals are at greater risk of cancer at older ages. Animals with tumors are given a weight of 1. The sum of the weights of all of the animals in an exposure group yields the effective survival-adjusted denominator.

derive the distribution of BMDs for the combined tumor load. For further details on the implementation of this approach and for the results of the analyses, see Appendix G.

5.2.1.2. Rodent Dose-Response Analyses: Dosimetry

In modeling the applied doses (or exposures), default dosimetry procedures were applied to convert applied rodent doses to HEDs. Essentially, for inhalation exposures, "ppm equivalence" across species was assumed, consistent with the recommendations of U.S. EPA (1994a) for deriving a human equivalent concentration for a Category 3 gas for which the blood:air partition coefficient in laboratory animals is greater than that in humans (e.g., the posterior population median estimate for the TCE blood:air partition coefficient was 14 in the mouse [Table 3-37], 19 in the rat [Table 3-38], and 9.2 in the human [Table 3-39]). For oral doses, ¾-power body-weight scaling was used, with a default average human body weight of 70 kg. See Appendix G for more details on the default dosimetry procedures.

In addition to applied doses, several internal dose-metrics were used in the dose-response modeling for each tumor type. Use of internal dose-metrics in dose-response modeling is described here briefly. For more details on the PBPK modeling used to estimate the levels of the dose-metrics corresponding to different exposure scenarios in rodents and humans, as well as a qualitative discussion of the uncertainties and limitations of the model, see Section 3.5; for a more detailed discussion of how the dose-metrics were used in dose-response modeling, see Appendix G. Quantitative analyses of the uncertainties and their implications for dose-response assessment, utilizing the results of the Bayesian analysis of the PBPK model, are discussed separately in Section 5.2.1.4.2.

5.2.1.2.1. Selection of dose-metrics for different cancer types

One area of scientific uncertainty in cancer dose-response assessment is the appropriate scaling between rodent and human doses for equivalent responses. As discussed above, for applied dose, the standard dosimetry assumptions for equal lifetime carcinogenic risk are, for inhalation exposure, the same lifetime exposure concentration in air, and, for oral exposure, the same lifetime daily dose scaled by body weight to the ¾ power. In this assessment, the cross-species scaling methodology, grounded in the principles of allometric variation of biologic processes, is used for describing pharmacokinetic equivalence (U.S. EPA, 1992, 2011a, 2005b; Allen and Fisher, 1993; Crump et al., 1989; Allen et al., 1987). Briefly, in the absence of adequate information to the contrary, the methodology determines pharmacokinetic equivalence across species through equal average lifetime concentrations or AUCs of the toxicant. Thus, in cases where the PBPK model can predict internal concentrations of the active moiety, equivalent daily AUCs are assumed to address cross-species pharmacokinetics. For cancer assessments, there is currently no adjustment for pharmacodynamic differences.

More detailed discussion of the cross-species scaling methodology, and its implications for dose-metric selection, was presented for the noncancer dose-response analyses in Section 5.1.3.1, and those details are not repeated here.

To summarize, the preferred dose-metric under this methodology is equivalent daily AUC of the active moiety (parent compound or metabolite). For metabolites, in cases where the rate of production, but not the rate of clearance, of the active moiety can be estimated, the preferred dose-metric is the rate of metabolism (through the appropriate pathway) scaled by body weight to the ¾ power. If there are sufficient data to consider the active metabolite moiety(ies) "active" and cleared through nonbiological processes, then the preferred dose-metric is the rate of metabolism (through the appropriate pathway) scaled by the tissue mass. Finally, if local metabolism is thought to be involved but cannot be estimated with the available data, then the AUC of the parent compound in blood is considered an appropriate surrogate and thus the preferred dose-metric.

Generally, an attempt was made to use tissue-specific dose-metrics representing particular pathways or metabolites identified from available data as having a likely role in the induction of a tissue-specific cancer. Where insufficient information was available to establish particular metabolites or pathways of likely relevance to a tissue-specific cancer, more general "upstream" metrics representing either parent compound or total metabolism had to be used. In addition, the selection of dose-metrics was limited to metrics that could be adequately estimated by the PBPK model (see Section 3.5). The (PBPK-based) dose-metrics used for the different cancer types are listed in Table 5-34. For each tumor type, the "primary" dose-metric referred to in Table 5-34 is the metric representing the particular metabolite or pathway whose involvement in carcinogenicity has the greatest biological support, whereas "alternative" dose-metrics represent upstream metabolic pathways (or TCE distribution, in the case of AUCCBld) that may be more generally involved.

5.2.1.2.1.1. Kidney

As discussed in Sections 4.4.6–4.4.7, there is sufficient evidence to conclude that TCE-induced kidney tumors in rats are primarily caused by GSH-conjugation metabolites either produced in situ in or delivered systemically to the kidney. As discussed in Section 3.3.3.2, bioactivation of these metabolites within the kidney, either by beta-lyase, FMO, or P450s, produces reactive species. Therefore, multiple lines of evidence support the conclusion that renal bioactivation of DCVC is the preferred basis for internal dose extrapolations of TCE-induced kidney tumors. However, uncertainties remain as to the relative contributions from each bioactivation pathway, and quantitative clearance data necessary to calculate the concentration of each species are lacking. Moreover, the estimates of the amount bioactivated are indirect, derived from the difference between overall GSH conjugation flux and NAcDCVC excretion (see Section 3.5.7.3.1).

The rationales for the dose-metrics for kidney tumors are the same as for kidney noncancer toxicity, discussed above in Section 5.1.3.1.1, and not repeated here. The primary internal dose-metric for TCE-induced kidney tumors is the weekly rate of DCVC bioactivation per unit body weight to the ¾ power (**ABioactDCVCBW34 [mg/kg^{¾}/week]**). Due to the larger relative kidney weight in rats as compared to humans, using the alternative scaling by kidney weight instead of body weight to the ¾ power would only change the quantitative interspecies extrapolation by about twofold,[43] so the sensitivity of the results to the scaling choice is relatively small. An alternative dose-metric that also involves the GSH conjugation pathway is the amount of GSH conjugation scaled by the ¾ power of body weight **(AMetGSHBW34 [mg/kg^{¾}/week])**. This dose-metric uses the total flux of GSH conjugation as the toxicologically-relevant dose, and, thus, incorporates any direct contributions from DCVG and DCVC, which are not addressed in the DCVC bioactivation metric. Another alternative dose-metric is the total amount of TCE metabolism (oxidation and GSH conjugation together) scaled by the ¾ power of body weight **(TotMetabBW34 [mg/kg^{¾}/week])**. This dose-metric uses the total flux of TCE metabolism as the toxicologically relevant dose, and, thus, incorporates the possible involvement of oxidative metabolites, acting either additively or interactively, in addition to GSH conjugation metabolites in nephrocarcinogenicity (see Section 4.4.6). While there is no evidence that TCE oxidative metabolites can on their own induce kidney cancer, some nephrotoxic effects attributable to oxidative metabolites (e.g., peroxisome proliferation) may modulate the nephrocarcinogenic potency of GSH metabolites. However, this dose-metric is given less weight than those involving GSH conjugation because, as discussed in Sections 4.4.6 and 4.4.7, the weight of evidence supports the conclusion that GSH conjugation metabolites play a predominant role in nephrocarcinogenicity.

5.2.1.2.1.2. Liver

As discussed in Section 4.5.6, there is substantial evidence that oxidative metabolism is involved in TCE hepatocarcinogenicity, based primarily on noncancer and cancer effects similar to those observed with TCE being observed with a number of oxidative metabolites of TCE (e.g., CH, TCA, and DCA). While TCA is a stable, circulating metabolite, CH and DCA are relatively short-lived, although enzymatically cleared (see Section 3.3.3.1). As discussed in Sections 4.5.6 and 4.5.7, there is now substantial evidence that TCA does not adequately account for the hepatocarcinogenicity of TCE; therefore, unlike in previous dose-response analyses (Clewell and Andersen, 2004; Rhomberg, 2000), the AUCs of TCA in plasma and in liver were not considered as dose-metrics. However, there are inadequate data across species to quantify the dosimetry of CH and DCA, and other intermediates of oxidative metabolism (such as TCE-oxide or

[43]The range of the difference is 2.1–2.4-fold using the posterior medians for the relative kidney weight in rats and humans from the PBPK model described in Section 3.5 (see Table 3-38) and body weights of 0.3–0.4 kg for rats and 60–70 kg for humans.

dichloroacetylchloride) also may be involved in carcinogenicity. Thus, due to uncertainties as to the active moiety(ies), but the strong evidence associating TCE liver effects with oxidative metabolism in the liver, hepatic oxidative metabolism is the preferred basis for internal dose extrapolations of TCE-induced liver tumors.

The rationales for the dose-metrics for liver tumors are the same as for liver noncancer toxicity, discussed above in Section 5.1.3.1.2, and not repeated here. The primary internal dose-metric for TCE-induced liver tumors is selected to be the weekly rate of hepatic oxidation per unit body weight to the ¾ power **(AMetLiv1BW34 [mg/kg^{¾}/week])**. Due to the larger relative liver weight in mice as compared to humans, scaling by liver weight instead of body weight to the ¾ power would only change the quantitative interspecies extrapolation by about fourfold,[44] so the sensitivity of the results to the scaling choice is relatively modest. The total amount of oxidative metabolism of TCE scaled by the ¾ power of body weight (**TotOxMetabBW34 [mg/kg^{¾}/week]**) was selected as an alternative dose-metric (the justification for the body weight to the ¾ power scaling is analogous to that for hepatic oxidative metabolism, above). This dose-metric accounts for the possible additional contributions of systemically delivered products of lung oxidation.

5.2.1.2.1.3. Lung

As discussed in Section 4.7.3, in situ oxidative metabolism in the respiratory tract may be more important to lung toxicity than systemically delivered metabolites, at least as evidenced by acute pulmonary toxicity. While chloral was originally implicated as the active metabolite, based on either acute toxicity or mutagenicity of chloral and/or CH, more recent evidence suggests that other oxidative metabolites may also contribute to lung toxicity. These data include the identification of dichloroacetyl lysine adducts in Clara cells (Forkert et al., 2006), and the induction of pulmonary toxicity by TCE in CYP2E1-null mice, which may generate a different spectrum of oxidative metabolites as compared to wild-type mice (respiratory tract tissue also contains P450s from the CYP2F family). Overall, the weight of evidence supports the selection of respiratory tract oxidation of TCE as the preferred basis for internal dose extrapolations of TCE-induced lung tumors. However, uncertainties remain as to the relative contributions from different oxidative metabolites, and quantitative clearance data necessary to calculate the concentration of each species are lacking.

Under the cross-species scaling methodology, the rate of respiratory tract oxidation would be scaled by body weight to the ¾ power. For chloral, as discussed in Section 4.7.3, the reporting of substantial TCOH but no detectable CH in blood following TCE exposure from experiments in isolated, perfused lungs (Dalbey and Bingham, 1978) support the conclusion that

[44]The range of the difference is 3.5–3.9-fold using the posterior medians for the relative liver weight in mice and humans from the PBPK model described in Section 3.5 (see Table 3-37) and body weights of 0.03–0.04 kg for mice and 60–70 kg for humans.

chloral does not leave the target tissue in substantial quantities, but that there is substantial clearance by enzyme-mediated biotransformation. DCAC is a relatively-short-lived intermediate from aqueous (nonenzymatic) decomposition of TCE-oxide that can be trapped with lysine or degrade further to form DCA, among other products (Cai and Guengerich, 1999). Cai and Guengerich (1999) reported a half-life of TCE-oxide under aqueous conditions of 12 s at 23°C, a time-scale that would be shorter at physiological conditions (37°C) and that includes formation of DCAC as well as its decomposition. Therefore, evidence for this metabolite suggests that its clearance both is sufficiently rapid so that it would remain at the site of formation and is nonenzymatically mediated so that its rate would be independent of body weight. Other oxidative metabolites may also play a role, but, because they have not been identified, no inferences can be made as to their clearance.

Therefore, because it is not clear what the contributions to TCE-induced lung tumors are from different oxidative metabolites produced in situ and the scaling by body weight to the ¾ power is supported for at least one of the possible active moieties, it was decided here to scale the rate of respiratory tract tissue oxidation of TCE by body weight to the ¾ power. The primary internal dose-metric for TCE-induced lung tumors is, thus, the weekly rate of respiratory tract oxidation per unit body weight to the ¾ power **(AMetLngBW34 [mg/kg^{¾}/week])**. It should be noted that, due to the larger relative respiratory tract tissue weight in mice as compared to humans, scaling by tissue weight instead of body weight to the ¾ power would change the quantitative interspecies extrapolation by less than twofold,[45] so the sensitivity of the results to the scaling choice is relatively small.

While there is substantial evidence that acute pulmonary toxicity is related to pulmonary oxidative metabolism, for carcinogenicity, it is possible that, in addition to locally produced metabolites, systemically-delivered oxidative metabolites also play a role. Therefore, total oxidative metabolism scaled by the ¾ power of body weight (**TotOxMetabBW34 [mg/kg^{¾}/week]**) was selected as an alternative dose-metric (the justification for the body weight to the ¾ power scaling is analogous to that for respiratory tract oxidative metabolism, above).

Another alternative dose-metric considered here is the AUC of TCE in blood **(AUCCBld [mg-hour/L/week])**. This dose-metric would account for the possibility that local metabolism is determined primarily by TCE delivered in blood via systemic circulation to pulmonary tissue (the flow rate of which scales as body weight to the ¾ power), as assumed in previous PBPK models, rather than TCE delivered in air via diffusion to the respiratory tract, as is assumed in the PBPK model described in Section 3.5. However, as discussed in Section 3.5 and Appendix A, the available pharmacokinetic data provide greater support for the updated model structure. This dose-metric also accounts for the possible role of TCE itself in pulmonary

[45]The range of the difference is 1.6–1.8-fold using the posterior medians for the relative respiratory tract tissue weight in mice and humans from the PBPK model described in Section 3.5 (see Table 3-37), and body weights of 0.03–0.04 kg for mice and 60–70 kg for humans.

carcinogenicity (consistent with the assumption that the same average concentration of TCE in blood will lead to a similar lifetime cancer risk across species).

5.2.1.2.1.4. Other sites

For all other sites listed in Table 5-34, there is insufficient information for site-specific determinations of appropriate dose-metrics. While TCE metabolites and/or metabolizing enzymes have been reported in some of these tissues (e.g., male reproductive tract), their roles in carcinogenicity for these specific sites have not been established. Although "primary" and "alternative" dose-metrics are defined, they do not differ appreciably in their degrees of plausibility.

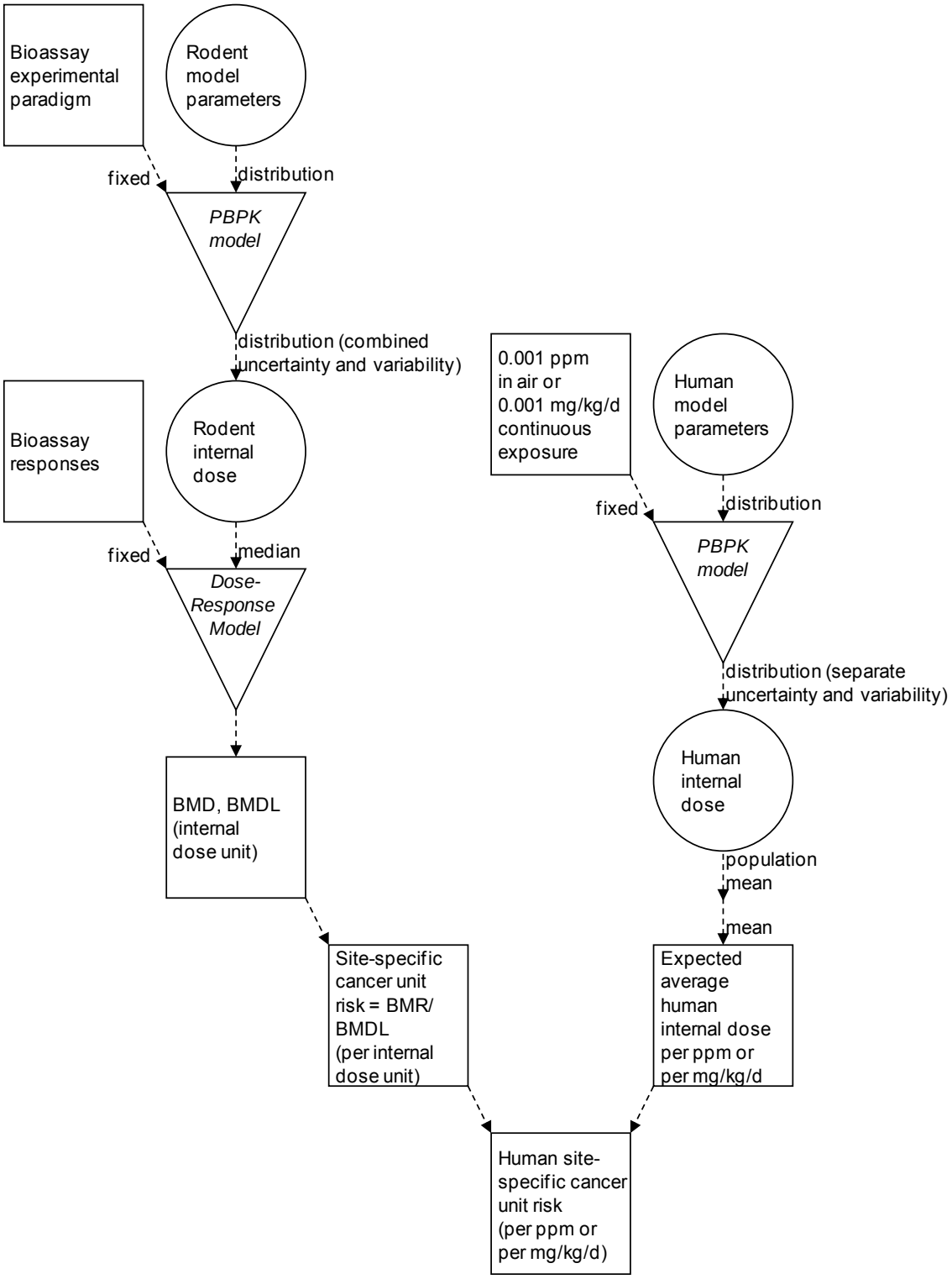
Given that the majority of the toxic and carcinogenic responses to TCE appear to be associated with metabolism, total metabolism of TCE scaled by the ¾ power of body weight was selected as the primary dose-metric **(TotMetabBW34 [mg/kg^{¾}/week])**. This dose-metric uses the total flux of TCE metabolism as the toxicologically-relevant dose, and, thus, incorporates the possible involvement of any TCE metabolite in carcinogenicity.

An alternative dose-metric considered here is the AUC of TCE in blood. This dose-metric would account for the possibility that the determinant of carcinogenicity is local metabolism, governed primarily by TCE delivered in blood via systemic circulation to the target tissue (the flow rate of which scales as body weight to the ¾ power). This dose-metric also accounts for the possible role of TCE itself in carcinogenicity (consistent with the assumption that the same average concentration of TCE in blood will lead to a similar lifetime cancer risk across species).

5.2.1.2.2. Methods for dose-response analyses using internal dose-metrics

As shown in Figure 5-5, the general approach taken for the use of internal dose-metrics in dose-response modeling was to first apply the rodent PBPK model to obtain rodent values for the dose-metrics corresponding to the applied doses in a bioassay. Then, dose-response modeling for a tumor response was performed using the internal dose-metrics and the multistage model or the survival-adjusted modeling approaches described above to obtain a BMD and BMDL in terms of the dose-metric. On an internal dose basis, humans and rodents are presumed to have similar lifetime cancer risks, and the relationship between human internal and external doses is essentially linear at low doses up to 0.1 mg/kg/day or 0.1 ppm, and nearly linear up to 10 mg/kg/day or 10 ppm. Therefore, the BMD and BMDL were then converted HEDs (or exposures) using conversion ratios estimated from the human PBPK model at 0.001 mg/kg/day or 0.001 ppm (see Table 5-35). Because the male and female conversions differed by <11%, the human BMDLs were derived using the mean of the sex-specific conversion factors (except for testicular tumors, for which only male conversion factors were used). Finally, a slope factor or unit risk estimate for that tumor response was derived from the human "BMDLs" as described

above (i.e., BMR/BMDL). Note that the converted "BMDs" and "BMDLs" are not actually human equivalent BMDs and BMDLs corresponding to the BMR because the conversion was not made in the dose range of the BMD; the converted BMDs and BMDLs are merely intermediaries to obtain a converted slope factor or unit risk estimate. In addition, it should be noted that median values of dose-metrics were used for rodents, whereas mean values were used for humans. Because the rodent population model characterizes study-to-study variation, animals of the same sex/species/strain combination within a study were assumed to be identical. Therefore, use of median dose-metric values for rodents can be interpreted as assuming that the animals in the bioassay were all "typical" animals and the dose-response model is estimating a "risk to the typical rodent." In practice, the use of median or mean internal doses for rodents did not make much difference except when the uncertainty in the dose-metric was high (e.g., AMetLungBW34 dose-metric in mice). A quantitative analysis of the impact of the uncertainty in the rodent PBPK dose-metrics is included in Section 5.2.1.4.2. On the other hand, the human population model characterizes individual-to-individual variation. Because the quantity of interest is the human population mean risk, the expected value (averaging over the uncertainty) of the population mean (averaging over the variability) dose-metric was used for the conversion to human slope factor or unit risks. Therefore, the extrapolated slope factor or unit risk estimates can be interpreted as the expected "average risk" across the population based on rodent bioassays.



Square nodes indicate point values, circular nodes indicate distributions, and the inverted triangles indicate a (deterministic) functional relationship.

Figure 5-5. Flow-chart for dose-response analyses of rodent bioassays using PBPK model-based dose-metrics.

Table 5-35. Mean PBPK model predictions for weekly internal dose in humans exposed continuously to low levels of TCE via inhalation (ppm) or orally (mg/kg/day)

Dose-metric[a]	0.001 ppm		0.001 mg/kg/d	
	Female	Male	Female	Male
ABioactDCVCBW34	0.00324	0.00324	0.00493	0.00515
AMetGSHBW34	0.00200	0.00200	0.00304	0.00318
AMetLiv1BW34	0.00703	0.00683	0.0157	0.0164
AMetLngBW34	0.00281	0.00287	6.60×10^{-5}	6.08×10^{-5}
AUCCBld	0.00288	0.00298	0.000411	0.000372
TotMetabBW34	0.0118	0.0117	0.0188	0.0196
TotOxMetabBW34	0.00984	0.00970	0.0157	0.0164

[a]See note to Table 5-34 for dose-metric abbreviations. Values represent the mean of the (uncertainty) distribution of population means for each sex and exposure scenario, generated from Monte Carlo simulation of 500 populations of 500 individuals each.

5.2.1.3. Rodent Dose-Response Analyses: Results

A summary of the PODs and slope factor and unit risk estimates for each sex/species/bioassay/tumor type is presented in Tables 5-36 (inhalation studies) and 5-37 (oral studies). The PODs for individual cancer types were extracted from the modeling results in the figures in Appendix G. For the applied dose (default dosimetry) analyses, the POD is the BMDL from the male human ("M") BMDL entry at the top of the figure for the selected model; male results were extracted because the default weight for males in the PBPK modeling is 70 kg, which is the overall human weight in EPA's default dosimetry methods (for inhalation, male and female results are identical). As described in Section 5.2.1.2, for internal dose-metrics, male and female results were averaged, and the converted human "BMDLs" are not true BMDLs because they were converted outside the linear range of the PBPK models. It can be seen in Appendix G that the male and female results were similar for all of the dose-metrics.

Table 5-36. Summary of PODs and unit risk estimates for each sex/species/bioassay/tumor type (inhalation)

Study	Tumor type	BMR	PODs (ppm, in HECs)[a]							
			Applied dose	AUC CBld	TotMetab BW34	TotOxMetab BW34	AMetLng BW34	AMetLiv1 BW34	AMetGSH BW34	ABioact DCVCBW34
Female mouse										
Fukuda et al. (1983)	Lung adenoma + carcinoma	0.1	26.3	55.5		31.3	38.8			
Henschler et al. (1980)	Lymphoma	0.1	11.0[b]	–[b]	9.84					
Maltoni et al. (1986)	Lung adenoma + carcinoma	0.1	44.6	96.6		51.4	55.7			
	Liver	0.05	37.1			45.8		41.9		
	Combined	0.05	15.7			20.7				
Male mouse										
Maltoni et al. (1986)	Liver	0.1	34.3			51		37.9		
Male rat										
Maltoni et al. (1986)	Leukemia	0.05	28.2[c]	–[b]	28.3					
	Kidney adenoma + carcinoma	0.01	22.7		13.7				0.197	0.121
	Leydig cell	0.1	18.6[c]	–[d]	18.1					
	Combined	0.01	1.44		1.37					
Study	Tumor type	Unit risk estimate (ppm^{-1})[e]								
		Applied dose		AUC CBld	TotMetab BW34	TotOxMetab BW34	AMetLng BW34	AMetLiv1 BW34	AMetGSH BW34	ABioact DCVCBW34
Female mouse										
Fukuda et al. (1983)	Lung adenoma + carcinoma	3.8×10^{-3}		1.8×10^{-3}		3.2×10^{-3}	**2.6×10^{-3}**			
Henschler et al. (1980)	Lymphoma	9.1×10^{-3}			**1.0×10^{-2}**					
Maltoni et al. (1986)	Lung adenoma + carcinoma	2.2×10^{-3}		1.0×10^{-3}		1.9×10^{-3}	**1.8×10^{-3}**			
	Liver	1.3×10^{-3}				1.1×10^{-3}		**1.2×10^{-3}**		
	Combined	3.2×10^{-3}				**2.4×10^{-3}**				

Table 5-36. Summary of PODs and unit risk estimates for each sex/species/bioassay/tumor type (inhalation) (continued)

Study	Tumor type	Unit risk estimate (ppm^{-1})[e]							
		Applied dose	AUC CBld	TotMetab BW34	TotOxMetab BW34	AMetLng BW34	AMetLiv1 BW34	AMetGSH BW34	ABioact DCVCBW34
Male mouse									
Maltoni et al. (1986)	Liver	2.9×10^{-3}			2.0×10^{-3}		**2.6×10^{-3}**		
Male rat									
Maltoni et al. (1986)	Leukemia	1.8×10^{-3}		**1.8×10^{-3}**					
	Kidney adenoma + carcinoma	4.4×10^{-4}		7.3×10^{-4}				5.1×10^{-2}	**8.3×10^{-2}**
	Leydig cell	5.4×10^{-3}		**5.5×10^{-3}**					
	Combined	7.0×10^{-3}		7.3×10^{-3}					

[a]For the applied doses, the PODs are BMDLs. However, for the internal dose-metrics, the PODs are not actually human equivalent BMDLs corresponding to the BMR because the interspecies conversion does not apply to the dose range of the BMDL; the converted BMDLs are merely intermediaries to obtain a converted unit risk estimate. The calculation that was done is equivalent to using linear extrapolation from the BMDLs in terms of the internal dose-metric to get a unit risk estimate for low-dose risk in terms of the internal dose-metric and then converting that estimate to a unit risk estimate in terms of human equivalent exposures. The PODs reported here are what one would get if one then used the unit risk estimate to calculate the human exposure level corresponding to a 10% extra risk, but the unit risk estimate is not intended to be extrapolated upward out of the low-dose range, e.g., above 10^{-4} risk. In addition, for the internal dose-metrics, the PODs are the average of the male and female human "BMDL" results presented in Appendix G.
[b]Inadequate fit to control group, but the primary metric, TotMetabBW34, fits adequately.
[c]Dropped highest-dose group to improve model fit.
[d]Inadequate overall fit.
[e]Unit risk estimate = BMR/POD. Results for the primary dose-metric are in bold.

Table 5-37. Summary of PODs and slope factor estimates for each sex/species/bioassay/tumor type (oral)

Study	Tumor type	BMR	PODs (mg/kg/d, in HEDs)[a]							
			Applied dose	AUC CBld	TotMetab BW34	TotOxMetab BW34	AMetLng BW34	AMetLiv1 BW34	AMetGSH BW34	ABioact DCVCBW34
Female mouse										
NCI (1976)	Liver carcinoma	0.1	26.5			17.6		14.1		
	Lung adenoma + carcinoma	0.1	41.1	682		24.7	757			
	Leukemias + sarcomas	0.1	43.1	733	20.6					
	Combined	0.05	7.43			5.38				
Male mouse										
NCI (1976)	Liver carcinoma	0.1	8.23			4.34		3.45		
Female rat										
NTP (1988)	Leukemia	0.05	72.3	3,220	21.7					
Male rat										
NTP (1990)[c]	Kidney adenoma + carcinoma	0.1	32		11.5				0.471	0.292
NTP (1988)										
Marshall[d]	Testicular	0.1	3.95	167	1.41					
August	Subcutaneous sarcoma	0.05	60.2	2,560	21.5					
Osborne-Mendel[c]	Kidney adenoma + carcinoma	0.1	41.5		14.3				0.648	0.402
Female mouse										
NCI (1976)	Liver carcinoma	3.8×10^{-3}				5.7×10^{-3}		**7.1×10^{-3}**		
	Lung adenoma + carcinoma	2.4×10^{-3}	1.5×10^{-4}			4.0×10^{-3}	**1.3×10^{-4}**			
	Leukemias + sarcomas	2.3×10^{-3}	1.4×10^{-4}	**4.9×10^{-3}**						
	Combined	6.7×10^{-3}				**9.3×10^{-3}**				

Table 5-37. Summary of PODs and slope factor estimates for each sex/species/bioassay/tumor type (oral) (continued)

Study	Tumor type	Slope factor estimate (mg/kg/d)$^{-1}$ [b]							
		Applied dose	AUC CBld	TotMetab BW34	TotOxMetab BW34	AMetLng BW34	AMetLiv1 BW34	AMetGSH BW34	ABioact DCVCBW34
Male mouse									
NCI (1976)	Liver carcinoma	1.2×10^{-2}			2.3×10^{-2}		**2.9×10^{-2}**		
Female rat									
NTP (1988)	Leukemia	6.9×10^{-4}	1.6×10^{-5}	**2.3×10^{-3}**					
Male rat									
NTP (1990)[c]	Kidney adenoma + carcinoma	1.6×10^{-3}		4.3×10^{-3}				1.1×10^{-1}	**1.7×10^{-1}**
NTP (1988)									
Marshall[d]	Testicular	2.5×10^{-2}	6.0×10^{-4}	**7.1×10^{-2}**					
August	Subcutaneous sarcoma	8.3×10^{-4}	2.0×10^{-5}	**2.3×10^{-3}**					
Osborne-Mendel[c]	Kidney adenoma + carcinoma	2.4×10^{-3}		7.0×10^{-3}				1.5×10^{-1}	**2.5×10^{-1}**

[a]For the applied doses, the PODs are BMDLs. However, for the internal dose-metrics, the PODs are not actually human equivalent BMDLs corresponding to the BMR because the interspecies conversion does not apply to the dose range of the BMDL; the converted BMDLs are merely intermediaries to obtain a converted slope factor estimate. The calculation that was done is equivalent to using linear extrapolation from the BMDLs in terms of the internal dose-metric to get a slope factor estimate for low-dose risk in terms of the internal dose-metric and then converting that estimate to a slope factor estimate in terms of HEDs. The PODs reported here are what one would get if one then used the slope factor estimate to calculate the human dose level corresponding to a 10% extra risk, but the slope factor estimate is not intended to be extrapolated upward out of the low-dose range, e.g., above 10^{-4} risk. In addition, for the internal dose-metrics, the PODs are the average of the male and female human "BMDL" results presented in Appendix G.
[b]Slope factor estimate = BMR/POD. Results for the primary dose-metric are in bold.
[c]Using MSW adjusted incidences (see text and Table 5-38).
[d]Using poly-3 adjusted incidences (see text and Table 5-38).

For two data sets, the highest dose (exposure) group was dropped to get a better fit when using applied doses. This technique can improve the fit when the response tends to plateau with increasing dose. Plateauing typically occurs when metabolic saturation alters the pattern of metabolite formation or when survival is impacted at higher doses, and it is assumed that these high-dose responses are less relevant to low-dose risk. The highest-dose group was not dropped to improve the fit for any of the internal dose-metrics because it was felt that if the dose-metric was an appropriate reflection of internal dose of the reactive metabolite(s), then use of the dose-metric should have ameliorated the plateauing in the dose-response relationship (note that survival-impacted data sets were addressed using survival adjustment techniques). For a 3rd data set (Henschler lymphomas), it might have helped to drop the highest exposure group, but there were only two exposure groups, so this was not done. As a result, the selected model, although it had an adequate fit overall, did not fit the control group very well (the model estimated a higher background response than was observed); thus, the BMD and BMDL were likely overestimated and the risk underestimated. The estimates from the NCI (1976) oral male mouse liver cancer data set are also somewhat more uncertain because the response rate was extrapolated down from a response rate of about 50% extra risk to the BMR of 10% extra risk.

Some general patterns can be observed in Tables 5-36 and 5-37. For inhalation, the unit risk estimates for different dose-metrics were generally similar (within about 2.5-fold) for most cancer types. The exception was for kidney cancer, where the estimates varied by over 2 orders of magnitude, with the AMetGSHBW34 and ABioactDCVCBW34 metrics yielding the highest estimates. This occurs because pharmacokinetic data indicate, and the PBPK model predicts, substantially more GSH conjugation (as a fraction of intake), and hence subsequent bioactivation, in humans relative to rats. The range of the risk estimates for individual cancer types overall (across cancer types and dose-metrics) was encompassed by the range of estimates across the dose-metrics for kidney cancer in the male rat, which was from 4.4×10^{-4} per ppm (applied dose) to 8.3×10^{-2} per ppm (ABioactDCVCBW34).

For oral exposure, the slope factor estimates are more variable across dose-metrics because of first-pass effects in the liver (median estimates for the fraction of TCE metabolized in *one* pass through the liver in mice, rats, and humans are >0.8). Here, the exception is for the risk estimates for cancer of the liver itself, which are also within about a 2.5-fold range, because the liver gets the full dose of all of the metrics during that ―first pass." For the other cancer types, the range of estimates across dose-metrics varies from about 30-fold to over 2 orders of magnitude, with the estimates based on AUCCBld and AMetLngBW34 being at the low end and those based on AMetGSHBW34 and ABioactDCVCBW34 again being at the high end. For AUCCBld, the PBPK model predicted the blood concentrations to scale more closely to body weight rather than the ¾ power of body weight, so the extrapolated human unit risks using this dose-metric are smaller than those obtained by applied dose or other dose-metrics that included ¾ power body weight scaling. For AMetLngBW34, pharmacokinetic data indicate, and the

PBPK model predicts, that the human respiratory tract metabolizes a lower fraction of total TCE intake than the mouse respiratory tract, so the extrapolated risk to humans based on this metric is lower than that obtained using applied dose or other dose-metrics. Overall, the oral slope factor estimates for individual cancer types ranged from 1.6×10^{-5} per mg/kg/day (female rat leukemia, AUCCBld) to 2.5×10^{-1} per mg/kg/day (male Osborne-Mendel rat kidney, ABioactDCVCBW34), a range of over 4 orders of magnitude. It must be recognized, however, that not all dose-metrics are equally credible, and, as will be presented below, the slope factor estimates for total cancer risk for the most sensitive bioassay response for each sex/species combination using the primary (preferred) dose-metrics fall within a very narrow range.

Results for survival-adjusted analyses are summarized in Table 5-38. For the time-independent (BMDS) multistage model, the risk estimates using poly-3 adjustment are higher than those without poly-3 adjustment. This is to be expected because the poly-3 adjustment decreases denominators when accounting for early mortality, and, for these data sets, the higher-dose groups had greater early mortality. The difference was fairly modest for the kidney cancer data sets (about 30% higher) but somewhat larger for the testicular cancer data set (about 150% higher).

Table 5-38. Comparison of survival-adjusted results for three oral male rat data sets[a]

Dose-metric	Adjustment method	BMR	POD (mg/kg/d)	BMD:BMDL	Slope factor estimate (per mg/kg/d)
NTP (1990) F344 rat kidney adenoma + carcinoma					
Applied dose	unadj BMDS	0.05	56.9	1.9	8.8×10^{-4}
	poly-3 BMDS	0.1	89.2	1.9	1.1×10^{-3}
	MSW	0.05	32.0	2.6	1.6×10^{-3}
TotMetabBW34	unadj BMDS	0.05	20.2	2.1	2.5×10^{-3}
	poly-3 BMDS	0.1	31.8	1.7	3.1×10^{-3}
	MSW	0.05	11.5	3.1	4.3×10^{-3}
AMetGSHBW34	unadj BMDS	0.05	0.841	1.9	5.9×10^{-2}
	poly-3 BMDS	0.1	1.32	1.9	7.6×10^{-2}
	MSW	0.05	0.471	2.4	1.1×10^{-1}
ABioactDCVCBW34	unadj BMDS	0.05	0.522	1.9	9.6×10^{-2}
	poly-3 BMDS	0.1	0.817	1.9	1.2×10^{-1}
	MSW	0.05	0.292	2.4	**1.7×10^{-1}**

Table 5-38. Comparison of survival-adjusted results for three oral male rat data sets[a]

Dose-metric	Adjustment method	BMR	POD (mg/kg/d)	BMD:BMDL	Slope factor estimate (per mg/kg/d)
NTP (1988) Osborne-Mendel rat kidney adenoma + carcinoma					
Applied dose	unadj BMDS	0.1	86.6	1.7	1.2×10^{-3}
	poly-3 BMDS	0.1	65.9	1.7	1.5×10^{-3}
	MSW	0.1	41.5	2.0	2.4×10^{-3}
TotMetabBW34	unadj BMDS	0.1	30.4	1.7	3.3×10^{-3}
	poly-3 BMDS	0.1	23.1	1.7	4.3×10^{-3}
	MSW	0.1	14.3	2.0	7.0×10^{-3}
AMetGSHBW34	unadj BMDS	0.1	1.35	1.7	7.4×10^{-2}
	poly-3 BMDS	0.1	1.03	1.7	9.7×10^{-2}
	MSW	0.1	0.648	2.0	1.5×10^{-1}
ABioactDCVCBW34	unadj BMDS	0.1	0.835	1.7	1.2×10^{-1}
	poly-3 BMDS	0.1	0.636	1.7	1.6×10^{-1}
	MSW	0.1	0.402	2.0	**2.5×10^{-1}**
NTP (1988) Marshall rat testicular tumors					
Applied dose	unadj BMDS	0.1	9.94	1.4	1.0×10^{-2}
	poly-3 BMDS	0.1	3.95	1.5	2.5×10^{-2}
	MSW	0.1	1.64	5.2	6.1×10^{-2}
AUCCBld	unadj BMDS	0.1	427	1.4	2.3×10^{-4}
	poly-3 BMDS	0.1	167	1.6	6.0×10^{-4}
	MSW	0.1	60.4	2.6	1.7×10^{-3}
TotMetabBW34	unadj BMDS	0.1	3.53	4.3	2.8×10^{-2}
	poly-3 BMDS	0.1	1.41	1.5	**7.1×10^{-2}**
	MSW	0.1	0.73	9.4	1.4×10^{-1}

[a]For the applied doses, the PODs are BMDLs. However, for the internal dose-metrics, the PODs are not actually human equivalent BMDLs corresponding to the BMR because the interspecies conversion does not apply to the dose range of the BMDL; the converted BMDLs are merely intermediaries to obtain a converted slope factor estimate. Results for the primary dose-metric are in bold.

In addition, the MSW time-to-tumor model generated higher risk estimates than the poly-3 adjustment technique. The MSW results were about 40% higher for the NTP F344 rat kidney cancer data sets and about 60% higher for the NTP Osborne-Mendel rat kidney cancer data sets. For the NTP Marshall rat testicular cancer data set, the discrepancies were greater; the results ranged from about 100 to 180% higher for the different dose-metrics. As discussed in Section 5.2.1.1, these two approaches differ in the way they take early mortality into account. The poly-3 technique merely adjusts the tumor incidence denominators, using a constant power 3 of time, to reflect the fact that animals are at greater risk of cancer at older ages. The MSW model estimates risk as a function of time (and dose), and it estimates the power (of time)

parameter for each data set.[46] For the NTP F344 rat kidney cancer and NTP Marshall rat testicular cancer data sets, the estimated power parameter was close to 3 in each case, ranging from 3.0 to 3.7; for the NTP Osborne-Mendel rat kidney cancer data sets, however, the estimated power parameter was about 10 for each of the dose-metrics, presumably reflecting the fact that these were late-occurring tumors (the earliest occurred at 92 weeks). Using a higher power parameter than 3 in the poly-3 adjustment would give even less weight to nontumor-bearing animals that die early and would, thus, increase the adjusted incidence even more in the highest-dose groups where the early mortality is most pronounced, increasing the slope factor estimate. Nonetheless, as noted above, the MSW results were only about 60% higher for the NTP Osborne-Mendel rat kidney cancer data sets for which MSW estimated a power parameter of about 10.

In general, the risk estimates from the MSW model would be preferred because, as discussed above, this model incorporates more information (e.g., tumor context) and estimates the power parameter rather than using a constant value of three. From Table 5-38, it can be seen that the results from MSW yielded higher BMD:BMDL ratios than the results from the poly-3 technique. These ratios were only slightly higher and not unusually large for MSW model analyses of the NTP (1990, 1988) kidney tumor estimates, and this, along with the adequate fit (assessed visually) of the MSW model, supports using the slope factor estimates from the MSW modeling of rat kidney tumor incidence. On the other hand, the BMD:BMDL ratio was relatively large for the applied dose analysis and, in particular, for the preferred dose-metric analysis (9.4-fold) of the NTP Marshall rat testicular tumor data set. Therefore, for this endpoint, the poly-3-adjusted results were used, although they may underestimate risk somewhat as compared to the MSW model.

In addition to the results from dose-response modeling of individual cancer types, the results of the combined tumor risk analyses for the three bioassays in which the rodents exhibited increased risks at multiple sites are also presented in Tables 5-36 and 5-37, in the rows labeled "combined" under the column heading "Tumor Type." These results were extracted from the detailed results in Appendix G. Note that, because of the computational complexity of the combined tumor analyses, dose-response modeling was only done using applied dose and a common upstream internal dose-metric, rather than using the different preferred dose-metrics for each tumor type within a combined tumor analysis.

For the Maltoni et al. (1986) female mouse inhalation bioassay, the combined tumor risk estimates are bounded by the highest individual tumor risk estimates and the sums of the individual tumor risks estimates (the risk estimates are upper bounds, so the combined risk estimate (i.e., the upper bound on the sum of the individual central tendency estimates) should be

[46]Conceptually, the approaches differ most when different tumor contexts (incidental or fatal) are considered, because the poly-3 technique only accounts for time of death, while the MSW model can account for the tumor context and attempt to estimate an induction time (t0), although this was not done for any of the data sets in this assessment.

less than the sum of the individual upper bound estimates), as one would expect. The common upstream internal dose-metric used for the combined analysis was TotOxMetabBW34, which is not the primary metric for either of the individual cancer types. For the liver tumors, the primary metric was AMetLiv1BW34, but as can be seen in Table 5-36, it yields results similar to those for TotOxMetabBW34. Likewise, for the lung tumors, the primary metric was AMetLngBW34, which yields a unit risk estimate slightly smaller than for TotOxMetabBW34. Thus, the results of the combined analysis using TotOxMetabBW34 as a common metric is not likely to substantially over- or underestimate the combined risk based on preferred metrics for each of the cancer types.

For the Maltoni et al. (1986) male rat inhalation bioassay, the combined risk estimates are also reasonably bounded, as expected. The common upstream internal dose-metric used for the combined analysis was TotMetabBW34, which is the primary metric for two of the three individual cancer types. However, as can be seen in Table 5-36, the risk estimate for the preferred dose-metric for the third tumor type, ABioactDCVCBW34 for the kidney tumors, is substantially higher than the risk estimates for the primary dose-metrics for the other two cancer types and would dominate a combined tumor risk estimate across primary dose-metrics; thus, the ABioactDCVCBW34-based kidney tumor risk estimate alone can reasonably be used to represent the total cancer risk for the bioassay using preferred internal dose-metrics, although it would underestimate the combined risk to some extent (e.g., the kidney-based estimate is 8.3×10^{-2} per ppm; the combined estimate would be about 9×10^{-2} per ppm, rounded to one significant figure).

For the third bioassay [NCI (1976) female mouse oral bioassay], the combined tumor risk estimates are once again reasonably bounded. The common upstream internal dose-metric used for the combined analysis was TotOxMetabBW34, which is not the primary metric for any of the three individual cancer types but was considered to be the most suitable metric to apply as a basis for combining risk across these different cancer types. The slope factor estimate for the lung based on the primary dose-metric for that site becomes negligible compared to the estimates for the other two cancer types (see Table 5-37). However, the slope factor estimates for the remaining two cancer types are both somewhat underestimated using the TotOxMetabBW34 metric rather than the primary metrics for those tumors (the TotOxMetabBW34-based estimate for leukemias + sarcomas, which is not presented in Table 5-30 because, in the absence of better mechanistic information, more upstream metrics were used for that individual tumor type, is 4.1×10^{-3} per mg/kg/day). Thus, overall, the combined estimate based on TotOxMetabBW34 is probably a reasonable estimate for the total tumor risk in this bioassay, although it might overestimate risk slightly.

The most sensitive sex/species results are extracted from Tables 5-29 and 5-30 and presented in Tables 5-39 (inhalation) and 5-40 (oral). The BMD:BMDL ratios for all of the results corresponding to the slope factor and unit risk estimates based on the preferred dose-

metrics ranged from 1.3 to 2.1. For inhalation, the most sensitive bioassay responses based on the preferred dose-metrics ranged from 2.6×10^{-3} to 8.3×10^{-2} per ppm across the sex/species combinations (with the exception of the female rat, which exhibited no apparent TCE-associated response in the 3 available bioassays). For oral exposure, the most sensitive bioassay responses based on the preferred dose-metrics ranged from 2.3×10^{-3} to 2.5×10^{-1} per mg/kg/day across the sex/species combinations. For both routes of exposure, the most sensitive sex/species response was (or was dominated by, in the case of the combined tumors in the male rat by inhalation) male rat kidney cancer based on the preferred dose-metric of ABioactDCVCBW34.

Table 5-39. Inhalation: most sensitive bioassay for each sex/species combination[a]

Sex/species	Endpoint (study)	Unit risk per ppm		
		Preferred dose-metric	Default methodology	Alternative dose-metrics, studies, or endpoints
Female mouse	Lymphoma (Henschler et al., 1980)	1.0×10^{-2}	9.1×10^{-3}	$1 \times 10^{-3} \sim 4 \times 10^{-3}$
Male mouse	Liver hepatoma (Maltoni et al., 1986)	2.6×10^{-3}	2.9×10^{-3}	2×10^{-3}
Female rat	–	–	–	–
Male rat	Leukemia+ Kidney adenoma and carcinoma+ Leydig cell tumors (Maltoni et al., 1986)	8.3×10^{-2}	7.0×10^{-3}	$4 \times 10^{-4} \sim 5 \times 10^{-2}$ [individual site results]

[a]Results extracted from Table 5-36.

Table 5-40. Oral: most sensitive bioassay for each sex/species combination[a]

Sex/species	Endpoint (study)	Unit risk per mg/kg/d		
		Preferred dose-metric	Default methodology	Alternative dose-metrics, studies, or endpoints
Female mouse	Liver carcinoma+ lung adenoma and carcinoma+ sarcomas + leukemias (NCI, 1976)	9.3×10^{-3}	6.7×10^{-3}	$1 \times 10^{-4} \sim 7 \times 10^{-3}$ [individual site results]
Male mouse	Liver carcinoma (NCI, 1976)	2.9×10^{-2}	1.2×10^{-2}	2×10^{-2}
Female rat	Leukemia (NTP, 1988)	2.3×10^{-3}	6.9×10^{-4}	2×10^{-5}
Male rat	Kidney adenoma + carcinoma (NTP, 1988, Osborne-Mendel)	2.5×10^{-1}	2.4×10^{-3} [b]	$2 \times 10^{-5} \sim 2 \times 10^{-1}$

[a]Results extracted from Table 5-37.
[b]Most sensitive male rat result using default methodology is 2.5×10^{-2} per mg/kg/day for NTP (1988) Marshall rat testicular tumors.

5.2.1.4. Uncertainties in Dose-Response Analyses of Rodent Bioassays

5.2.1.4.1. Qualitative discussion of uncertainties

All risk assessments involve uncertainty, as study data are extrapolated to make inferences about potential effects in humans from environmental exposure. The largest sources of uncertainty in the TCE rodent-based cancer risk estimates are interspecies extrapolation and low-dose extrapolation. Some limited human (occupational) data from which to estimate human cancer risk are available, and cancer risk estimates based on these data are developed in Section 5.2.2 below. In addition, some quantitative uncertainty analyses of the interspecies differences in pharmacokinetics were conducted and are presented in Section 5.2.1.4.2.

The rodent bioassay data offer conclusive evidence of carcinogenicity in both rats and mice, and the available epidemiologic and mechanistic data support the relevance to humans of the TCE-induced carcinogenicity observed in rodents. The epidemiologic data provide sufficient evidence that TCE is "carcinogenic to humans" (see Section 4.11). There is even some evidence of site concordance with the rodent findings, although site concordance is not essential to human relevance and, in fact, is not observed across TCE-exposed rats and mice. The strongest evidence in humans is for TCE-induced kidney tumors, with fairly strong evidence for lymphomas and some lesser support for liver tumors; each of these cancer types has also been observed in TCE rodent bioassays. Furthermore, the mechanistic data are supportive of human relevance because, while the exact reactive species associated with TCE-induced cancers are not known, the metabolic pathways for TCE are qualitatively similar for rats, mice, and humans (see Section 3.3). The impact of uncertainties with respect to quantitative differences in TCE metabolism is discussed in Section 5.2.1.4.2.

Typically, the cancer risk estimated is for the total cancer burden from all sites that demonstrate an increased tumor incidence for the most sensitive experimental species and sex. It is expected that this approach is protective of the human population, which is more diverse but is exposed to lower exposure levels.

For the inhalation unit risk estimates, the preferred estimate from the most sensitive species and sex was the estimate of 8.3×10^{-2} per ppm for the male rat, which was based on multiple tumors observed in this sex/species but was dominated by the kidney tumor risk estimated with the dose-metric for bioactivated DCVC. This estimate was the high end of the range of estimates (see Table 5-39) but was within an order of magnitude of other estimates, such as the preferred estimate for the female mouse and the male rat kidney estimate based on the GSH conjugation dose-metric, which provide additional support for an estimate of this magnitude. The preferred estimate for the male mouse was about an order of magnitude and a half lower. The female rat showed no apparent TCE-associated tumor response in the three available inhalation bioassays; however, this apparent absence of response is inconsistent with the observations of increased cancer risk in occupationally exposed humans and in female rats in

oral bioassays. In Section 5.2.2.2, an inhalation unit risk estimate based on the human data is derived and can be compared to the rodent-based estimate.

For the oral slope factor estimate, the preferred estimate from the most sensitive species and sex was the estimate of 2.5×10^{-1} per mg/kg/day, again for the male rat, based on the kidney tumor risk estimated with the dose-metric for bioactivated DCVC. This estimate was at the high end of the range of estimates (see Table 5-40) but was within an order of magnitude of other estimates, such as the preferred male mouse estimate and the male rat kidney estimate based on the GSH conjugation dose-metric, which provide additional support for an estimate of this magnitude. The preferred estimates for the female mouse and the female rat were about another order of magnitude lower. Some of the oral slope factor estimates based on the alternative dose-metric of AUC for TCE in the blood were as much as three orders of magnitude lower, but these estimates were considered less credible than those based on the preferred dose-metrics. In Section 5.2.2.3, an oral slope factor estimate based on the human (inhalation) data is derived using the PBPK model for route-to-route extrapolation; this estimate can be compared to the rodent-based estimate.

Furthermore, the male rat kidney tumor estimates from the inhalation (Maltoni et al., 1986) and oral (NTP, 1988) studies were consistent on the basis of internal dose using the dose-metric for bioactivated DCVC. In particular, the linearly extrapolated slope (i.e., the BMR/BMDL) per unit of internal dose derived from Maltoni et al. (1986) male rat kidney tumor data was 2.4×10^{-1} per weekly mg DCVC bioactivated per unit body weight$^{3/4}$, while the analogous slope derived from NTP (1988) male rat kidney tumor data was 9.3×10^{-2} per weekly mg DCVC bioactivated per unit body weight$^{3/4}$ (MSW-modeled results), a difference of less than threefold.[47] These results also suggest that differences between routes of administration are adequately accounted for by the PBPK model using this dose-metric.

Regarding low-dose extrapolation, a key consideration in determining what extrapolation approach to use is the mode(s) of action. However, mode-of-action data are lacking or limited for each of the cancer responses associated with TCE exposure, with the exception of the kidney tumors (see Section 4.11). For the kidney tumors, the weight of the available evidence supports the conclusion that a mutagenic mode of action is operative (see Section 4.4); this mode of action supports linear low-dose extrapolation. The weight of evidence also supports involvement of processes of cytotoxicity and regenerative proliferation in the carcinogenicity of TCE, although not with the extent of support as for a mutagenic mode of action. In particular, data linking

[47]For the Maltoni et al. (1986) male rat kidney tumors, the unit risk estimate of 8.3×10^{-2} per ppm using the ABioactDCVCBW34 dose metric, from Table 5-36, is divided by the average male and female internal doses at 0.001 ppm (0.0034/0.001) from Table 5-35, to yield a unit risk in internal dose units of 2.4×10^{-2}. For the NTP (1988) male rat kidney tumors, the unit risk estimate of 2.5×10^{-1} per mg/kg/day using the ABioactDCVCBW34 dose metric, from Table 5-37, is divided by the average male and female internal doses at 0.001 mg/kg/day (0.0027/0.001) from Table 5-35, to yield a unit risk in internal dose units of 9.3×10^{-2}. Note that the original BMDLs and unit risks from BMD modeling were in internal dose units that were then converted to applied dose units using the values in Table 5-35, so this calculation reverses that conversion.

TCE-induced proliferation to increased mutation or clonal expansion are lacking, as are data informing the quantitative contribution of cytotoxicity. Moreover, it is unlikely that any contribution from cytotoxicity leads to a non-linear dose-response relationship near the POD for rodent kidney tumors, since maximal levels of toxicity are reached before the onset of tumors. Finally, because any possible involvement of a cytotoxicity mode of action would be additional to mutagenicity, the dose-response relationship would nonetheless be expected to be linear at low doses. Therefore, the additional involvement of a cytotoxicity mode of action does not provide evidence against the use of linear extrapolation from the POD.

For the other TCE-induced cancers, the mode(s) of action is unknown. When the mode(s) of action cannot be clearly defined, EPA generally uses a linear approach to estimate low-dose risk (U.S. EPA, 2005b), based on the following general principles:

- A chemical's carcinogenic effects may act additively to ongoing biological processes, given that diverse human populations are already exposed to other agents and have substantial background incidences of various cancers.
- A broadening of the dose-response curve (i.e., less rapid fall-off of response with decreasing dose) in diverse human populations and, accordingly, a greater potential for risks from low-dose exposures (Lutz et al., 2005; Zeise et al., 1987) is expected for two reasons: First, even if there is a "threshold" concentration for effects at the cellular level, that threshold is expected to differ across individuals. Second, greater variability in response to exposures would be anticipated in heterogeneous populations than in inbred laboratory species under controlled conditions (due to, e.g., genetic variability, disease status, age, nutrition, and smoking status).
- The general use of linear extrapolation provides reasonable upper-bound estimates that are believed to be health-protective (U.S. EPA, 2005b) and also provides consistency across assessments.

Additional uncertainties arise from the specific dosimetry assumptions, the model structures and parameter estimates in the PBPK models, the dose-response modeling of data in the observable range, and the application of the results to potentially sensitive human populations. As discussed in Section 5.2.1.2.1, one uncertainty in the tissue-specific dose-metrics used here is whether to scale the rate of metabolism by tissue mass or body weight to the ¾ in the absence of specific data on clearance; however, in the cases where this is an issue (the lung, liver, and kidney), the impact of this choice is relatively modest (less than twofold to about fourfold). An additional dosimetry assumption inherent in this analysis is that equal concentrations of the active moiety over a lifetime yield equivalent lifetime risk of cancer across species, and the extent to which this is true for TCE is unknown. Furthermore, it should be noted that use of tissue-specific dosimetry inherently presumes site concordance of tumors across species.

With respect to uncertainties in the estimates of internal dose themselves, a quantitative analysis of the uncertainty and variability in the PBPK model-predicted dose-metric estimates and their impacts on cancer risk estimates is presented in Section 5.2.1.4.2. Additional uncertainties in the PBPK model were discussed in Section 3.5. Furthermore, this assessment examined a variety of dose-metrics for the different cancer types using PBPK models for rats, mice, and humans, so the impact of dose-metric selection can be assessed. As discussed in Section 5.2.1.2.1, there is strong support for the primary dose-metrics selected for kidney, liver, and, to a lesser extent, lung. For the other tumor sites, there is more uncertainty about dose-metric selection. The cancer slope factor and unit risk estimates obtained using the preferred dose-metrics were generally similar (within about threefold) to those derived using default dosimetry assumptions (e.g., equal risks result from equal cumulative equivalent exposures or doses), with the exception of the bioactivated DCVC dose-metric for rat kidney tumors and the metric for the amount of TCE oxidized in the respiratory tract for mouse lung tumors occurring from oral exposure (see Tables 5-39 and 5-40). The higher risk estimates for kidney tumors based on the bioactivated DCVC dose-metric are to be expected because pharmacokinetic data indicate, and the PBPK model predicts, substantially more GSH conjugation (as a fraction of intake), and hence subsequent bioactivation, in humans relative to rats. Nonetheless, there is substantial uncertainty in the quantitative extrapolation of GSH conjugation from rodents to humans due to limitations in the available data. The lower risk estimates for lung tumors from oral TCE exposure based on the metric for the amount of TCE oxidized in the respiratory tract are because there is a greater first-pass effect in human liver relative to mouse liver following oral exposure and because the gavage dosing used in rodent studies leads to a large bolus dose that potentially overwhelms liver metabolism to a greater extent than a more graded oral exposure. Both of these effects result in relatively more TCE being available for metabolism in the lung for mice than for humans. In addition, mice have greater respiratory metabolism relative to humans. However, because oxidative metabolites produced in the liver may contribute to respiratory tract effects, using respiratory tract metabolism alone as a dose-metric may underestimate lung tumor risk. The slope factor or unit risk estimates obtained using the alternative dose-metrics were also generally similar to those derived using default dosimetry assumptions, with the exception of the metric for the amount of TCE conjugated with GSH for rat kidney tumors, again because humans have greater GSH conjugation, and the AUC of TCE in blood for all of the cancer types resulting from oral exposure, again because of first-pass effects.

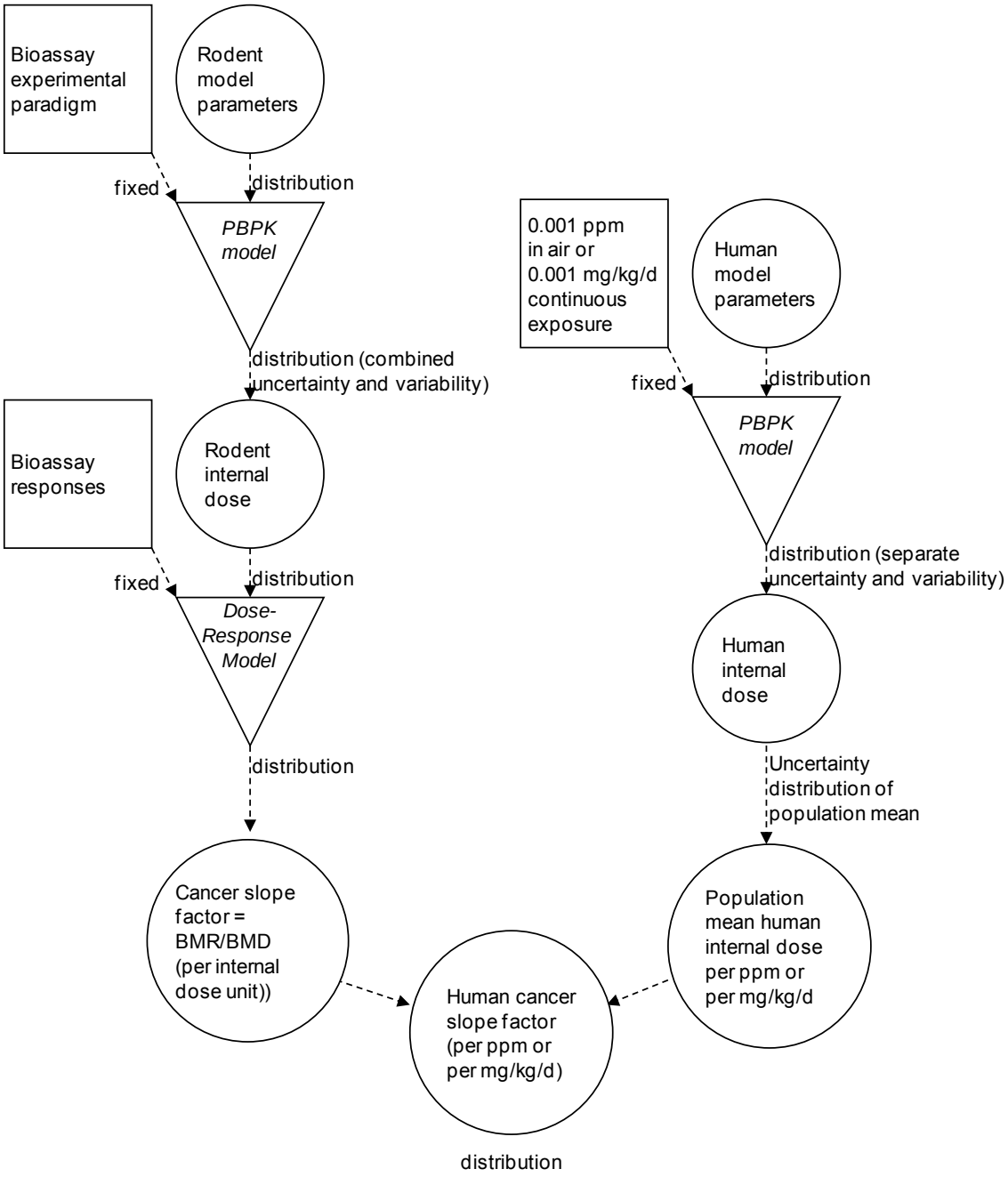
With respect to uncertainties in the dose-response modeling, the two-step approach of modeling only in the observable range, as put forth in EPA's cancer assessment guidelines (U.S. EPA, 2005b), is designed in part to minimize model dependence. The ratios of the BMDs to the BMDLs give some indication of the statistical uncertainties in the dose-response modeling. These ratios did not exceed a value of 2.5 for any of the primary analyses used in this assessment. Thus, overall, modeling uncertainties in the observable range are considered to be

minimal. Some additional uncertainty is conveyed by uncertainties in the survival adjustments made to some of the bioassay data; however, their impact is also believed to be minimal relative to the uncertainties already discussed (i.e., interspecies and low-dose extrapolations).

Regarding the cancer risks to potentially sensitive human populations or lifestages, pharmacokinetic data on 42 individuals were used in the Bayesian population analysis of the PBPK model discussed in Section 3.5. The impacts of these data on the predicted population mean are incorporated in the quantitative uncertainty analyses presented in Section 5.2.1.4.2. These data do not, however, reflect the full range of metabolic variability in the human population (they are all from healthy, mostly male, volunteers) and do not address specific potentially sensitive subgroups (see Section 4.10). Moreover, there is inadequate information about disease status, co-exposures, and other factors that make humans vary in their responses to TCE. It will be a challenge for future research to quantify the differential risk indicated by different risk factors or exposure scenarios.

5.2.1.4.2. Quantitative uncertainty analysis of PBPK model-based dose-metrics

The Bayesian analysis of the PBPK model for TCE generates distributions of uncertainty and variability in the internal dose-metrics than can be readily fed into dose-response analysis. As shown in Figure 5-6, the overall approach taken for the uncertainty analysis is similar to that used for the point estimates except that distributions are carried through the analysis rather than median or expected values. In particular, the PBPK model-based rodent internal doses are carried through to a distribution of BMDs (which also includes sampling variance from the number of responding and at risk animals in the bioassay). This distribution of BMDs generates a distribution of cancer slope factors based on internal dose, which then is combined with the (uncertainty) distribution of the human population mean conversion to applied dose or exposure. The resulting distribution for the human population mean risk per unit dose or exposure accounts for uncertainty in the PBPK model parameters (rodent and human) and the binomial sampling error in the bioassays. These distributions can then be compared with the point estimates, based on median rodent dose-metrics and mean human population dose-metrics, reported in Tables 5-36 and 5-37. Details of the implementation of this uncertainty analysis, which used the WinBugs software in conjugation with the R statistical package, are reported in Appendix G.



Square nodes indicate point values, circular nodes indicate distributions, and the inverted triangles indicate a (deterministic) functional relationship.

Figure 5-6. Flow-chart for uncertainty analysis of dose-response analyses of rodent bioassays using PBPK model-based dose-metrics.

Overall, as shown in Tables 5-41 and 5-42, the 95% confidence upper bound of the distributions for the linearly extrapolated risk per unit dose or exposure ranged from one- to eightfold higher than the point slope factors and unit risks derived using the BMDLs reported in Tables 5-36 and 5-37. The largest differences, up to fourfold, for rat kidney tumors and

eightfold for mouse lung tumors, primarily reflect the substantial uncertainty in the internal dose-metrics for rat kidney DCVC and GSH conjugation and for mouse lung oxidation (see Section 3.5). Additionally, despite the differences in the degree of uncertainty due to the PBPK model across endpoints and dose-metrics, the only case where the choice of the most sensitive bioassay for each sex/species combination would change based on the 95% confidence upper bounds reported in Tables 5-41 and 5-42 would be for female mouse inhalation bioassays. Even in this case, the difference between slope factor or unit risk estimate for the most sensitive and next most sensitive study/endpoint was only twofold.

Table 5-41. Summary of PBPK model-based uncertainty analysis of unit risk estimates for each sex/species/bioassay/tumor type (inhalation)

Study	Tumor type	BMR	Dose-metric	Unit risk estimates $(ppm)^{-1}$)				
				From	Summary statistics of unit risk distribution			
				Table 5-36	Mean	5% lower bound	Median	95% upper bound
Female mouse								
Fukuda et al. (1983)	Lung adenoma + carcinoma[a]	0.1	**AMetLngBW34**	**2.6×10^{-3}**	5.65×10^{-3}	2.34×10^{-4}	1.49×10^{-3}	2.18×10^{-2}
			TotOxMetabBW34	3.2×10^{-3}	1.88×10^{-3}	3.27×10^{-4}	1.52×10^{-3}	4.59×10^{-3}
			AUCCBld	1.8×10^{-3}	1.01×10^{-3}	1.54×10^{-4}	8.36×10^{-4}	2.44×10^{-3}
Henschler et al. (1980)	Lymphoma[b]	0.1	**TotMetabBW34**	**1.0×10^{-2}**	4.38×10^{-3}	6.06×10^{-4}	3.49×10^{-3}	1.11×10^{-2}
Maltoni et al. (1986)	Lung adenoma + carcinoma[a]	0.1	**AMetLngBW34**	**1.8×10^{-3}**	3.88×10^{-3}	1.48×10^{-4}	1.04×10^{-3}	1.52×10^{-2}
			TotOxMetabBW34	1.9×10^{-3}	1.10×10^{-3}	3.73×10^{-4}	9.52×10^{-4}	2.32×10^{-3}
			AUCCBld	1.0×10^{-3}	5.25×10^{-4}	1.63×10^{-4}	4.64×10^{-4}	1.10×10^{-3}
	Liver	0.05	**AMetLiv1BW34**	**1.2×10^{-3}**	6.27×10^{-4}	2.18×10^{-4}	5.39×10^{-4}	1.32×10^{-3}
			TotOxMetabBW34	1.1×10^{-3}	5.98×10^{-4}	1.81×10^{-4}	5.07×10^{-4}	1.31×10^{-3}
Male mouse								
Maltoni et al. (1986)	Liver	0.1	**AMetLiv1BW34**	**2.6×10^{-3}**	1.35×10^{-3}	4.28×10^{-4}	1.16×10^{-3}	2.93×10^{-3}
			TotOxMetabBW34	2.0×10^{-3}	1.23×10^{-3}	4.24×10^{-4}	1.06×10^{-3}	2.60×10^{-3}
Male rat								
Maltoni et al. (1986)	Leukemia[b]	0.05	**TotMetabBW34**	**1.8×10^{-3}**	9.38×10^{-4}	1.26×10^{-4}	7.86×10^{-4}	2.25×10^{-3}
	Kidney adenoma + carcinoma	0.01	**ABioactDCVCBW34**	**8.3×10^{-2}**	9.07×10^{-2}	3.66×10^{-3}	3.64×10^{-2}	3.21×10^{-1}
			AMetGSHBW34	5.1×10^{-2}	3.90×10^{-2}	2.71×10^{-3}	2.20×10^{-2}	1.30×10^{-1}
			TotMetabBW34	7.3×10^{-4}	3.94×10^{-4}	8.74×10^{-5}	3.42×10^{-4}	8.74×10^{-4}
	Leydig cell[b]	0.1	**TotMetabBW34**	**5.5×10^{-3}**	4.34×10^{-3}	1.99×10^{-3}	3.98×10^{-3}	7.87×10^{-3}

[a]WinBUGS dose-response analyses did not adequately converge for the AMetLngBW34 dose-metric using the 3rd-order multistage model (used for results in Table 5-36), but did converge when the 2nd-order model was used. Summary statistics reflect results of 2nd-order model calculations.
[b]Poor dose-response fits in point estimates for AUCCBld, so not included in uncertainty analysis.

Table 5-42. Summary of PBPK model-based uncertainty analysis of slope factor estimates for each sex/species/bioassay/tumor type (oral)

Study	Tumor type	BMR	Dose-metric	Slope factor estimates $(mg/kg/d)^{-1}$				
				From	Summary statistics of slope factor distribution			
				Table 5-37 or 5-38	Mean	5% lower bound	Median	95% upper bound
Female mouse								
NCI (1976)	Liver carcinoma	0.1	**AMetLiv1BW34**	**7.1×10^{-3}**	3.26×10^{-3}	9.35×10^{-4}	2.44×10^{-3}	8.35×10^{-3}
			TotOxMetabBW34	5.7×10^{-3}	2.63×10^{-3}	8.76×10^{-4}	2.01×10^{-3}	6.60×10^{-3}
	Lung adenoma + carcinoma[a]	0.1	**AMetLngBW34**	**1.3×10^{-4}**	1.28×10^{-4}	6.73×10^{-6}	4.12×10^{-5}	4.62×10^{-4}
			TotOxMetabBW34	4.0×10^{-3}	1.84×10^{-3}	5.29×10^{-4}	1.39×10^{-3}	4.73×10^{-3}
			AUCCBld	1.5×10^{-4}	7.16×10^{-5}	4.40×10^{-6}	3.39×10^{-5}	2.18×10^{-4}
	Leukemias + sarcomas	0.1	**TotMetabBW34**	**4.9×10^{-3}**	1.60×10^{-3}	1.42×10^{-4}	1.13×10^{-3}	4.65×10^{-3}
			AUCCBld	1.4×10^{-4}	6.36×10^{-5}	3.10×10^{-6}	2.90×10^{-5}	1.94×10^{-4}
Male mouse								
NCI (1976)	Liver carcinoma	0.1	**AMetLiv1BW34**	**2.9×10^{-2}**	1.65×10^{-2}	4.70×10^{-3}	1.25×10^{-2}	4.25×10^{-2}
			TotOxMetabBW34	2.3×10^{-2}	1.32×10^{-2}	4.41×10^{-3}	1.01×10^{-2}	3.29×10^{-2}
Female rat								
NTP (1988)	Leukemia	0.05	**TotMetabBW34**	**2.3×10^{-3}**	1.89×10^{-3}	5.09×10^{-4}	1.43×10^{-3}	4.69×10^{-3}
			AUCCBld	1.6×10^{-5}	1.56×10^{-5}	3.39×10^{-6}	1.07×10^{-5}	3.98×10^{-5}
Male rat								
NTP (1990)	Kidney adenoma + carcinoma[b]	0.1	**ABioactDCVCBW34**	**1.2×10^{-1}**	1.40×10^{-1}	5.69×10^{-3}	5.24×10^{-2}	5.18×10^{-1}
			AMetGSHBW34	7.6×10^{-2}	6.18×10^{-2}	4.00×10^{-3}	3.27×10^{-2}	2.11×10^{-1}
			TotMetabBW34	3.1×10^{-3}	2.49×10^{-3}	7.14×10^{-4}	1.96×10^{-3}	5.96×10^{-3}

Table 5-42. Summary of PBPK model-based uncertainty analysis of slope factor estimates for each sex/species/bioassay/tumor type (oral) (continued)

Study	Tumor type	BMR	Dose-metric	Slope factor estimates $(mg/kg/d)^{-1}$				
				From	Summary statistics of slope factor distribution			
				Table 5-37 or 5-38	Mean	5% lower bound	Median	95% upper bound
NTP (1988)								
Marshall	Testicular[b]	0.1	**TotMetabBW34**	**7.1×10^{-2}**	6.18×10^{-2}	1.92×10^{-2}	4.89×10^{-2}	1.45×10^{-1}
			AUCCBld	6.0×10^{-4}	5.45×10^{-4}	1.18×10^{-4}	3.70×10^{-4}	1.44×10^{-3}
August	Subcut sarcoma	0.05	**TotMetabBW34**	**2.3×10^{-3}**	1.65×10^{-3}	4.58×10^{-4}	1.27×10^{-3}	4.04×10^{-3}
			AUCCBld	2.0×10^{-5}	1.35×10^{-5}	1.53×10^{-6}	8.34×10^{-6}	3.73×10^{-5}
Osborne-Mendel	Kidney adenoma + carcinoma[b]	0.1	**ABioactDCVCBW34**	**1.6×10^{-1}**	1.61×10^{-1}	5.45×10^{-3}	6.35×10^{-2}	6.02×10^{-1}
			AMetGSHBW34	9.7×10^{-2}	7.47×10^{-2}	3.90×10^{-3}	3.85×10^{-2}	2.54×10^{-1}
			TotMetabBW34	4.3×10^{-3}	2.73×10^{-3}	5.40×10^{-4}	2.10×10^{-3}	6.89×10^{-3}

[a]WinBUGS dose-response analyses did not adequately converge for AMetLngBW34 dose-metric using the 3rd-order multistage model (used for results in Table 5-37), but did converge when the 2nd-order model was used. Summary statistics reflect results of 2nd-order model calculations.
[b]Using poly-3 adjusted incidences from Table 5-38 (software for WinBUGS-based analyses using the MSW model was not developed).

5.2.2. Dose-Response Analyses: Human Epidemiologic Data

Of the epidemiological studies of TCE and cancer, only two had sufficient exposure-response information for potential dose-response analysis. The two studies, Charbotel et al. (2006) and Moore et al. (2010), were both case-control studies of TCE and kidney cancer, and both had quantitative cumulative exposure estimates for the individual subjects. In the study by Moore et al. (2010), however, the cumulative exposure estimates were assessed by experts based on categorical metrics for frequency and intensity of exposure and not continuous measures. Moore et al. (2010) also used a categorical confidence-of-exposure metric to classify different jobs because of the potential for exposure misclassification from this approach. While the detailed approach used by Moore et al. (2010) should be fairly reliable for general rankings, the resulting estimates are not expected to be as quantitatively accurate as those in the Charbotel et al. (2006) study, which relied on a task-exposure matrix based on decades of measurements from the Arve Valley workshops (Fevotte et al., 2006; see also Section 4.4 for more discussion of the exposure assessments). Thus, the Charbotel et al. (2006) study was selected as the sole basis for the derivation of an inhalation unit risk estimate for kidney cancer (see Section 5.2.2.1). Other epidemiological studies were used in Section 5.2.2.2 below to provide information for a comparison of RR estimates across cancer types. These epidemiologic data were used to derive an adjusted inhalation unit risk estimate for the combined risk of developing kidney cancer, NHL, or liver cancer. The human PBPK model was then used to perform route-to-route extrapolation to derive an oral slope factor estimate for the combined risk of kidney cancer, NHL, or liver cancer (see Section 5.2.2.3).

5.2.2.1. Inhalation Unit Risk Estimate for RCC Derived from Charbotel et al. (2006) Data

The Charbotel et al. (2006) case-control study of 86 incident RCC cases and 316 age-and sex-matched controls, with individual cumulative exposure estimates for TCE for each subject, provides a sufficient human data set for deriving quantitative cancer risk estimates for RCC in humans. The study is a high-quality study that used a detailed exposure assessment (Fevotte et al., 2006) and took numerous potential confounding factors, including exposure to other chemicals, into account (see Section 4.4). A significant dose-response relationship was reported for cumulative TCE exposure and RCC (Charbotel et al., 2006).

The derivation of an inhalation unit risk estimate, defined as the plausible upper bound lifetime risk of cancer from chronic inhalation of TCE per unit of air concentration, for RCC incidence in the U.S. population, based on results of the Charbotel et al. (2006) case-control study, is presented in the following subsections.

5.2.2.1.1. RCC results from the Charbotel et al. (2006) study

Charbotel et al. (2006) analyzed their data using conditional logistic regression, matching on sex and age, and reported results (ORs) for cumulative TCE exposure categories, adjusted for tobacco smoking and BMI (Charbotel et al., 2006, Table 6). The exposure categories were constructed as tertiles based on the cumulative exposure levels in the exposed control subjects. The results are summarized in Table 5-43, with mean exposure levels kindly provided by Dr. Charbotel (2008).

For additional details and discussion of the Charbotel et al. (2006) study, see Section 4.4 and Appendix B.

Table 5-43. Results from Charbotel et al. (2006) on relationship between TCE exposure and RCC

Cumulative exposure category	Mean cumulative exposure (ppm × yrs)	Adjusted OR (95% CI)
Nonexposed		1
Low	62.4	1.62 (0.75, 3.47)
Medium	253.2	1.15 (0.47, 2.77)
High	925.0	2.16 (1.02, 4.60)

5.2.2.1.2. Prediction of lifetime extra risk of RCC incidence from TCE exposure

The categorical results summarized in Table 5-43 were used for predicting the extra risk of RCC incidence from continuous environmental exposure to TCE. Extra risk is defined as:

$$\text{Extra risk} = (Rx - Ro)/(1 - Ro),$$

where *Rx* is the lifetime risk in the exposed population and R*o* is the lifetime risk in an unexposed population (i.e., the background risk). Because kidney cancer is a rare event, the ORs in Table 5-43 can be used as estimates of the RR ratio = *Rx*/*Ro* (Rothman and Greenland, 1998). A weighted linear regression model was used to model the dose-response data in Table 5-43 to obtain a slope estimate (regression coefficient) for RR of RCC versus cumulative exposure, under the commonly employed assumption that exposure was measured without error. Use of a linear model in the observable range of the data is often a good general approach for epidemiological data because such data are frequently too limited (i.e., imprecise), as is the case here, to clearly identify an alternate model (U.S. EPA, 2005b). This linear dose-response function was then used to calculate lifetime extra risks in an actuarial program (life-table analysis) that accounts for age-specific rates of death and background disease, under the common

assumption that the RR is independent of age.[48] In addition, it is generally assumed that RR estimates transfer across populations, independent of background disease rates—in this case, the RR estimates based on the Charbotel et al. (2006) study, which was conducted in France, are assumed to apply to the U.S. population.[49]

For the weighted linear regression, the weights used for the RR estimates were the inverses of the variances, which were calculated from the CIs. Using this approach,[50] a linear regression coefficient of 0.001205 per ppm × year (SE = 0.0008195 per ppm × year) was obtained from the categorical results.

For the life-table analysis, U.S. age-specific all-cause mortality rates for 2004 for both sexes and all race groups combined (CDC, 2007) were used to specify the all-cause background mortality rates in the actuarial program. Because the goal is to estimate the unit risk for extra risk of cancer incidence, not mortality, and because the Charbotel et al. (2006) data are incidence data, RCC incidence rates were used for the cause-specific background "mortality" rates in the life-table analysis.[51] SEER 2001–2005 cause-specific background incidence rates for RCC were obtained from the SEER public-use database.[52] SEER collects good-quality cancer incidence data from a variety of geographical areas in the United States. The incidence data used here are from SEER 17, a registry of 17 states, cities, or regions covering about 26% of the United States population (http://seer.cancer.gov). The risks were computed up to age 85 years for continuous exposures to TCE.[53] Conversions between occupational TCE exposures and continuous environmental exposures were made to account for differences in the number of days exposed per year (240 vs. 365 days) and in the amount of air inhaled per day (10 vs. 20 m^3; U.S. EPA, 1994a). The SE for the regression coefficient from the weighted linear regression calculation described above was used to compute the 95% upper confidence limit (UCL) for the slope estimate, and this value was used to derive 95% UCLs for risk estimates (or 95% lower

[48]This program is an adaptation of the approach previously used by the Committee on the Biological Effects of Ionizing Radiation (BEIR, 1988). The same methodology was also used in U.S. EPA's 1,3-butadiene health risk assessment (U.S. EPA, 2002d). A spreadsheet illustrating the extra risk calculation for the derivation of the LEC_{01} for RCC incidence is presented in Appendix H.

[49]In any event, background kidney cancer rates between the United States and France are similar, with estimated age-adjusted incidence rates of 14.1 per 100,000 in the United States (Surveillance, Epidemiology, and End Results: http://seer.cancer.gov/statfacts/htmp/kidrp.html) and 10.4 per 100,000 in France (European Cancer Observatory: http://eu-cancer.iarc.fr/cancer-19-kidney.html.en).

[50]Equations for this weighted linear regression approach are presented in Rothman (1986) and summarized in Appendix H.

[51]No adjustment was made for using RCC incidence rates rather than mortality rates to represent cause-specific mortality in the actuarial program because the RCC incidence rates are negligible in comparison to the all-cause mortality rates. Otherwise, all-cause mortality rates for each age interval would have been adjusted to reflect people dying of a cause other than RCC *or* being diagnosed with RCC.

[52]In accordance with the "SEER Program Coding and Staging Manual 2007" (http://seer.cancer.gov/manuals/2007/SPCSM_2007_AppendixC_p6.pdf), pages C-831 to C-833, RRC was specified as ICD-0-3 histological types coded 8312, 8260, 8310, 8316-8320, 8510, 8959, and 8255 (mixed types).

[53]Rates above age 85 years are not included because cause-specific disease rates are less stable for those ages. Note that 85 years is not employed here as an average lifespan but, rather, as a cut-off point for the life-table analysis, which uses actual age-specific mortality rates.

confidence limits [LCLs] for corresponding exposure estimates), based on a normal approximation.

Point estimates and one-sided 95% UCLs for the extra risk of RCC incidence associated with varying levels of environmental exposure to TCE based on linear regression of the Charbotel et al. (2006) categorical results were determined by the actuarial program; the results are presented in Section 5.2.1.3. The models based on cumulative exposure yield extra risk estimates that are fairly linear for exposures up to approximately 1 ppm.

Consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), the same data and methodology were also used to estimate the exposure level (EC_x: "effective concentration corresponding to an extra risk of x%") and the associated 95% lower confidence limit of the effective concentration corresponding to an extra risk of 1% (LEC_x [lowest effective concentration], $x = 0.01$). A 1% extra risk level is commonly used for the determination of the POD for epidemiological data. Use of a 1% extra risk level for these data is supported by the fact that, based on the actuarial program, the risk ratio (i.e., Rx/Ro) for an extra risk of 1% for RCC incidence is 1.9, which is in the range of the ORs reported by Charbotel et al. (see Table 5-43). Thus, 1% extra risk was selected for determination of the POD, and, consistent with the *Guidelines for Carcinogen Risk Assessment*, the LEC value corresponding to that risk level was used as the actual POD. For the linear model that was selected, the unit risk is independent of the benchmark risk level used to determine the POD (at low exposures/risk levels; see Table 5-44); however, selection of a benchmark risk level is generally useful for comparisons across models.

Table 5-44. Extra risk estimates for RCC incidence from various levels of lifetime exposure to TCE, using linear cumulative exposure model

Exposure concentration (ppm)	MLE of extra risk	95% UCL on extra risk
0.001	2.603×10^{-6}	5.514×10^{-6}
0.01	2.603×10^{-5}	5.514×10^{-5}
0.1	2.602×10^{-4}	5.512×10^{-4}
1.0	2.598×10^{-3}	5.496×10^{-3}
10.0	2.562×10^{-2}	5.333×10^{-2}

As discussed in Section 4.4, there is sufficient evidence to conclude that a mutagenic mode of action is operative for TCE-induced kidney tumors, which supports the use of linear low-dose extrapolation from the POD. The EC_{01}, LEC_{01}, and inhalation unit risk estimates for RCC incidence using the linear cumulative exposure model are presented in Table 5-45. Converting the units, 5.49×10^{-3} per ppm corresponds to a unit risk of 1.02×10^{-6} per µg/m^3 for RCC incidence.

Table 5-45. EC_{01}, LEC_{01}, and unit risk estimates for RCC incidence, using linear cumulative exposure model

EC_{01} (ppm)	LEC_{01} (ppm)	unit risk (per ppm)[a]
3.87	1.82	5.49×10^{-3}

[a]Unit risk = $0.01/LEC_{01}$.

5.2.2.1.3. Uncertainties in the RCC unit risk estimate

The two major sources of uncertainty in quantitative cancer risk estimates are generally interspecies extrapolation and high-dose to low-dose extrapolation. The unit risk estimate for RCC incidence derived from the Charbotel et al. (2006) results is not subject to interspecies uncertainty because it is based on human data. A major uncertainty remains in the extrapolation from occupational exposures to lower environmental exposures. There was some evidence of a contribution to increased RCC risk from peak exposures; however, there remained an apparent dose-response relationship for RCC risk with increasing cumulative exposure without peaks, and the OR for exposure with peaks compared to exposure without peaks was not significantly elevated (Charbotel et al., 2006). Although the actual exposure-response relationship at low exposure levels is unknown, the conclusion that a mutagenic mode of action is operative for TCE-induced kidney tumors supports the linear low-dose extrapolation that was used (U.S. EPA, 2005b). The weight of evidence also supports involvement of processes of cytotoxicity and regenerative proliferation in the carcinogenicity of TCE, although not with the extent of support as for a mutagenic mode of action. In particular, data linking TCE-induced proliferation to increased mutation or clonal expansion are lacking, as are data informing the quantitative contribution of cytotoxicity. Because any possible involvement of a cytotoxicity mode of action would be additional to mutagenicity, the dose-response relationship would nonetheless be expected to be linear at low doses. Therefore, the additional involvement of a cytotoxicity mode of action does not provide evidence against the use of linear extrapolation from the POD.

Another notable source of uncertainty in the cancer unit risk estimate is the dose-response model used to model the study data to estimate the POD. A weighted linear regression across the categorical ORs was used to obtain a slope estimate; use of a linear model in the observable range of the data is often a good general approach for human data because epidemiological data are frequently too limited (i.e., imprecise) to clearly identify an alternate model (U.S. EPA, 2005b). The Charbotel et al. (2006) study is a relatively small case-control study, with only 86 RCC cases, 37 of which had TCE exposure; thus, the dose-response data upon which to specify a model are indeed limited.

In accordance with EPA's *Guidelines for Carcinogen Risk Assessment*, the lower bound on the EC_{01} is used as the POD; this acknowledges some of the uncertainty in estimating the POD from the available dose-response data. In this case, the statistical uncertainty associated with the EC_{01} is relatively small, as the ratio between the EC_{01} and the LEC_{01} is about twofold.

The inhalation unit risk estimate of 5.49×10^{-3} per ppm presented above, which is calculated based on a linear extrapolation from the POD (LEC_{01}), is expected to provide an upper bound on the risk of cancer incidence. However, for certain applications, such as benefit-cost analyses, estimates of "central tendency" for the risk below the POD are desired. Because a linear dose-response model was used in the observable range of the human data and the POD was within the low-dose linear range for extra risk as a function of exposure, linear extrapolation below the LEC_{01} has virtually the same slope as the 95% UCL on the actual (linear) dose-response model in the low-dose range (i.e., below the POD). This is illustrated in Table 5-44, where the 95% UCL on extra risk for RCC incidence predicted by the dose-response model is about 5.51×10^{-3} per ppm for exposures at or below about 0.1 ppm, which is virtually equivalent to the unit risk estimate of 5.49×10^{-3} per ppm derived from the LEC_{01} (see Table 5-45). The same holds for the central tendency (weighted least squares) estimates of the extra risk from the (linear) dose-response model (i.e., the dose-response model prediction of 2.60×10^{-3} per ppm from Table 5-44 is virtually identical to the value of 2.58×10^{-3} per ppm obtained from linear extrapolation below the EC_{01}, i.e., by dividing 0.01 extra risk by the EC_{01} of 3.87 from Table 5-45). In other words, because the dose-response model that was used to model the data in the observable range is already low-dose linear near the POD, if one assumes that the same linear model is valid for the low-dose range, one can use the central tendency (weighted least squares) estimate from the model to derive a statistical "best estimate" of the slope rather than relying on an extrapolated risk estimate ($0.01/EC_{01}$). (The extrapolated risk estimates are not generally central tendency estimates in any statistical sense because once risk is extrapolated below the EC_{01} using the formulation $0.01/EC_{01}$, it is no longer a function of the original model that generated the EC_{01} and LEC_{01} estimates.)

An important source of uncertainty in the underlying Charbotel et al. (2006) study is the retrospective estimation of TCE exposures in the study subjects. This case-control study was conducted in the Arve Valley in France, a region with a high concentration of workshops devoted to screw cutting, which involves the use of TCE and other degreasing agents. Since the 1960s, occupational physicians of the region have collected a large quantity of well-documented measurements, including TCE air concentrations and urinary metabolite levels (Fevotte et al., 2006). The study investigators conducted a comprehensive exposure assessment to estimate cumulative TCE exposures for the individual study subjects, using a detailed occupational questionnaire with a customized task-exposure matrix for the screw-cutting workers and a more general occupational questionnaire for workers exposed to TCE in other industries (Fevotte et al., 2006). The exposure assessment even attempted to take dermal exposure from hand-dipping practices into account by equating it with an equivalent airborne concentration based on biological monitoring data. Despite the appreciable effort of the investigators, considerable uncertainty associated with any retrospective exposure assessment is inevitable, and some exposure misclassification is unavoidable. Such exposure misclassification was most likely for

the 19 deceased cases and their matched controls, for which proxy respondents were used, and for exposures outside the screw-cutting industry (295 of 1,486 identified job periods involved TCE exposure; 120 of these were not in the screw-cutting industry).

Although the exposure estimates from Moore et al. (2010) were not considered to be as quantitatively accurate as those of Charbotel et al. (2006), as discussed at the beginning of Section 5.2.2, it is worth noting, in the context of uncertainty in the exposure assessment, that the exposure estimates in Moore et al. (2010) are substantially lower than those of Charbotel et al. (2006) for comparable OR estimates. For example, for all subjects and high-confidence assessments only, respectively, Moore et al. (2010) reported OR estimates of 1.19 and 1.77 for cumulative exposures <1.58 ppm × years and 2.02 and 2.23 for cumulative exposures ≥1.58 ppm × years. Charbotel et al. (2006), on the other hand, reported OR estimates for all subjects of 1.62, 1.15, and 2.16 for mean cumulative exposures of 62.4, 253.2, and 925.0 ppm × years, respectively. If the exposure estimates for Charbotel et al. (2006) are overestimated, as suggested by the exposure estimates from Moore et al. (2010), then the slope of the linear regression model, and hence the unit risk estimate, would be correspondingly underestimated.

Another noteworthy source of uncertainty in the Charbotel et al. (2006) study is the possible influence of potential confounding or modifying factors. This study population, with a high prevalence of metal-working, also had relatively high prevalences of exposure to petroleum oils, cadmium, petroleum solvents, welding fumes, and asbestos (Fevotte et al., 2006). Other exposures assessed included other solvents (including other chlorinated solvents), lead, and ionizing radiation. None of these exposures was found to be significantly associated with RCC at a $p = 0.05$ significance level. Cutting fluids and other petroleum oils were associated with RCC at a $p = 0.1$ significance level; however, further modeling suggested no association with RCC when other significant factors were taken into account (Charbotel et al., 2006). Moreover, a review of other studies suggested that potential confounding from cutting fluids and other petroleum oils is of minimal concern (see Section 4.4.2.3). Nonetheless, a sensitivity analysis was conducted using the OR estimates further adjusted for cutting fluids and other petroleum oils from the unpublished report by Charbotel et al. (2005), and an essentially identical unit risk estimate of 5.46×10^{-3} per ppm was obtained.[54] In addition, the medical questionnaire included familial kidney disease and medical history, such as kidney stones, infection, chronic dialysis, hypertension, and use of antihypertensive drugs, diuretics, and analgesics. BMI was also calculated, and lifestyle information such as smoking habits and coffee consumption was collected. Univariate analyses found high levels of smoking and BMI to be associated with

[54]The OR estimates further adjusted for cutting fluids and other petroleum oils were 1.52 (95% CI: 0.66, 3.49), 1.07 (0.39, 2.88), and 1.96 (0.71, 5.37) for the low, medium, and high cumulative exposure groups, respectively (Charbotel et al., 2005). For the linear regression model, these OR estimates yielded a shallower slope estimate of 0.0009475 per ppm × year but a larger SE of 0.0009709 per ppm × year. In the lifetable analysis, these latter estimates in turn yielded a slightly higher EC_{01} estimate (4.92 versus 3.87 ppm), because of the shallower slope estimate, but an essentially identical LEC_{01}, because of the larger SE.

increased odds of RCC, and these two variables were included in the conditional logistic regressions. Thus, although impacts of other factors are possible, this study took great pains to attempt to account for potential confounding or modifying factors.

Some other sources of uncertainty associated with the epidemiological data are the dose-metric and lag period. As discussed above, there was some evidence of a contribution to increased RCC risk from peak TCE exposures; however, there appeared to be an independent effect of cumulative exposure without peaks. Cumulative exposure is considered a good measure of total exposure because it integrates exposure (levels) over time. If there is a contributing effect of peak exposures, not already taken into account in the cumulative exposure metric, the linear slope may be overestimated to some extent. Sometimes, cancer data are modeled with the inclusion of a lag period to discount more recent exposures not likely to have contributed to the onset of cancer. In an unpublished report, Charbotel et al. (2005) also present the results of a conditional logistic regression with a 10-year lag period, and these results are very similar to the unlagged results reported in their published paper, suggesting that the lag period might not be an important factor in this study.

Some additional sources of uncertainty are not so much inherent in the exposure-response modeling or in the epidemiologic data themselves but, rather, arise in the process of obtaining more general Agency risk estimates from the epidemiologic results. EPA cancer risk estimates are typically derived to represent an upper bound on increased risk of cancer incidence for all sites affected by an agent for the general population. From experimental animal studies, this is accomplished by using tumor incidence data and summing across all of the tumor sites that demonstrate significantly increased incidences, customarily for the most sensitive sex and species, to attempt to be protective of the general human population. However, in estimating comparable risks from the Charbotel et al. (2006) epidemiologic data, certain limitations are encountered. For one thing, these epidemiology data represent a geographically limited (Arve Valley, France), and likely not very diverse, population of working adults. Thus, there is uncertainty about the applicability of the results to a more diverse general population. Additionally, the Charbotel et al. (2006) study was a study of RCC only, and so the risk estimate derived from it does not represent all of the tumor sites that may be affected by TCE. The issue of cancer risk at other sites is addressed in the next section (see Section 5.2.2.2).

5.2.2.1.4. Conclusions regarding the RCC unit risk estimate

An EC_{01} of 3.9 ppm was calculated using a life-table analysis and linear modeling of the categorical conditional logistic regression results for RCC incidence reported in a high-quality case-control study. Linear low-dose extrapolation from the LEC_{01} yielded a lifetime extra RCC incidence unit risk estimate of 5.5×10^{-3} per ppm (1.0×10^{-6} per $\mu g/m^3$) of continuous TCE exposure. The assumption of low-dose linearity is supported by the conclusion that a mutagenic mode of action is operative for TCE-induced kidney tumors.

The inhalation unit risk estimate is expected to provide an upper bound on the risk of RCC incidence; however, this is just the risk estimate for RCC. A risk estimate for total cancer risk to humans would need to include the risk for other potential TCE-associated cancers.

5.2.2.2. Adjustment of the Inhalation Unit Risk Estimate for Multiple Sites

Human data on TCE exposure and cancer risk sufficient for dose-response modeling are only available for RCC, yet human and rodent data suggest that TCE exposure increases the risk of other cancers as well. In particular, there is evidence from human (and rodent) studies for increased risks of NHL and liver cancer (see Section 4.11). Therefore, the inhalation unit risk estimate derived from human data for RCC incidence was adjusted to account for potential increased risk of those cancer types. To make this adjustment, a factor accounting for the relative contributions to the extra risk for cancer incidence from TCE exposure for these three cancer types combined versus the extra risk for RCC alone was estimated, and this factor was applied to the unit risk estimate for RCC to obtain a unit risk estimate for the three cancer types combined (i.e., lifetime extra risk for developing *any* of the three types of cancer). This estimate is considered a better estimate of total cancer risk from TCE exposure than the estimate for RCC alone.

Although only the Charbotel et al. (2006) study was found adequate for direct estimation of inhalation unit risks, the available epidemiologic data provide sufficient information for estimating the *relative* potency of TCE across tumor sites. In particular, the relative contributions to extra risk (for cancer incidence) were calculated from two different data sets to derive the adjustment factor for adjusting the unit risk estimate for RCC to a unit risk estimate for the three types of cancers (RCC, NHL, and liver) combined. The first calculation is based on the results of the meta-analyses of human epidemiologic data for the three cancer types (see Appendix C); the second calculation is based on the results of the Raaschou-Nielsen et al. (2003) study, the largest single human epidemiologic study by far with RR estimates for all three cancer types. The approach for each calculation was to use the RR estimates and estimates of the lifetime background risk in an unexposed population, Ro, to calculate the lifetime risk in the exposed population, Rx, where Rx = RR × Ro, for each tumor type. Then, the extra risk from TCE exposure for each tumor type could be calculated using the equation in Section 5.2.2.1.2. Finally, the extra risks were summed across the three cancer types and the ratio of the sum of the extra risks to the extra risk for RCC was derived. For the first calculation, the RRm estimates from the meta-analyses for NHL, kidney cancer, and liver (and biliary) cancer were used as the RR estimates. For the second calculation, the SIR estimates from the Raaschou-Nielsen et al. (2003) study were used. For both calculations, Ro for RCC was taken from the life-table analysis described in Section 5.2.2.1.2 and presented in Appendix H, which estimated a lifetime risk for RCC incidence up to age 85 years. For Ro values for the other two sites, SEER statistics for the lifetime risk of developing cancer were used

(http://seer.cancer.gov/statfacts/html/nhl.html and http://seer.cancer.gov/statfacts/html/livibd.html).

In both cases, an underlying assumption in deriving the relative potencies is that the relative values of the age-specific background incidence risks for the person-years from the epidemiologic studies for each tumor type approximate the relative values of the lifetime background incidence risks for those cancer types. In other words, at least on a proportional basis, the lifetime background incidence risks (for the U.S. population) for each site approximate the age-specific background incidence risks for the study populations. A further assumption is that the lifetime risk of RCC up to 85 years is an adequate approximation to the full lifetime risk, which is what was used for the other two cancer types. The first calculation, based on the results of the meta-analyses for the three cancer types, has the advantage of being based on a large data set, incorporating data from many different studies. However, this calculation relies on a number of additional assumptions. First, it is assumed that the RRm estimates from the meta-analyses, which are based on different groups of studies, reflect similar overall TCE exposures (i.e., that the overall TCE exposures are similar across the different groups of studies that went into the different meta-analyses for the three cancer types). Second, it is assumed that the RRm estimates, which incorporate RR estimates for both mortality and incidence, represent good estimates for cancer incidence risk from TCE exposure. In addition, it is assumed that the RRm for kidney cancer, for which RCC estimates from individual studies were used when available, is a good estimate for the overall RR for RCC and that the RRm estimate for NHL, for which different studies used different classification schemes, is a good estimate for the overall RR for NHL. The second calculation, based on the results of the Raaschou-Nielsen et al. (2003) study, the largest single study with RR estimates for all three cancer types, has the advantage of having RR estimates that are directly comparable. In addition, the Raaschou-Nielsen et al. study provided data for the precise cancer types of interest for the calculation (i.e., RCC, NHL, and liver [and biliary] cancer).

The input data and results of the calculations are presented in Table 5-46. The value for the ratio of the sum of the extra risks to the extra risk for RCC alone was 3.28 in calculation #1 and 4.36 in calculation #2, which together suggest that 4 is a reasonable factor to use to adjust the inhalation unit risk estimate based on RCC for multiple sites to obtain a total cancer unit risk estimate.[55] Using this factor to adjust the unit risk estimate based on RCCs entails the further fundamental assumption that the dose-response relationships for the other two cancer types (NHL and liver cancer) are similarly linear (i.e., that the relative potencies are roughly maintained at lower exposure levels). This assumption is consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), which recommends low-dose linear extrapolation in the absence of sufficient evidence to support a nonlinear mode of action.

[55]Both the geometric and arithmetic means of the two values for the ratio are 3.8, which rounds to 4, in keeping with the imprecise nature of the adjustment factor. The factor of 4 is within 25% of either calculated ratio.

Table 5-46. Relative contributions to extra risk for cancer incidence from TCE exposure for multiple cancer types

	RR	Ro	Rx	Extra risk	Ratio to kidney value
Calculation #1: using RR estimates from the meta-analyses					
Kidney (RCC)	1.27	0.0107	0.01359	0.002920	1
NHL	1.23	0.0202	0.02485	0.004742	1.62
Liver (and biliary) cancer	1.29	0.0066	0.008514	0.001927	0.66
			sum	0.009589	**3.28**
Kidney + NHL only			**sum**	0.007662	2.62
Calculation #2: using RR estimates from Rasschou-Nielsen et al. (2003)					
Kidney (RCC)	1.20	0.0107	0.01284	0.002163	1
NHL	1.24	0.0202	0.02505	0.004948	2.29
Liver (and biliary) cancer	1.35	0.0066	0.008910	0.002325	1.07
			sum	0.009436	**4.36**
Kidney + NHL only			**sum**	0.007111	3.29

Applying the factor of 4 to the lifetime extra RCC incidence unit risk estimate of 5.49×10^{-3} per ppm (1.0×10^{-6} per $\mu g/m^3$) of continuous TCE exposure yields a cancer unit risk estimate of 2.2×10^{-2} per ppm (4.1×10^{-6} per $\mu g/m^3$). Table 5-46 also presents calculations for just kidney and NHL extra risks combined, because the strongest human evidence is for those two cancer types. For those two cancer types, the calculations support a factor of 3.[56] Applying this factor to the RCC unit risk estimate yields an estimate of 1.6×10^{-2} per ppm, which results in the same estimate as for the three cancer types combined when finally rounded to one significant figure (i.e., 2×10^{-2} per ppm [or 3×10^{-6} per $\mu g/m^3$, which is still similar to the three-tumor-type estimate in those units]).

In addition to the uncertainties in the underlying RCC estimate, there are uncertainties related to the assumptions inherent in these calculations for adjusting to multiple sites, as detailed above. Nonetheless, the fact that the calculations based on two different data sets yielded comparable values for the adjustment factor (both within 25% of the selected factor of 4) provides more robust support for the use of the factor of 4. Additional uncertainties pertain to the weight of evidence supporting the association of TCE exposure with increased risk of cancer for the three cancer types. As discussed in Section 4.11.2, it was found that the weight of evidence for kidney cancer was sufficient to classify TCE as ~~"~~carcinogenic to humans." It was also concluded that there was strong evidence that TCE causes NHL as well, although the evidence for liver cancer was more limited. In addition, the rodent studies demonstrate clear

[56]The geometric and mean of the two values for the ratio, 2.62 and 3.29, is 2.96, and the arithmetic mean is 2.94, which both round to 3, in keeping with the imprecise nature of the adjustment factor. The factor of 3 is within 15% of either calculated ratio.

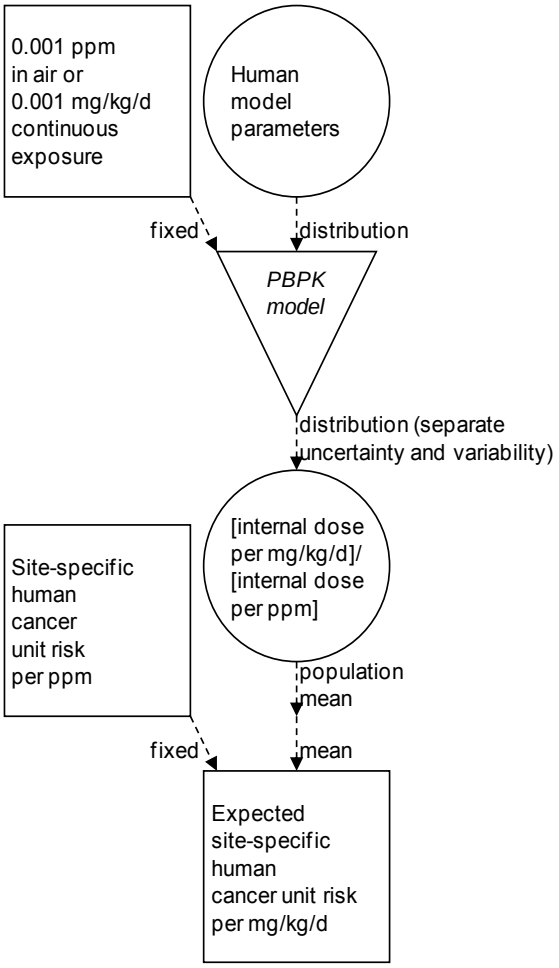
evidence of multisite carcinogenicity, with cancer types including those for which associations with TCE exposure are observed in human studies (i.e., liver and kidney cancers and NHLs). Overall, the evidence was found to be sufficiently persuasive to support the use of the adjustment factor of 4 based on these three cancer types, resulting in a cancer inhalation unit risk estimate of 2.2×10^{-2} per ppm (4.1×10^{-6} per $\mu g/m^3$). Alternatively, if one were to use the factor based only on the two cancer types with the strongest human evidence, the cancer inhalation unit risk estimate would be only slightly reduced (25%).

5.2.2.3. Route-to-Route Extrapolation Using PBPK Model

Route-to-route extrapolation of the inhalation unit risk estimate was performed using the PBPK model described in Section 3.5. The (partial) unit risk estimates for NHL and liver cancer were derived as for the total cancer inhalation unit risk estimate in Section 5.2.2.2, except that the ratios of extra risk for the individual cancer types relative to kidney cancer were used as adjustment factors rather than the ratio of the sum. As presented in Table 5-46, for NHL, the ratios from the two different calculations were 1.62 and 2.29, so a factor of 2 was used; for liver cancer, the ratios were 0.66 and 1.07, so a factor of 1 was used. (With the ratio of 1 for kidney cancer itself, the combined adjustment factor is 4, reproducing the factor of 4 used to estimate the total cancer unit risk from the multiple sites in Section 5.2.2.2)

Because different internal dose-metrics are preferred for each target tissue site, a separate route-to-route extrapolation was performed for each site-specific unit risk estimate calculated in Sections 5.2.2.1 and 5.2.2.2. As shown in Figure 5-7, the approach taken to apply the human PBPK model in the low-dose range where external and internal doses are linearly related to derive a conversion that is the ratio of internal dose per mg/kg/day to internal dose per ppm. The expected value of the population mean for this conversion factor (in ppm per mg/kg/day) was used to extrapolate each inhalation unit risk in units of risk per ppm to an oral slope factor in units of risk per mg/kg/day. Note that this conversion is the *mean of the ratio* of internal dose predictions, and is not the same as taking the *ratio of the mean* of internal dose predictions in Table 5-35.[57]

[57]For route-to-route extrapolation based on dose-response analysis performed on internal dose, as is the case for rodent bioassays, it would be appropriate to use the values in Table 5-35 to first "unconvert" the unit risk based on one route, and then recovert to a unit risk based on the other route.



Square nodes indicate point values, circle nodes indicate distributions, and the inverted triangle indicates a (deterministic) functional relationship.

Figure 5-7. Flow-chart for route-to-route extrapolation of human site-specific cancer inhalation unit risks to oral slope factors.

Table 5-47 shows the results of this route-to-route extrapolation for the "primary" and "alternative" dose-metrics. For reference, route-to-route extrapolation based on total intake (i.e., ventilation rate × air concentration = oral dose × body weight) using the parameters in the PBPK model would yield an expected population average conversion of 0.95 ppm per mg/kg/day. For TotMetabBW34, TotOxMetabBW34, and AMetLiv1BW34, the conversion is 2.0–2.8 ppm per mg/kg/day, greater than that based on intake. This is because of the greater metabolic first pass in the liver, which leads to a higher percentage of intake being metabolized via oral exposure relative to inhalation exposure for the same intake. Conversely, for the AUC in blood, the conversion is 0.14 ppm per mg/kg/day, less than that based on intake—the greater first pass in the liver means lower blood levels of parent compound via oral exposure relative to inhalation for the same intake. The conversion for the primary dose-metric for the kidney, ABioactDCVCBW34, is 1.7 ppm per mg/kg/day, less than that for total, oxidative, or liver

oxidative metabolism. This is because the majority of metabolism in first pass through the liver is via oxidation, whereas with inhalation exposure, more parent compound reaches the kidney, in which metabolism is via GSH conjugation.

Table 5-47. Route-to-route extrapolation of site-specific inhalation unit risks to oral slope factors

	Kidney	NHL	Liver
Inhalation unit risk (risk per ppm)	5.49×10^{-3}	1.10×10^{-2}	5.49×10^{-3}
Primary dose-metric	ABioactDCVCBW34[a]	TotMetabBW34	AMetLiv1BW34
ppm per mg/kg/d[b]	1.70	1.97	2.82
Oral slope factor (risk per mg/kg/d)	9.33×10^{-3}	2.16×10^{-2}	1.55×10^{-2}
Alternative dose-metric	TotMetabBW34	AUCCBld	TotOxMetabBW34
ppm per mg/kg/d[b]	1.97	0.137	2.04
Oral slope factor (risk per mg/kg/d)	1.08×10^{-2}	1.50×10^{-3}	1.12×10^{-2}

[a]The AMetGSHBW34 dose-metric gives the same route-to-route conversion because there is no route dependence in the pathway between GSH conjugation and DCVC bioactivation.
[b]Average of expected population mean of males and females. Male and female estimates differed by <1% for ABioactDCVCBW34; TotMetabBW34, AMetLiv1BW34, and TotOxMetabBW34, and <15% for AUCCBld. Uncertainty on the population mean route-to-route conversion, expressed as the ratio between the 97.5% quantile the 2.5% quantile, is about 2.6-fold for ABioactDCVCBW34, 1.5-fold for TotMetabBW34, AMetLiv1BW34, and TotOxMetabBW34, and about 3.4-fold for AUCCBld.

When one sums the oral slope factor estimates based on the primary (preferred) dose-metrics for the three individual cancer types shown in Table 5-47, the resulting total cancer oral slope factor estimate is **4.64×10^{-2} per mg/kg/day**. In the case of the oral route-extrapolated results, the ratio of the risk estimate for the three cancer types combined to the risk estimate for kidney cancer alone is 5.0. This value differs from the factor of 4 used for the total cancer inhalation unit risk estimate because of the different dose-metrics used for the different cancer types when the route-to-route extrapolation is performed. If only the kidney cancer and NHL results, for which the evidence is strongest, were combined, the resulting total cancer oral slope factor estimate would be 3.09×10^{-2} per mg/kg/day, and the ratio of this risk estimate to that for kidney cancer alone would be 3.3.

If one were to use some of the risk estimates based on alternative dose-metrics in Table 5-40, the total cancer risk estimate would vary depending on for which tumor type(s) an alternative metric was used. The most extreme difference would occur when the alternative metric is used for NHL and liver tumors; in that case, the resulting total cancer oral slope factor estimate would be 2.20×10^{-2} per mg/kg/day, and the ratio of this risk estimate to that for kidney cancer alone (based on the primary dose-metric of ABioactDCVCBW34) would be 2.4.

The uncertainties in these conversions are relatively modest. As discussed in the note to Table 5-47, the 95% confidence range for the route-to-route conversions at its greatest spans 3.4-fold. The greatest uncertainty is in the selection of the dose-metric for NHL, since the use of the alternative dose-metric of AUCCBld yields a converted oral slope factor that is 14-fold lower than that using the primary dose-metric of TotMetabBW34. However, for the other two tumor sites, the range of conversions is tighter, and lies within threefold of the conversion based solely on intake.

5.2.3. Summary of Unit Risk Estimates

5.2.3.1. Inhalation Unit Risk Estimate

The inhalation unit risk for TCE is defined as a plausible upper bound lifetime extra risk of cancer from chronic inhalation of TCE per unit of air concentration. The preferred estimate of the inhalation unit risk for TCE is 2.20×10^{-2} per ppm (**2×10^{-2} per ppm [4×10^{-6} per µg/m^3]** rounded to one significant figure), based on human kidney cancer risks reported by Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer. This estimate is based on good-quality human data, thus avoiding the uncertainties inherent in interspecies extrapolation.

This value is supported by inhalation unit risk estimates from multiple rodent bioassays, the most sensitive of which range from **1×10^{-2} to 2×10^{-1} per ppm [2×10^{-6} to 3×10^{-5} per µg/m^3]**. From the inhalation bioassays selected for analysis in Section 5.2.1.1, and using the preferred PBPK model-based dose-metrics, the inhalation unit risk estimate for the most sensitive sex/species is 8×10^{-2} per ppm [2×10^{-5} per µg/m^3], based on kidney adenomas and carcinomas reported by Maltoni et al. (1986) for male Sprague-Dawley rats. Leukemias and Leydig cell tumors were also increased in these rats, and, although a combined analysis for these cancer types that incorporated the different site-specific preferred dose-metrics was not performed, the result of such an analysis is expected to be similar, about 9×10^{-2} per ppm [2×10^{-5} per µg/m^3]. The next most sensitive sex/species from the inhalation bioassays is the female mouse, for which lymphomas were reported by Henschler et al. (1980); these data yield a unit risk estimate of 1.0×10^{-2} per ppm [2×10^{-6} per µg/m^3]. In addition, the 90% CIs reported in Table 5-41 for male rat kidney tumors from Maltoni et al. (1986) and female mouse lymphomas from Henschler et al. (1980), derived from the quantitative analysis of PBPK model uncertainty, both included the estimate based on human data of 2×10^{-2} per ppm. Furthermore, PBPK model-based route-to-route extrapolation of the results for the most sensitive sex/species from the oral bioassays, kidney tumors in male Osborne-Mendel rats and testicular tumors in Marshall rats (NTP, 1988), leads to inhalation unit risk estimates of 2×10^{-1} per ppm [3×10^{-5} per µg/m^3] and 4×10^{-2} per ppm [8×10^{-6} per µg/m^3], respectively, with the preferred estimate based on human data falling within the route-to-route extrapolation of the 90%

CIs reported in Table 5-42.[58] Finally, for all of these estimates, the ratios of BMDs to the BMDLs did not exceed a value of 3, indicating that the uncertainties in the dose-response modeling for determining the POD in the observable range are small.

Although there are uncertainties in these various estimates, as discussed in Sections 5.2.1.4, 5.2.2.1.3, and 5.2.2.2, confidence in the proposed inhalation unit risk estimate of 2×10^{-2} per ppm [4×10^{-6} per µg/m^3], based on human kidney cancer risks reported by Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer (as discussed in Section 5.2.2.2), is further increased by the similarity of this estimate to estimates based on multiple rodent data sets.

5.2.3.2. Oral Slope Factor Estimate

The oral slope factor for TCE is defined as a plausible upper bound lifetime extra risk of cancer from chronic ingestion of TCE per mg/kg/day oral dose. The preferred estimate of the oral slope factor is 4.64×10^{-2} per mg/kg/day (**5×10^{-2} per mg/kg/day** rounded to one significant figure), resulting from PBPK model-based route-to-route extrapolation of the inhalation unit risk estimate based on the human kidney cancer risks reported in Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer. This estimate is based on good-quality human data, thus avoiding uncertainties inherent in interspecies extrapolation. In addition, uncertainty in the PBPK model-based route-to-route extrapolation is relatively low (Chiu, 2006; Chiu and White, 2006). In this particular case, extrapolation using different dose-metrics yielded expected population mean risks within about a twofold range, and, for any particular dose-metric, the 95% CI for the extrapolated population mean risks for each site spanned a range of no more than about threefold.

This value is supported by oral slope factor estimates from multiple rodent bioassays, the most sensitive of which range from **3×10^{-2} to 3×10^{-1} per mg/kg/day**. From the oral bioassays selected for analysis in Section 5.2.1.1, and using the preferred PBPK model-based dose-metrics, the oral slope factor estimate for the most sensitive sex/species is 3×10^{-1} per mg/kg/day, based on kidney tumors in male Osborne-Mendel rats (NTP, 1988). The oral slope factor estimate for testicular tumors in male Marshall rats (NTP, 1988) is somewhat lower at 7×10^{-2} per mg/kg/day. The next most sensitive sex/species result from the oral studies is for male mouse liver tumors (NCI, 1976), with an oral slope factor estimate of 3×10^{-2} per mg/kg/day. In addition, the 90% CIs reported in Table 5-42 for male Osborne-Mendel rat kidney tumors (NTP,

[58]For oral-to-inhalation extrapolation of NTP (1988) male rat kidney tumors, the unit risk estimate of 2.5 × 10-1 per mg/kg/day using the ABioactDCVCBW34 dose metric, from Table 5-37, is divided by the average male and female internal doses at 0.001 mg/kg/day, (0.00504/0.001), and then multiplied by the average male and female internal doses at 0.001 ppm (0.00324/0.001), both from Table 5-35, to yield a unit risk of 1.6 × 10-1 [3.0 × 10–5 per µg/m^3]. For oral-to-inhalation extrapolation of NTP (1988) male rat testicular tumors, the unit risk estimate of 7.1×10^{-2} per mg/kg/day using the TotMetabBW34 dose metric, from Table 5-37, is divided by the male internal dose at 0.001 mg/kg/day, (0.0192/0.001), and then multiplied by the male internal doses at 0.001 ppm (0.0118/0.001), both from Table 5-35, to yield a unit risk of 4.4×10^{-2} [8.1×10^{-6} per µg/m^3].

1988), male F344 rat kidney tumors (NTP, 1990), and male Marshall rat testicular tumors (NTP, 1988), derived from the quantitative analysis of PBPK model uncertainty, all included the estimate based on human data of 5×10^{-2} per mg/kg/day, while the upper 95% confidence bound for male mouse liver tumors from NCI (1976) was slightly below this value at 4×10^{-2} per mg/kg/day. Furthermore, PBPK model-based route-to-route extrapolation of the most sensitive endpoint from the inhalation bioassays, male rat kidney tumors from Maltoni et al. (1986), leads to an oral slope factor estimate of 1×10^{-1} per mg/kg/day, with the preferred estimate based on human data falling within the route-to-route extrapolation of the 90% CI reported in Table 5-41.[59] Finally, for all of these estimates, the ratios of BMDs to the BMDLs did not exceed a value of 3, indicating that the uncertainties in the dose-response modeling for determining the POD in the observable range are small.

Although there are uncertainties in these various estimates, as discussed in Sections 5.2.1.4, 5.2.2.1.3, 5.2.2.2, and 5.2.2.3, confidence in the proposed oral slope factor estimate of 5×10^{-2} per mg/kg/day, resulting from PBPK model-based route-to-route extrapolation of the inhalation unit risk estimate based on the human kidney cancer risks reported in Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer (as discussed in Section 5.2.2.2), is further increased by the similarity of this estimate to estimates based on multiple rodent data sets.

5.2.3.3. Application of ADAFs

When there is sufficient weight of evidence to conclude that a carcinogen operates through a mutagenic mode of action, and in the absence of chemical-specific data on age-specific susceptibility, EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005e) advises that increased early-life susceptibility be assumed and recommends that default ADAFs be applied to adjust for this potential increased susceptibility from early-life exposure. As discussed in Section 4.4, there is sufficient evidence to conclude that a mutagenic mode of action is operative for TCE-induced kidney tumors. The weight of evidence also supports involvement of processes of cytotoxicity and regenerative proliferation in the carcinogenicity of TCE, although not with the extent of support as for a mutagenic mode of action. In particular, data linking TCE-induced proliferation to increased mutation or clonal expansion are lacking, as are data informing the quantitative contribution of cytotoxicity. Because any possible involvement of a cytotoxicity mode of action would be additional to mutagenicity, the mutagenic mode of action would be expected to dominate at low doses. Therefore, the additional involvement of a cytotoxicity mode of action does not provide evidence against the application of ADAFs. In addition, as described in Section 4.10, TCE-

[59]For the Maltoni et al. (1986) male rat kidney tumors, the unit risk estimate of 8.3×10^{-2} per ppm using the ABioactDCVCBW34 dose metric, from Table 5-36, is divided by the average male and female internal doses at 0.001 ppm (0.00324/0.001) and then multiplied by the average male and female internal doses at 0.001 mg/kg/day, (0.00504/0.001), both from Table 5-35, to yield a unit risk of 1.3×10^{-1} per mg/kg/day.

specific data are inadequate for quantification of early-life susceptibility to TCE carcinogenicity. Therefore, as recommended in the *Supplemental Guidance,* the default ADAFs are applied.

See the *Supplemental Guidance* for detailed information on the general application of these adjustment factors. In brief, the *Supplemental Guidance* establishes ADAFs for three specific age groups. The current ADAFs and their age groupings are 10 for <2 years, 3 for 2–<16 years, and 1 for ≥16 years (U.S. EPA, 2005e). For risk assessments based on specific exposure assessments, the 10- and 3-fold adjustments to the slope factor or unit risk estimates are to be combined with age-specific exposure estimates when estimating cancer risks from early-life (<16-years-of-age) exposure. Currently, due to lack of appropriate data, no ADAFs are used for other lifestages, such as the elderly. However, the ADAFs and their age groups may be revised over time. The most current information on the application of ADAFs for cancer risk assessment can be found at www.epa.gov/cancerguidelines.

In the case of TCE, the inhalation unit risk and oral slope factor estimates reflect lifetime risk for cancer at multiple sites, and a mutagenic mode of action has been established for one of these sites, the kidney. The following subsections illustrate how one might apply the default ADAFs to the *kidney-cancer component* of the inhalation unit risk and oral slope factor estimates for TCE. These are **sample calculations,** and individual risk assessors should use exposure-related parameters (e.g., age-specific water ingestion rates) that are appropriate for their particular risk assessment applications.

In addition to the uncertainties discussed above for the inhalation and oral total cancer unit risk or slope factor estimates, there are uncertainties in the application of ADAFs to adjust for potential increased early-life susceptibility. For one thing, the adjustment is made only for the kidney cancer component of total cancer risk because that is the tumor type for which the weight of evidence was sufficient to conclude that TCE-induced carcinogenesis operates through a mutagenic mode of action. However, it may be that TCE operates through a mutagenic mode of action for other cancer types as well or that it operates through other modes of action that might also convey increased early-life susceptibility. Additionally, the ADAFs are general default factors, and it is uncertain to what extent they reflect increased early-life susceptibility for exposure to TCE, if increased early-life susceptibility occurs.

Furthermore, the assumption of increased early-life susceptibility, invoked by the finding of a mutagenic mode of action for kidney cancer, is in contradiction to the assumption that RR is independent of age that was used to derive the unit risk estimates in the life-table analysis. In some other assessments faced with a similar situation, a small modification has been made to the derivation of the unit risk estimate to avoid the contradictory assumptions (by calculating an adult-exposure-only unit risk estimate for the application of ADAFs). This has the effect of slightly reducing the unit risk estimate to which the ADAFs are applied. Because there are multiple cancer types for TCE but the finding of a mutagenic mode of action applies to only one of them, and because under these circumstances application of the ADAFs already has a minimal

impact on the total risk for most exposure scenarios, as discussed with respect to the examples in Sections 5.2.3.3.1 and 5.2.3.3.2 below, no attempt was made to modify the kidney cancer unit risk estimate for this assessment. Such a modification would have substantially increased the complexity of the calculations, which are already more elaborate than the standard ADAF applications, without having much quantitative impact on the final risk estimates.

5.2.3.3.1. Example application of ADAFs for inhalation exposures.

A calculation template for application of the ADAFs is provided in Table 5-48, with an Excel spreadsheet version available on the HERO database (U.S. EPA, 2011e). In the example provided, it is assumed that an individual is exposed to 1 $\mu g/m^3$ in air from birth through age 70 years. Using the template, risk estimates for different exposure scenarios can be obtained by changing the exposure concentrations (including possibly zero for some age groups). The steps in the calculation are as follows:

(1) Separate the kidney cancer contribution from the NHL + liver cancer contribution to the inhalation unit risk estimate. From Section 5.2.2.1.4, the kidney lifetime unit risk is 1.0×10^{-6} per $\mu g/m^3$ in air. Subtracting this from the total lifetime unit risk of 4.1×10^{-6} per $\mu g/m^3$ from Section 5.2.2.2 results in the estimated contribution of NHL + liver cancer being 3.1×10^{-6} per $\mu g/m^3$.

(2) Assign a lifetime unit risk estimate for each age group. The template shows the recommended age groupings from U.S. EPA (2005c) in Column A (augmented by additional age groups from U.S. EPA, 2008c, and for assessing 30 year exposures), along with the age group duration (Column D), and the fraction of lifetime each age group represents (Column E; used as a duration adjustment). For each age group, the (unadjusted) lifetime unit risk estimates for kidney cancer, total cancer, and NHL + liver cancer are shown in Column F, I, and J, respectively.

(3) For each age group, the kidney cancer inhalation unit risk estimate (Column F) is multiplied by the risk per $\mu g/m^3$ equivalence (Column B), the exposure concentration (Column C), the duration adjustment (Column E), and the ADAF (Column G), to obtain the partial risk from exposure during those ages (Column H). For inhalation exposures, a "risk per $\mu g/m^3$ equivalence" of 1 is assumed across age groups (i.e., equivalent risk from equivalent exposure levels in air, independent of body size), as shown in Column B. In this calculation, a unit lifetime exposure of 1 $\mu g/m^3$ is assumed, as shown in Column C.

(4) For each age group, the NHL + liver cancer unit risk estimate (Column J) is multiplied by the risk per $\mu g/m^3$ equivalence (Column B), the exposure concentration (Column C), and the duration adjustment (Column E), to obtain the partial risk from exposure during those ages (Column K).

(5) For each age group, the ADAF-adjusted partial risk for kidney cancer (Column H) is added to the partial risk for NHL + liver cancer (Column K), resulting in the total partial risk (Column L).

(6) The age-group-specific partial risks are added together to obtain the estimated total lifetime risk (bottom of Column L).

Table 5-48. Sample calculation for total lifetime cancer risk based on the kidney unit risk estimate, potential risk for NHL and liver cancer, and potential increased early-life susceptibility, assuming a constant lifetime exposure to 1 µg/m^3 of TCE in air

Column A	Column B	Column C	Column D	Column E	Column F	Column G	Column H	Column I	Column J	Column K	Column L
	Exposure scenario parameters				Dose-response assessment calculations						
Units:		**(µg/m^3)**	**yr**	**-**	**(µg/m^3)$^{-1}$**	**-**		**(µg/m^3)$^{-1}$**	**(µg/m^3)$^{-1}$**		
Age group	Risk per µg/m^3 air equivalence	Exposure concentration	Age group duration	Duration adjustment (Column D/70 yr)	Kidney cancer unadjusted lifetime unit risk (see Section 5.2.2.1.4)	Default ADAF	**Kidney cancer ADAF-adjusted partial risk (Column B × Column C × Column E × Column F × Column G)**	Kidney cancer+NHL+ liver cancer unadjusted lifetime unit risk (see Section 5.2.2.2)	NHL+ liver cancer lifetime unit risk (Column I – Column F)	**NHL and liver cancer partial risk (Column B × Column C × Column E × Column J)**	**Total partial risk (Column H + Column K)**
Birth to <1 mo	1	1.000	0.083	0.0012	1×10^{-6}	10	**1.2×10^{-8}**	4.1×10^{-6}	3.1×10^{-6}	**3.7×10^{-9}**	**1.6×10^{-8}**
1–<3 mo	1	1.000	0.167	0.0024	1×10^{-6}	10	**2.4×10^{-8}**	4.1×10^{-6}	3.1×10^{-6}	**7.4×10^{-9}**	**3.1×10^{-8}**
3–<6 mo	1	1.000	0.250	0.0036	1×10^{-6}	10	**3.6×10^{-8}**	4.1×10^{-6}	3.1×10^{-6}	**1.1×10^{-8}**	**4.7×10^{-8}**
6–<12 mo	1	1.000	0.500	0.0071	1×10^{-6}	10	**7.1×10^{-8}**	4.1×10^{-6}	3.1×10^{-6}	**2.2×10^{-8}**	**9.4×10^{-8}**
1–<2 yrs	1	1.000	1.000	0.0143	1×10^{-6}	10	**1.4×10^{-7}**	4.1×10^{-6}	3.1×10^{-6}	**4.4×10^{-8}**	**1.9×10^{-7}**
2–<3 yrs	1	1.000	1.000	0.0143	1×10^{-6}	3	**4.3×10^{-8}**	4.1×10^{-6}	3.1×10^{-6}	**4.4×10^{-8}**	**8.7×10^{-8}**
3–<6 yrs	1	1.000	3.000	0.0429	1×10^{-6}	3	**1.3×10^{-7}**	4.1×10^{-6}	3.1×10^{-6}	**1.3×10^{-7}**	**2.6×10^{-7}**
6–<11 yrs	1	1.000	5.000	0.0714	1×10^{-6}	3	**2.1×10^{-7}**	4.1×10^{-6}	3.1×10^{-6}	**2.2×10^{-7}**	**4.4×10^{-7}**
11–<16 yrs	1	1.000	5.000	0.0714	1×10^{-6}	3	**2.1×10^{-7}**	4.1×10^{-6}	3.1×10^{-6}	**2.2×10^{-7}**	**4.4×10^{-7}**
16–<18 yrs	1	1.000	2.000	0.0286	1×10^{-6}	1	**2.9×10^{-8}**	4.1×10^{-6}	3.1×10^{-6}	**8.9×10^{-8}**	**1.2×10^{-7}**
18-<21	1	1.000	3.000	0.0429	1×10^{-6}	1	**4.3×10^{-8}**	4.1×10^{-6}	3.1×10^{-6}	**1.3×10^{-7}**	**1.8×10^{-7}**
21-<30	1	1.000	9.000	0.1286	1×10^{-6}	1	**1.3×10^{-7}**	4.1×10^{-6}	3.1×10^{-6}	**4.0×10^{-7}**	**5.3×10^{-7}**
30–70 yrs	1	1.000	40.000	0.5714	1×10^{-6}	1	**5.7×10^{-7}**	4.1×10^{-6}	3.1×10^{-6}	**1.8×10^{-6}**	**2.3×10^{-6}**
										Total unit risk	**4.8×10^{-6}**

From the example calculation, based on continuous exposure to 1 μg/m^3 from birth to age 70, the estimated total lifetime risk is 4.8×10^{-6}, which corresponds to a lifetime unit risk estimate of 4.8×10^{-6} per μg/m^3. The risk-specific air concentrations at risk levels of 10^{-6}, 10^{-5}, and 10^{-4} are 0.21, 2.1, and 21 μg/m^3, respectively.

This total cancer unit risk estimate of 4.8×10^{-6} per μg/m^3 (2.6×10^{-2} per ppm), adjusted for potential increased early-life susceptibility, is only minimally (17.5%) increased over the unadjusted total cancer unit risk estimate because the kidney cancer risk estimate that gets adjusted for potential increased early-life susceptibility is only part of the total cancer risk estimate. Thus, foregoing the ADAF adjustment in the case of full lifetime calculations will not seriously impact the resulting risk estimate. For less-than-lifetime exposure calculations, the impact of applying the ADAFs will increase as the proportion of time at older ages decreases. The maximum impact will be when exposure is for only the first 2 years of life, in which case, the partial lifetime total cancer risk estimate for exposure to 1 μg/m^3 adjusted for potential increased early-life susceptibility is $10 \times$ (1 μg/m^3) $\times$ (1.0×10^{-6} per μg/m^3) $\times$ (2 / 70) for the kidney cancer risk + (1 μg/m^3) $\times$ (3.1×10^{-6} per μg/m^3) $\times$ (2 / 70) for the NHL and liver cancer, or 3.7×10^{-7}, which is over 3 times greater than the unadjusted partial lifetime total cancer risk estimate for exposure to 1 μg/m^3 of (1 μg/m^3) $\times$ (4.1×10^{-6} per μg/m^3) $\times$ (2 / 70), or 1.2×10^{-7}.

5.2.3.3.2. Example application of ADAFs for oral drinking water exposures

For oral exposures, the calculation of risk estimates adjusted for potential increased early-life susceptibility is complicated by the fact that for a constant exposure level (e.g., a constant concentration of TCE in drinking water) doses will vary by age because of different age-specific uptake rates (e.g., drinking water consumption rates). Different EPA Program or Regional Offices may have different default age-specific uptake rates that they use for risk assessments for specific exposure scenarios, and the calculations presented below are merely to illustrate the general approach to applying ADAFs for oral TCE exposures, using exposure to 1 μg/L of TCE in drinking water from birth through age 70 years as an example. Using the template, risk estimates for different exposure scenarios can be obtained by changing the intake rates and exposure concentrations (including possibly zero for some age groups). The steps in the calculation, illustrated in the template in Table 5-49 (available as an Excel spreadsheet version on the HERO database, U.S. EPA, 2011e), are as follows:

(1) Separate the kidney cancer contribution from the NHL + liver cancer contribution to the oral slope factor estimate. From Section 5.2.2.3, the kidney lifetime oral slope factor is 9.3×10^{-3} per mg/kg/day. Subtracting this from the total lifetime oral slope factor of 4.6×10^{-2} per mg/kg/day from Section 5.2.2.3 results in an estimated contribution from NHL + liver cancer of 3.7×10^{-2} per mg/kg/day.

(2) Assign a lifetime oral slope factor estimate for each age group. The template shows the recommended age groupings from U.S. EPA (2005c) in Column A (augmented by additional age groups from U.S. EPA, 2008c, and for assessing 30 year exposures), along with the age group duration (Column D), and the fraction of lifetime each age group represents (Column E; used as a duration adjustment). For each age group, the (unadjusted) lifetime oral slope factor estimates for kidney cancer, total cancer, and NHL + liver cancer are shown in Columns F, I, and J, respectively.

(3) For each age group, the kidney cancer oral slope factor estimate (Column F) is multiplied by the drinking water ingestion rate (Column B), the exposure concentration (Column C), the duration adjustment (Column E), and the ADAF (Column G), to obtain the partial risk from exposure during those ages (Column H). Age-specific water ingestion rates in L/kg/day, taken from the EPA Office of Water Policy Document *Age Dependent Adjustment Factor (ADAF) Application* are shown in Column B.[60] In this calculation, a lifetime unit exposure of 1 µg/L is assumed, as shown in Column C.

(4) For each age group, the NHL + liver cancer oral slope factor estimate (Column J) is multiplied by the drinking water ingestion rate (Column B), the exposure concentration (Column C), and the duration adjustment (Column E), to obtain the partial risk from exposure during those ages (Column K).

(5) For each age group, the ADAF-adjusted partial risk for kidney cancer (Column H) is added to the partial risk for NHL + liver cancer (Column K), resulting in the total partial risk (Column L).

(6) The age-group-specific partial risks are added together to obtain the estimated total lifetime risk (bottom of Column L).

[60]Values for the 90th percentile were taken from Table 3-19 of U.S. EPA (2008a) (consumers-only estimates of combined direct and indirect water ingestion from community water) and U.S. EPA (2004) (Table A1). The 90th percentile was based on the policy in the U.S. EPA Office of Water for determining risk through direct and indirect consumption of drinking water (U.S. EPA, 2011f). Community water was used in the illustration because U.S. EPA only regulates community water sources and not private wells and cisterns or bottled water. Data for "consumers only" (i.e., excluding individuals who did not ingest community water) were used because formula-fed infants (as opposed to breast-fed infants, who consume very little community water), children, and young adolescents are often the population of concern with respect to water consumption.

Table 5-49. Sample calculation for total lifetime cancer risk based on the kidney cancer slope factor estimate, potential risk for NHL and liver cancer, and potential increased early-life susceptibility, assuming a constant lifetime exposure to 1 µg/L of TCE in drinking water

Column A	Column B	Column C	Column D	Column E	Column F	Column G	Column H	Column I	Column J	Column K	Column L
	Exposure scenario parameters				Dose-response assessment calculations						
Units:	L water/kg/d	mg/L water	yr	-	$(mg/kg/d)^{-1}$	-	-	$(mg/kg/d)^{-1}$	$(mg/kg/d)^{-1}$	-	-
Age group	Ingestion rate	Exposure concentration	Age group duration	Duration adjustment (Column D/ 70 yr)	Kidney cancer unadjusted lifetime slope factor (see Table 5-40)	Default ADAF	**Kidney cancer ADAF adjusted partial risk (Column B × Column C × Column E × Column F × Column G)**	Kidney cancer+NHL+ liver cancer unadjusted lifetime unit risk (see Section 5.2.2.3)	NHL+ liver cancer lifetime unit risk (Column I – Column F)	**NHL and liver cancer partial risk (Column B × Column C × Column E × Column J)**	**Total partial risk (Column H + Column K)**
Birth to <1 mo	0.235	0.001	0.083	0.0012	9.3×10^{-3}	10	$\mathbf{2.6 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{1.0 \times 10^{-8}}$	$\mathbf{3.6 \times 10^{-8}}$
1–<3 mo	0.228	0.001	0.167	0.0024	9.3×10^{-3}	10	$\mathbf{5.0 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{2.0 \times 10^{-8}}$	$\mathbf{7.0 \times 10^{-8}}$
3–<6 mo	0.148	0.001	0.250	0.0036	9.3×10^{-3}	10	$\mathbf{4.9 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{1.9 \times 10^{-8}}$	$\mathbf{6.9 \times 10^{-8}}$
6–<12 mo	0.112	0.001	0.500	0.0071	9.3×10^{-3}	10	$\mathbf{7.4 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{2.9 \times 10^{-8}}$	$\mathbf{1.0 \times 10^{-7}}$
1–<2 yrs	0.056	0.001	1.000	0.0143	9.3×10^{-3}	10	$\mathbf{7.4 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{2.9 \times 10^{-8}}$	$\mathbf{1.0 \times 10^{-7}}$
2–<3 yrs	0.052	0.001	1.000	0.0143	9.3×10^{-3}	3	$\mathbf{2.1 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{2.7 \times 10^{-8}}$	$\mathbf{4.8 \times 10^{-8}}$
3–<6 yrs	0.049	0.001	3.000	0.0429	9.3×10^{-3}	3	$\mathbf{5.9 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{7.7 \times 10^{-8}}$	$\mathbf{1.4 \times 10^{-7}}$
6–<11 yrs	0.035	0.001	5.000	0.0714	9.3×10^{-3}	3	$\mathbf{7.0 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{9.2 \times 10^{-8}}$	$\mathbf{1.6 \times 10^{-7}}$
11–<16 yrs	0.026	0.001	5.000	0.0714	9.3×10^{-3}	3	$\mathbf{5.2 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{6.8 \times 10^{-8}}$	$\mathbf{1.2 \times 10^{-7}}$
16–<18 yrs	0.024	0.001	2.000	0.0286	9.3×10^{-3}	1	$\mathbf{6.4 \times 10^{-9}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{2.8 \times 10^{-8}}$	$\mathbf{3.2 \times 10^{-8}}$
18–<21 yrs	0.029	0.001	3.000	0.0429	9.3×10^{-3}	1	$\mathbf{1.2 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{4.6 \times 10^{-8}}$	$\mathbf{5.7 \times 10^{-8}}$
21–<30 yrs	0.032	0.001	9.000	0.1286	9.3×10^{-3}	1	$\mathbf{3.8 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{1.5 \times 10^{-7}}$	$\mathbf{1.9 \times 10^{-7}}$
30–70 yrs	0.032	0.001	40.000	0.5714	9.3×10^{-3}	1	$\mathbf{1.7 \times 10^{-7}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{6.7 \times 10^{-7}}$	$\mathbf{8.4 \times 10^{-7}}$
										Total unit risk:	$\mathbf{2.0 \times 10^{-6}}$

Because the TCE intake is not constant across age groups, one does not calculate a lifetime unit risk estimate in terms of risk per mg/kg/day adjusted for potential increased early-life susceptibility. One could calculate a unit risk estimate for TCE in drinking water in terms of μg/L from the result in Table 5-49, but this is dependent on the water ingestion rates used. Based on the example calculation assuming continuous exposure to 1 μg/L of TCE in drinking water from birth to age 70 years and using the drinking water intake rates shown, estimated total lifetime risk is 2.0×10^{-6}, which corresponds to a lifetime drinking water unit risk estimate of 2.0×10^{-6} per μg/L. The corresponding risk-specific drinking water concentrations at risk levels of 10^{-6}, 10^{-5}, and 10^{-4} are 0.51, 5.1, and 51 μg/L, respectively. For different exposure and intake parameters, the risk-specific drinking water concentrations would need to be recalculated.

As with the adjusted inhalation risk estimate in Section 5.2.3.3.1, the lifetime total cancer risk estimate of 2.0×10^{-6} calculated for lifetime exposure to 1 μg/L of TCE in drinking water adjusted for potential increased early-life susceptibility is only minimally (25%) increased over the unadjusted total cancer unit risk estimate. (This calculation is not shown, but if one omits the ADAFs for each of the age groups in Table 5-49, the resulting total lifetime risk estimate is 1.6×10^{-6}.) Unlike with inhalation exposure under the assumption of ppm equivalence, which is generally assumed to extend across age groups as well as species, the oral intake rates are higher in the potentially more susceptible younger age groups. This would tend to yield a larger relative impact of adjusting for potential increased early-life susceptibility for oral risk estimates compared to inhalation risk estimates. In the case of TCE, however, this impact is partially offset by the lesser proportion of the total oral cancer risk that is accounted for by the kidney cancer risk, which is the component of total risk that is being adjusted for potential increased early-life susceptibility, based on the primary dose-metrics (1/5 vs. 1/4 for inhalation). Thus, as with lifetime inhalation risk, foregoing the ADAF adjustment in the case of full lifetime calculations will not seriously impact the resulting risk estimate. For less-than-lifetime exposure calculations, the impact of applying the ADAFs will increase as the proportion of time at older ages decreases. The maximum impact will be when exposure is for only the first 2 years of life, in which case the partial lifetime total cancer risk estimate for exposure to 1 μg/L adjusted for potential increased early-life susceptibility is 3.8×10^{-7} (adding partial risks from Table 5-49 for the appropriate ages groups), which is almost 3 times greater than the unadjusted partial lifetime total cancer risk estimate for exposure to 1 μg/L of $5 \times (0.001$ mg/L) $\times (0.103$ L/kg/day) $\times (9.33 \times 10^{-3}$ per mg/kg/day) $\times (2/70)$, or 1.4×10^{-7}, where 5 is the factor for the multiple cancer types for oral exposure, 0.103 L/kg/day is the time-weighted ingestion rate for the 1st two years of life using the rates in Table 5-49, 9.33×10^{-3} per mg/kg/day is the unadjusted oral slope factor estimate for kidney cancer, and 2/70 is the duration adjustment.

5.3. KEY RESEARCH NEEDS FOR TCE DOSE-RESPONSE ANALYSES

For noncancer dose-response assessment, key research that would substantially improve the accuracy or utility of TCE noncancer risk estimates includes:

- Research to obtain toxicokinetic data to better quantify the amount of bioactivation of DCVC to toxic moiety(ies) in rats and humans, including data on human variability in DCVC bioactivation.

- Research to obtain mechanistic data that would identify the active moiety(ies) for TCE-induced immunological effects and developmental cardiac defects. As a corollary, data on human variability pharmacokinetics of the active moiety after TCE exposure would also be informative.

- Research to obtain mechanistic data that would quantitatively inform the pharmacodynamic factors that would make individuals more or less susceptible to kidney, immunological, and developmental cardiac defects induced by TCE.

- Research to obtain TCE dose-response data on kidney effects, immunological effects, and developmental cardiac defects at a larger number of doses at and below the current LOAELs, so as to better describe the dose-response shape at low effect levels. Ideally, studies would be based on human epidemiologic data with good quantitative exposure assessment. Studies in laboratory animals would need to address the limitations in the currently available studies. For example, studies of cardiac defects would need to address limitations of the Johnson et al. (2003) study described in Section 4.8.3.3.2.

- Development of a probabilistic approach to noncancer dose-response analysis that would enable calculation of a risk-specific dose for noncancer effects, while capturing uncertainty and variability quantitatively.

For cancer dose-response assessment, key research that would substantially improve the accuracy or utility of TCE cancer risk estimates includes:

- Research to obtain toxicokinetic data to better quantify the amount of bioactivation of DCVC to toxic moiety(ies) in humans, including data on human variability in DCVC bioactivation.

- Research to obtain mechanistic data that would identify the active moiety(ies) for TCE-induced liver tumors and NHL. As a corollary, data on human variability pharmacokinetics of the active moiety after TCE exposure would also be informative.

- Research to obtain mechanistic data that would quantitatively inform the pharmacodynamic factors that would make individuals more or less susceptible to kidney tumors, liver tumors, and NHL induced by TCE. This includes data on life-stage-specific susceptibility that would replace the default ADAFs for kidney tumors and the assumption of no life-stage-specific susceptibility for liver tumors and NHL.

- Research to obtain human epidemiologic dose-response data on TCE-induced kidney tumors, liver tumors, and NHL with good quantitative exposure assessment.

- Research to obtain additional human epidemiologic data on TCE exposure and other tumors, so as to better estimate the total risk of cancer from TCE exposure.

- Development of a probabilistic approach to cancer dose-response analysis that would enable calculation of a differential susceptibility to carcinogenic effects, while capturing uncertainty and variability quantitatively.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

This section summarizes the human hazard potential for TCE. For extensive discussions and references, see Chapter 2 for exposure information, Chapter 3 for toxicokinetics and PBPK modeling, and Sections 4.1–4.9 for the epidemiologic and experimental studies of TCE noncancer and cancer toxicity. Section 4.10 summarizes information on susceptibility, and Section 4.11 provides a more detailed summary and references for noncancer toxicity and carcinogenicity.

6.1.1. Exposure (see Chapter 2)

TCE is a volatile compound with moderate water solubility. Most TCE produced today is used for metal degreasing. The highest environmental releases are to the air. Ambient air monitoring data suggest that mean levels have remained fairly constant since 1999 at about 0.3 μg/m^3 (0.06 ppb). As discussed in Chapter 2, in 2006, ambient air monitors (n = 258) had annual means ranging from 0.03 to 7.73 μg/m^3 with a median of 0.13 μg/m^3 and an overall average of 0.23 μg/m^3. Indoor levels are commonly ≥3 times higher than outdoor levels due to releases from building materials and consumer products. Vapor intrusion is a likely significant source in situations where residences are located near soils or groundwater with high contamination levels and sparse indoor air sampling had detected TCE levels ranging from 1 to 140 μg/m^3. TCE is among the most common groundwater contaminants and the one present in the highest concentration in a summary of groundwater analyses reported in 1982. The median level of TCE in groundwater, based on a large survey by the USGS for 1985–2001, is 0.15 μg/L. It has also been detected in a wide variety of foods in the 1–100 μg/kg range. None of the environmental sampling has been done using statistically based national surveys. However, a substantial amount of air and groundwater data have been collected allowing reasonably well-supported estimates of typical daily intakes by the general population: inhalation—13 μg/day and water ingestion—0.2 μg/day. The limited food data suggest an intake of about 5 μg/day, but this must be considered preliminary. Higher exposures have occurred to various occupational groups, particularly with vapor degreasing that has the highest potential for exposure because vapors can escape into the work place. For example, past studies of aircraft workers have shown short-term peak exposures in the hundreds of ppm (>500,000 μg/m^3) and long-term exposures in the low tens of ppm (>50,000 μg/m^3). Occupational exposures have likely decreased in recent

years due to better release controls, improvements in worker protection, and substituting other solvents for TCE.

Exposure to a variety of TCE-related compounds, which include metabolites of TCE and other parent compounds that produce similar metabolites, can alter or enhance TCE metabolism and toxicity by generating higher internal metabolite concentrations than would result from TCE exposure by itself. Available estimates suggest that exposures to most of these TCE-related compounds are comparable to or greater than TCE itself.

6.1.2. Toxicokinetics and PBPK Modeling (see Chapter 3 and Appendix A)

TCE is a lipophilic compound that readily crosses biological membranes. Exposures may occur via the oral, dermal, and inhalation routes, with evidence for systemic availability from each route. TCE can also be transferred transplacentally and through breast milk ingestion. TCE is rapidly and nearly completely absorbed from the gut following oral administration, and animal studies indicate that exposure vehicle may impact the time course of absorption: oily vehicles may delay absorption, whereas aqueous vehicles result in a more rapid increase in blood concentrations. See Section 3.1 for additional discussion of TCE absorption.

Following absorption to the systemic circulation, TCE distributes from blood to solid tissues by each organ‘s solubility. This process is mainly determined by the blood:tissue partition coefficients, which are largely determined by tissue lipid content. Adipose partitioning is high, so adipose tissue may serve as a reservoir for TCE, and accumulation into adipose tissue may prolong internal exposures. TCE attains high concentrations relative to blood in the brain, kidney, and liver—all of which are important target organs of toxicity. TCE is cleared via metabolism mainly in three organs: the kidney, liver, and lungs. See Section 3.2 for additional discussion of TCE distribution.

The metabolism of TCE is an important determinant of its toxicity. Metabolites are generally thought to be responsible for toxicity-especially for the liver and kidney. Initially, TCE may be oxidized via CYP isoforms or conjugated with GSH by GST enzymes. While CYP2E1 is generally accepted to be the CYP isoform most responsible for TCE oxidation, others forms may also contribute. There are conflicting data as to which GST isoforms are responsible for TCE conjugation, with one rat study indicating alpha-class GSTs and another rat study indicating mu and pi-class GST. The balance between oxidative and conjugative metabolites generally favors the oxidative pathway, especially at lower concentrations, and inhibition of CYP-dependent oxidation in vitro increases GSH conjugation in renal preparations. However, different investigators have reported considerably different rates for TCE conjugation in human liver and kidney cell fractions, perhaps due to different analytical methods. The inferred flux through the GSH pathway differs by >4 orders of magnitude across data sets. While the

available data are consistent with the higher values being overestimates, the degree of overestimation is unclear, and differing results may be attributable to true interindividual variation. Overall, there remains significant uncertainty in the quantitative estimation of TCE GSH conjugation. See Section 3.3 for additional discussion of TCE metabolism.

Once absorbed, TCE is excreted primarily either in breath as unchanged TCE or carbon dioxide [CO_2], or in urine as metabolites. Minor pathways of elimination include excretion of metabolites in saliva, sweat, and feces. Following oral administration or upon cessation of inhalation exposure, exhalation of unmetabolized TCE is a major elimination pathway. Initially, elimination of TCE upon cessation of inhalation exposure demonstrates a steep concentration-time profile: TCE is rapidly eliminated in the minutes and hours postexposure, and then the rate of elimination via exhalation decreases. Following oral or inhalation exposure, urinary elimination of parent TCE is minimal, with urinary elimination of the metabolites, TCA and TCOH, accounting for the bulk of the absorbed dose of TCE. See Section 3.4 for additional discussion of TCE excretion.

As part of this assessment, a comprehensive Bayesian PBPK model-based analysis of the population toxicokinetics of TCE and its metabolites was developed in mice, rats, and humans (also reported in Chiu et al., 2009). This analysis considered a wider range of physiological, chemical, in vitro, and in vivo data than any previously published analysis of TCE. The toxicokinetics of the "population average," its population variability, and their uncertainties are characterized and estimates of experimental variability and uncertainty are included in this analysis. The experimental database included separate sets for model calibration and evaluation for rats and humans; fewer data were available in mice, and were all used for model calibration. Local sensitivity analyses confirm that the calibration data inform the value of most model parameters, with the remaining parameters either informed by substantial prior information or having little sensitivity with respect to dose metric predictions. The total combination of these approaches and PBPK analysis substantially supports the model predictions. In addition, the approach employed yields an accurate characterization of the uncertainty in metabolic pathways for which available data were sparse or relatively indirect, such as GSH conjugation and respiratory tract metabolism. Key conclusions from the model predictions include: (1) as expected, TCE is substantially metabolized, primarily by oxidation at doses below saturation; (2) GSH conjugation and subsequent bioactivation in humans appear to be 10–100-fold greater than previously estimated; and (3) mice had the greatest rate of respiratory tract oxidative metabolism compared to rats and humans. However, there are uncertainties as to the accuracy of the analytical method used for some of the available in vivo data on GSH conjugation. Because these data are highly influential, the PBPK modeling results for the flux of GSH conjugation should be interpreted with caution. Thus, there is lower confidence in the accuracy of GSH

conjugation predictions as compared to other dose-metrics, such as those related to the parent compound, total metabolism, or oxidative metabolites. The predictions of the PBPK model are subsequently used in noncancer and cancer dose-response analyses for inter- and intraspecies extrapolation of toxicokinetics (see Section 6.2, below). See Section 3.5 and Appendix A for additional discussion of and details about PBPK modeling of TCE and metabolites.

6.1.3. Noncancer Toxicity

This section summarizes the weight of evidence for TCE noncancer toxicity. Based on the available human epidemiologic data and experimental and mechanistic studies, it is concluded that TCE poses a potential human health hazard for noncancer toxicity to the CNS, kidney, liver, immune system, male reproductive system, and developing fetus. The evidence is more limited for TCE toxicity to the respiratory tract and female reproductive system. The conclusions pertaining to specific endpoints within these tissues and systems are summarized below.

6.1.3.1. Neurological Effects (see Sections 4.3 and 4.11.1.1 and Appendix D)

Both human and animal studies have associated TCE exposure with effects on several neurological domains. Multiple epidemiologic studies in different populations have reported abnormalities in trigeminal nerve function in association with TCE exposure. Two small studies did not report an association between TCE exposure and trigeminal nerve function. However, statistical power was limited, exposure misclassification was possible, and, in one case, methods for assessing trigeminal nerve function were not available. As a result, these studies do not provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment. Laboratory animal studies have also demonstrated TCE-induced changes in the morphology of the trigeminal nerve following short-term exposures in rats. However, one study reported no significant changes in TSEP in rats exposed to TCE for 13 weeks. See Section 4.3.1 for additional discussion of studies of alterations in nerve conduction and trigeminal nerve effects. Human chamber, occupational, and geographic-based/drinking water studies have consistently reported subjective symptoms such as headaches, dizziness, and nausea, which are suggestive of vestibular system impairments. One study reported changes in nystagmus threshold (a measure of vestibular system function) following an acute TCE exposure. There are only a few laboratory animal studies relevant to this neurological domain, with reports of changes in nystagmus, balance, and handling reactivity. See Section 4.3.3 for additional discussion of TCE effects on vestibular function. Fewer and more limited epidemiologic studies are suggestive of TCE exposure being associated with delayed motor function, and changes in auditory, visual, and cognitive function or performance (see

Sections 4.3.2, 4.3.4, 4.3.5, and 4.3.6). Acute and subchronic animal studies show disruption of the auditory system, changes in visual evoked responses to patterns or flash stimulus, and neurochemical and molecular changes. Animal studies suggest that while the effects on the auditory system lead to permanent function impairments and histopathology, effects on the visual system may be reversible with termination of exposure. Additional acute studies reported structural or functional changes in hippocampus, such as decreased myelination or decreased excitability of hippocampal CA1 neurons, although the relationship of these effects to overall cognitive function is not established (see Section 4.3.9). An association between TCE exposure and sleep changes has also been demonstrated in rats (see Section 4.3.7). Some evidence exists for motor-related changes in rats/mice exposed acutely/subchronically to TCE, but these effects have not been reported consistently across all studies (see Section 4.3.6). Gestational exposure to TCE in humans has been reported to be associated with neurodevelopmental abnormalities including neural tube defects, encephalopathy, impaired cognition, aggressive behavior, and speech and hearing impairment. Developmental neurotoxicological changes have also been observed in animals including aggressive behaviors following an in utero exposure to TCE and a suggestion of impaired cognition as noted by decreased myelination in the CA1 hippocampal region of the brain. See Section 4.3.8 for additional discussion of developmental neurological effects of TCE. Therefore, overall, the strongest neurological evidence of human toxicological hazard is for changes in trigeminal nerve function or morphology and impairment of vestibular function, based on both human and experimental studies, while fewer and more limited evidence exists for delayed motor function, changes in auditory, visual, and cognitive function or performance, and neurodevelopmental outcomes.

6.1.3.2. Kidney Effects (see Sections 4.4.1, 4.4.4, 4.4.6, and 4.11.1.2)

Kidney toxicity has also been associated with TCE exposure in both human and animal studies. There are few human data pertaining to TCE-related noncancer kidney toxicity; however, several available studies reported elevated excretion of urinary proteins, considered nonspecific markers of nephrotoxicity, among TCE-exposed subjects compared to unexposed controls. While some of these studies include subjects previously diagnosed with kidney cancer, other studies report similar results in subjects who are disease free. Some additional support for TCE nephrotoxicity in humans is provided by two studies of ESRD; a study reporting a greater incidence of ESRD in TCE-exposed workers as compared to unexposed controls and a second study reporting a greater risk for progression from IgA or membranous nephropathy glomerulonephritis to ESRD and TCE-exposure. See Section 4.4.1 for additional discussion of human data on the noncancer kidney effects of TCE. Laboratory animal and in vitro data provide additional support for TCE nephrotoxicity. TCE causes renal toxicity in the form of

cytomegaly and karyomegaly of the renal tubules in male and female rats and mice following either oral or inhalation exposure. In rats, the pathology of TCE-induced nephrotoxicity appears distinct from age-related nephropathy. Increased kidney weights have also been reported in some rodent studies. See Section 4.4.4 for additional discussion of laboratory animal data on the noncancer kidney effects of TCE. Further studies with TCE metabolites have demonstrated a potential role for DCVC, TCOH, and TCA in TCE-induced nephrotoxicity. Of these, available data suggest that DCVC-induced renal effects are most similar to those of TCE and that DCVC is formed in sufficient amounts following TCE exposure to account for these effects. TCE or DCVC have also been shown to be cytotoxic to primary cultures of rat and human renal tubular cells. See Section 4.4.6 for additional discussion on the role of metabolism in the noncancer kidney effects of TCE. Overall, multiple lines of evidence support the conclusion that TCE causes nephrotoxicity in the form of tubular toxicity, mediated predominantly through the TCE GSH conjugation product DCVC.

6.1.3.3. Liver Effects (see Sections 4.5.1, 4.5.3, 4.5.4, 4.5.6, and 4.11.1.3, and Appendix E)

Liver toxicity has also been associated with TCE exposure in both human and animal studies. Although there are few human studies on liver toxicity and TCE exposure, several available studies have reported TCE exposure to be associated with significant changes in serum liver function tests, widely used in clinical settings in part to identify patients with liver disease, or changes in plasma or serum bile acids. Additional, more limited human evidence for TCE induced liver toxicity includes reports suggesting an association between TCE exposure and liver disorders, and case reports of liver toxicity including hepatitis accompanying immune-related generalized skin diseases, jaundice, hepatomegaly, hepatosplenomegaly, and liver failure in TCE-exposed workers. Cohort studies examining cirrhosis mortality and either TCE exposure or solvent exposure are generally null, but these studies cannot rule out an association with TCE because of their use of death certificates where there is a high degree (up to 50%) of underreporting. Overall, while some evidence exists of liver toxicity as assessed from liver function tests, the data are inadequate for making conclusions regarding causality. See Section 4.5.1 for additional discussion of human data on the noncancer liver effects of TCE. In rats and mice, TCE exposure causes hepatomegaly without concurrent cytotoxicity. Like humans, laboratory animals exposed to TCE have been observed to have increased serum bile acids, although the toxicological importance of this effect is unclear. Other effects in the rodent liver include small transient increases in DNA synthesis, cytomegaly in the form of "swollen" or enlarged hepatocytes, increased nuclear size probably reflecting polyploidization, and proliferation of peroxisomes. Available data also suggest that TCE does not induce substantial

cytotoxicity, necrosis, or regenerative hyperplasia, since only isolated, focal necroses and mild to moderate changes in serum and liver enzyme toxicity markers have been reported. These effects are consistently observed across rodent species and strains, although the degree of response at a given mg/kg/day dose appears to be highly variable across strains, with mice on average appearing to be more sensitive. See Sections 4.5.3 and 4.5.4 for additional discussion of laboratory animal data on the noncancer liver effects of TCE. While it is likely that oxidative metabolism is necessary for TCE-induced effects in the liver, the specific metabolite or metabolites responsible is less clear. However, the available data are strongly inconsistent with TCA being the sole or predominant active moiety for TCE-induced liver effects, particularly with respect to hepatomegaly. See Section 4.5.6 for additional discussion on the role of metabolism in the noncancer liver effects of TCE. Overall, TCE, likely through its oxidative metabolites, clearly leads to liver toxicity in laboratory animals, with mice appearing to be more sensitive than other laboratory animal species, but there is only limited epidemiologic evidence of hepatotoxicity being associated with TCE exposure.

6.1.3.4. Immunological Effects (see Sections 4.6.1.1, 4.6.2, and 4.11.1.4)

Effects related the immune system have also been associated with TCE exposure in both human and animal studies. A relationship between systemic autoimmune diseases, such as scleroderma, and occupational exposure to TCE has been reported in several recent studies, and a meta-analysis of scleroderma studies resulted in a statistically significant combined OR for any exposure in men (OR [OR]: 2.5, 95% CI: 1.1, 5.4), with a lower RR seen in women (OR: 1.2, 95% CI: 0.58, 2.6). The human data at this time do not allow a determination of whether the difference in effect estimates between men and women reflects the relatively low background risk of scleroderma in men, gender-related differences in exposure prevalence or in the reliability of exposure assessment, a gender-related difference in susceptibility to the effects of TCE, or chance. Additional human evidence for the immunological effects of TCE includes studies reporting TCE-associated changes in levels of inflammatory cytokines in occupationally-exposed workers and infants exposed via indoor air at air concentrations typical of such exposure scenarios (see Section 6.1.1); a large number of case reports (mentioned above) of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis; and a reported association between increased history of infections and exposure to TCE contaminated drinking water. See Section 4.6.1.1 for additional discussion of human data on the immunological effects of TCE. Immunotoxicity has also been reported in experimental rodent studies of TCE. Numerous studies have demonstrated accelerated autoimmune responses in autoimmune-prone mice, including changes in cytokine levels similar to those reported in human studies, with more severe effects, including autoimmune hepatitis, inflammatory skin lesions,

and alopecia, manifesting at longer exposure periods. Immunotoxic effects have been also reported in B6C3F$_1$ mice, which do not have a known particular susceptibility to autoimmune disease. Developmental immunotoxicity in the form of hypersensitivity responses have been reported in TCE-treated guinea pigs and mice via drinking water pre- and postnatally. Evidence of localized immunosuppression has also been reported in mice and rats. See Section 4.6.2 for additional discussion of laboratory animal data on the immunological effects of TCE. Overall, the human and animal studies of TCE and immune-related effects provide strong evidence for a role of TCE in autoimmune disease and in a specific type of generalized hypersensitivity syndrome, while there are less data pertaining to immunosuppressive effects.

6.1.3.5. Respiratory Tract Effects (see Sections 4.7.1.1, 4.7.2.1, 4.7.3, and 4.11.1.5)

The very few human data on TCE and pulmonary toxicity are too limited for drawing conclusions (see Section 4.7.1.1), but laboratory studies in mice and rats have shown toxicity in the bronchial epithelium, primarily in Clara cells, following acute exposures to TCE (see Section 4.7.2.1). A few studies of longer duration have reported more generalized toxicity, such as pulmonary fibrosis in mice and pulmonary vasculitis in rats. However, respiratory tract effects were not reported in other longer-term studies. Acute pulmonary toxicity appears to be dependent on oxidative metabolism, although the particular active moiety is not known. While earlier studies implicated chloral produced in situ by CYP enzymes in respiratory tract tissue in toxicity, the evidence is inconsistent and several other possibilities are viable. Although humans appear to have lower overall capacity for enzymatic oxidation in the lung relative to mice, CYP enzymes do reside in human respiratory tract tissue, suggesting that, qualitatively, the respiratory tract toxicity observed in rodents is biologically plausible in humans. See Section 4.7.3 for additional discussion of the role of metabolism in the noncancer respiratory tract toxicity of TCE. Therefore, overall, data are suggestive of TCE causing respiratory tract toxicity, based primarily on short-term studies in mice and rats, with available human data too few and limited to add to the weight of evidence for pulmonary toxicity.

6.1.3.6. Reproductive Effects (see Sections 4.8.1 and 4.11.1.6)

A number of human and laboratory animal studies suggest that TCE exposure has the potential for male reproductive toxicity, with a more limited number of studies examining female reproductive toxicity. Human studies have reported TCE exposure to be associated (in all but one case statistically-significantly) with increased sperm density and decreased sperm quality, altered sexual drive or function, or altered serum endocrine levels. Measures of male fertility, however, were either not reported or were reported to be unchanged with TCE exposure, though the statistical power of the available studies is quite limited. Epidemiologic studies have

identified possible associations of TCE exposure with effects on female fertility and with menstrual cycle disturbances, but these data are fewer than those available for male reproductive toxicity. See Section 4.8.1.1 for additional discussion of human data on the reproductive effects of TCE. Evidence of similar effects, particularly for male reproductive toxicity, is provided by several laboratory animal studies that reported effects on sperm, libido/copulatory behavior, and serum hormone levels, although some studies that assessed sperm measures did not report treatment-related alterations. Additional adverse effects on male reproduction have also been reported, including histopathological lesions in the testes or epididymides and altered in vitro sperm-oocyte binding or in vivo fertilization due to TCE or metabolites. While reduced fertility in rodents was only observed in one study, this is not surprising given the redundancy and efficiency of rodent reproductive capabilities. In addition, although the reduced fertility observed in the rodent study was originally attributed to systemic toxicity, the database as a whole suggests that TCE does induce reproductive toxicity independent of systemic effects. Fewer data are available in rodents on female reproductive toxicity. While in vitro oocyte fertilizability has been reported to be reduced as a result of TCE exposure in rats, a number of other laboratory animal studies did not report adverse effects on female reproductive function. See Section 4.8.1.2 for additional discussion of laboratory animal data on the reproductive effects of TCE. Very limited data are available to elucidate the mode of action for these effects, though some aspects of a putative mode of action (e.g., perturbations in testosterone biosynthesis) appear to have some commonalities between humans and animals (see Section 4.8.1.3.2). Together, the human and laboratory animal data support the conclusion that TCE exposure poses a potential hazard to the male reproductive system, but are more limited with regard to the potential hazard to the female reproductive system.

6.1.3.7. Developmental Effects (see Sections 4.8.3 and 4.11.1.7)

The relationship between TCE exposure (direct or parental) and developmental toxicity has been investigated in a number of epidemiologic and laboratory animal studies. Postnatal developmental outcomes examined include developmental neurotoxicity (addressed above with neurotoxicity), developmental immunotoxicity (addressed above with immunotoxicity), and childhood cancers. Prenatal effects examined include death (spontaneous abortion, perinatal death, pre- or postimplantation loss, resorptions), decreased growth (low birth weight, SGA, IUGR, decreased postnatal growth), and congenital malformations, in particular cardiac defects. Some epidemiological studies have reported associations between parental exposure to TCE and spontaneous abortion or perinatal death, and decreased birth weight or SGA, although other studies reported mixed or null findings. While comprising both occupational and environmental exposures, these studies are overall not highly informative due to the small numbers of cases and

limited exposure characterization or to the fact that exposures were to a mixture of solvents. See Section 4.8.3.1 for additional discussion of human data on the developmental effects of TCE. However, multiple well-conducted studies in rats and mice show analogous effects of TCE exposure: pre- or postimplantation losses, increased resorptions, perinatal death, and decreased birth weight. Interestingly, the rat studies reporting these effects used F344 or Wistar rats, while several other studies, all of which used Sprague-Dawley rats, reported no increased risk in these developmental measures, suggesting a strain difference in susceptibility. See Section 4.8.3.2 for additional discussion of laboratory animal data on the developmental effects of TCE. Therefore, overall, based on weakly suggestive epidemiologic data and fairly consistent laboratory animal data, it can be concluded that TCE exposure poses a potential hazard for prenatal losses and decreased growth or birth weight of offspring.

With respect to congenital malformations, epidemiology and experimental animal studies of TCE have reported increases in total birth defects, CNS defects, oral cleft defects, eye/ear defects, kidney/urinary tract disorders, musculoskeletal birth anomalies, lung/respiratory tract disorders, skeletal defects, and cardiac defects. Human occupational cohort studies, while not consistently reporting positive results, are generally limited by the small number of observed or expected cases of birth defects. While only one of the epidemiological studies specifically reported observations of eye anomalies, studies in rats have identified increases in the incidence of fetal eye defects following oral exposures during the period of organogenesis with TCE or its oxidative metabolites, DCA and TCA. The epidemiological studies, while individually limited, as a whole show relatively consistent elevations, some of which were statistically significant, in the incidence of cardiac defects in TCE-exposed populations compared to reference groups. In laboratory animal models, avian studies were the first to identify adverse effects of TCE exposure on cardiac development, and the initial findings have been confirmed multiple times. Additionally, administration of TCE and its metabolites, TCA and DCA, in maternal drinking water during gestation has been reported to induce cardiac malformations in rat fetuses. It is notable that a number of other studies, several of which were well-conducted, did not report induction of cardiac defects in rats, mice, or rabbits in which TCE was administered by inhalation or gavage. However, many of these studies used a traditional free-hand section technique on fixed fetal specimens, and a fresh dissection technique that can enhance detection of anomalies was used in the positive studies by Dawson et al. (1993) and Johnson et al. (2005, 2003). Nonetheless, two studies that used the same or similar fresh dissection technique did not report cardiac anomalies. Differences in other aspects of experimental design may have been contributing factors to the differences in observed response. In addition, mechanistic studies, such as the treatment-related alterations in endothelial cushion development observed in avian in ovo and in vitro studies, provide a plausible mechanistic basis for defects in septal and valvular

morphogenesis observed in rodents, and consequently support the plausibility of cardiac defects induced by TCE in humans. Therefore, while the studies by Dawson et al. (1993) and Johnson et al. (2003) 2005) have significant limitations, including the lack of clear dose-response relationship for the incidence of any specific cardiac anomaly and the pooling of data collected over an extended period, there is insufficient reason to dismiss their findings. See Section 4.8.3.3.2 for additional discussion of the conclusions with respect to TCE-induced cardiac malformations. Therefore, overall, based on weakly suggestive, but overall consistent, epidemiologic data, in combination with evidence from experimental animal and mechanistic studies, it can be concluded that TCE exposure poses a potential hazard for congenital malformations, including cardiac defects, in offspring.

6.1.4. Carcinogenicity (see Sections 4.1, 4.2, 4.4.2, 4.4.5, 4.4.7, 4.5.2, 4.5.5, 4.5.6, 4.5.7, 4.6.1.2, 4.6.2.4, 4.7.1.2, 4.7.2.2, 4.7.4, 4.8.2, 4.9, and 4.11.2, and Appendices B and C)

Following EPA (2005b) *Guidelines for Carcinogen Risk Assessment*, based on the available data as of 2010, TCE is characterized as ―carcinogenic to humans" by all routes of exposure. This conclusion is based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer. The consistency of increased kidney cancer RR estimates across a large number of independent studies of different designs and populations from different countries and industries provides compelling evidence given the difficulty, a priori, in detecting effects in epidemiologic studies when the RRs are modest and the cancers are relatively rare, and therefore, individual studies have limited statistical power. This strong consistency of the epidemiologic data on TCE and kidney cancer argues against chance, bias, and confounding as explanations for the elevated kidney cancer risks. In addition, statistically significant exposure-response trends were observed in high-quality studies. These studies were conducted in populations with high TCE exposure intensity or had the ability to identify TCE-exposed subjects with high confidence. These studies addressed important potential confounders and biases, further supporting the observed associations with kidney cancer as causal. See Section 4.4.2 for additional discussion of the human epidemiologic data on TCE exposure and kidney cancer. In a meta-analysis of 15 studies with high exposure potential, a statistically significant RRm estimate was observed for overall TCE exposure (RRm: 1.27 [95% CI: 1.13, 1.43]). The RRm estimate was greater for the highest TCE exposure groups (RRm: 1.58 [95% CI: 1.28, 1.96]; n = 13 studies). Meta-analyses investigating the influence of individual studies and the sensitivity of the results to alternate RR estimate selections found the RRm estimates to be highly robust. Furthermore, there was no indication of publication bias or significant heterogeneity across the 15 studies. It would require a substantial amount of negative data from informative studies (i.e., studies having a high likelihood of TCE exposure in individual study

subjects and which meet, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review) to contradict this observed association. See Section 4.4.2.5 and Appendix C for additional discussion of the kidney cancer meta-analysis.

The human evidence of carcinogenicity from epidemiologic studies of TCE exposure is strong for NHL but less convincing than for kidney cancer. Studies with high exposure potential generally reported excess RR estimates, with statistically significant increases in three studies with overall TCE exposure, and a statistically significant increase in the high TCE exposure group and statistically significant trend in a fourth study (see Section 4.6.1.2). The consistency of the association between TCE exposure and NHL is further supported by the results of meta-analyses (see Section 4.6.1.2.2 and Appendix C). A statistically significant RRm estimate was observed for overall TCE exposure (RRm: 1.23 [95% CI: 1.07, 1.42]; n = 17 studies), and, as with kidney cancer, the RRm estimate was greater for the highest TCE exposure groups (RRm: 1.43 [95% CI: 1.13, 1.82]; n = 13 studies) than for overall TCE exposure. Sensitivity analyses indicated that these results and their statistical significance were not overly influenced by any single study or choice of individual (study-specific) risk estimates, and in all of the influence and sensitivity analyses, the RRm estimate was statistically significantly increased. Some heterogeneity was observed, particularly between cohort and case-control studies, but it was not statistically significant. In addition, there was some evidence of potential publication bias. Thus, while the evidence is strong for NHL, issues of study heterogeneity, potential publication bias, and weaker exposure-response results contribute greater uncertainty.

The evidence is more limited for liver and biliary tract cancer mainly because only cohort studies are available and most of these studies have small numbers of cases due the comparative rarity of liver and biliary tract cancer. While most studies with high exposure potential reported excess RR estimates, they were generally based on small numbers of cases or deaths, with the result of wide CIs on the estimates. The low number of liver cancer cases in the available studies made assessing exposure-response relationships difficult. See Section 4.5.2 for additional discussion of the human epidemiologic data on TCE exposure and liver cancer. Consistency of the association between TCE exposure and liver cancer is supported by the results of meta-analyses (see Section 4.5.2 and Appendix C). These meta-analyses found a statistically significant increased RRm estimate for liver and biliary tract cancer of 1.29 (95% CI: 1.07, 1.56; n = 9 studies) with overall TCE exposure; but the meta-analyses using only the highest exposure groups yielded a lower, and nonstatistically significant, summary estimate for primary liver cancer (1.28 [95% CI: 0.93, 1.77], n = 8 studies). Although there was no evidence of heterogeneity or publication bias and the summary estimates were fairly insensitive to the use of alternative RR estimates, the statistical significance of the summary estimates depends heavily on the one large study by Raaschou-Nielsen et al. (2003). There were fewer adequate studies

with high exposure potential available for meta-analysis of liver cancer (9 vs. 17 for NHL and 15 for kidney), leading to lower statistical power, even with pooling. Thus, while there is epidemiologic evidence of an association between TCE exposure and liver cancer, the much more limited database, both in terms of number of available studies and number of cases within studies, contributes to greater uncertainty as compared to the evidence for kidney cancer or NHL.

In addition to the body of evidence pertaining to kidney cancer, NHL, and liver cancer, the available epidemiologic studies also provide more limited evidence of an association between TCE exposure and other types of cancer, including bladder, esophageal, prostate, cervical, breast, and childhood leukemia. Differences between these sets of data and the data for kidney cancer, NHL, and liver cancer are observations from fewer numbers of studies, a mixed pattern of observed risk estimates, and the general absence of exposure-response data from the studies using a quantitative TCE-specific exposure measure.

There are several other lines of supporting evidence for TCE carcinogenicity in humans by all routes of exposure. First, multiple chronic bioassays in rats and mice have reported increased incidences of tumors with TCE treatment via inhalation and gavage, including tumors in the kidney, liver, and lymphoid tissues − target tissues of TCE carcinogenicity also seen in epidemiological studies. Of particular note is the site-concordant finding of low, but biologically and sometimes statistically significant, increases in the incidence of kidney tumors in multiple strains of rats treated with TCE by either inhalation or corn oil gavage (see Section 4.4.5). The increased incidences were only detected at the highest tested doses, and were greater in male than female rats; although, notably, pooled incidences in females from five rat strains tested by NTP (NTP, 1990, 1988) resulted in a statistically significant trend. Although these studies have shown limited increases in kidney tumors, and several individual studies have a number of limitations, given the rarity of these tumors as assessed by historical controls and the repeatability of this result across studies and strains, these are considered biologically significant. Therefore, while individual studies provide only suggestive evidence of renal carcinogenicity, the database as a whole supports the conclusion that TCE is a kidney carcinogen in rats, with males being more sensitive than females. No other tested laboratory species (i.e., mice and hamsters) have exhibited increased kidney tumors, with no adequate explanation for these species differences (particularly with mice, which have been extensively tested). With respect to the liver, TCE and its oxidative metabolites CH, TCA, and DCA are clearly carcinogenic in mice, with strain and sex differences in potency that appear to parallel, qualitatively, differences in background tumor incidence. Data in other laboratory animal species are limited; thus, except for DCA which is carcinogenic in rats, inadequate evidence exists to evaluate the hepatocarcinogenicity of these compounds in rats or hamsters. However, to the extent that there is hepatocarcinogenic potential in rats, TCE is clearly less potent in the strains tested in this

species than in B6C3F$_1$ and Swiss mice. See Section 4.5.5 for additional discussion of laboratory animal data on TCE-induced liver tumors. Additionally, there is more limited evidence for TCE-induced lymphohematopoetic cancers in rats and mice, lung tumors in mice, and testicular tumors in rats. With respect to the lymphohematopoietic cancers, two studies in mice reported increased incidences of lymphomas in females of two different strains, and two studies in rats reported leukemias in males of one strain and females of another. However, these tumors had relatively modest increases in incidence with treatment, and were not reported to be increased in other studies. See Section 4.6.2.4 for additional discussion of laboratory animal data on TCE-induced lymphohematopoetic tumors. With respect to lung tumors, rodent bioassays have demonstrated a statistically significant increase in pulmonary tumors in mice following chronic inhalation exposure to TCE, and nonstatistically significant increases in mice exposed orally; but pulmonary tumors were not reported in other species tested (i.e., rats and hamsters) (see Section 4.7.2.2). Finally, increased testicular (interstitial or Leydig cell) tumors have been observed in multiple studies of rats exposed by inhalation and gavage, although in some cases, high (> 75%) control rates of testicular tumors in rats limited the ability to detect a treatment effect. See Section 4.8.2.2 for additional discussion of laboratory animal data on TCE-induced tumors of the reproductive system. Overall, TCE is clearly carcinogenic in rats and mice. The apparent lack of site concordance across laboratory animal studies may be due to limitations in design or conduct in a number of rat bioassays and/or genuine interspecies differences in qualitative or quantitative sensitivity (i.e., potency). Nonetheless, these studies have shown carcinogenic effects across different strains, sexes, and routes of exposure, and site-concordance is not necessarily expected for carcinogens. Of greater import is the finding that there is site-concordance between the main cancers observed in TCE-exposed humans and those observed in rodent studies—in particular, cancers of the kidney, liver, and lymphoid tissues.

A second line of supporting evidence for TCE carcinogenicity in humans consists of toxicokinetic data indicating that TCE is well absorbed by all routes of exposure, and that TCE absorption, distribution, metabolism, and excretion are qualitatively similar in humans and rodents. As summarized above, there is evidence that TCE is systemically available, distributes to organs and tissues, and undergoes systemic metabolism from all routes of exposure. Therefore, although the strongest evidence from epidemiologic studies largely involves inhalation exposures, the evidence supports TCE carcinogenicity being applicable to all routes of exposure. In addition, there is no evidence of major qualitative differences across species in TCE absorption, distribution, metabolism, and excretion. Extensive in vivo and in vitro data show that mice, rats, and humans all metabolize TCE via two primary pathways: oxidation by CYPs and conjugation with GSH via GSTs. Several metabolites and excretion products from both pathways have been detected in blood and urine from exposed humans as well as from at

least one rodent species. In addition, the subsequent distribution, metabolism, and excretion of TCE metabolites are qualitatively similar among species. Therefore, humans possess the metabolic pathways that produce the TCE metabolites thought to be involved in the induction of rat kidney and mouse liver tumors, and internal target tissues of both humans and rodents experience a similar mix of TCE and metabolites. See Sections 3.1–3.4 for additional discussion of TCE toxicokinetics. Quantitative interspecies differences in toxicokinetics do exist, and are addressed through PBPK modeling (see Section 3.5 and Appendix A). Importantly, these quantitative differences affect only interspecies extrapolations of carcinogenic potency, and do not affect inferences as to the carcinogenic hazard for TCE.

Finally, available mechanistic data do not suggest a lack of human carcinogenic hazard from TCE exposure. In particular, these data do not suggest qualitative differences between humans and test animals that would preclude any of the hypothesized key events in the carcinogenic mode of action in rodents from occurring in humans. For the kidney, the predominance of positive genotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular DCVC), together with toxicokinetic data consistent with their systemic delivery to and in situ formation in the kidney, supports the conclusion that a mutagenic mode of action is operative in TCE-induced kidney tumors. While supporting the biological plausibility of this hypothesized mode of action, available data on the *VHL* gene in humans or transgenic animals do not conclusively elucidate the role of *VHL* mutation in TCE-induced renal carcinogenesis. Cytotoxicity and compensatory cell proliferation, similarly presumed to be mediated through metabolites formed after GSH-conjugation of TCE, have also been suggested to play a role in the mode of action for renal carcinogenesis, as high incidences of nephrotoxicity have been observed in animals at doses that induce kidney tumors. Human studies have reported markers for nephrotoxicity at current occupational exposures, although data are lacking at lower exposures. Nephrotoxicity is observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but kidney tumors are only observed at low incidences in rats at the highest tested doses. Therefore, nephrotoxicity alone appears to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis, since maximal levels of toxicity are reached before the onset of tumors. In addition, nephrotoxicity has not been shown to be necessary for kidney tumor induction by TCE in rodents. In particular, there is a lack of experimental support for causal links, such as compensatory cellular proliferation or clonal expansion of initiated cells, between nephrotoxicity and kidney tumors induced by TCE. Furthermore, it is not clear if nephrotoxicity is one of several key events in a mode of action, if it is a marker for an "upstream" key event (such as oxidative stress) that may contribute independently to both nephrotoxicity and renal carcinogenesis, or if it is incidental to kidney tumor induction. Therefore, although the data are

consistent with the hypothesis that cytotoxicity and regenerative proliferation contribute to TCE-induced kidney tumors, the weight of evidence is not as strong as the support for a mutagenic mode of action. Moreover, while toxicokinetic differences in the GSH conjugation pathway along with their uncertainty are addressed through PBPK modeling, no data suggest that any of the proposed key events for TCE-induced kidney tumors in rats are precluded in humans. See Section 4.4.7 for additional discussion of the mode of action for TCE-induced kidney tumors. Therefore, TCE-induced rat kidney tumors provide additional support for the convincing human evidence of TCE-induced kidney cancer, with mechanistic data supportive of a mutagenic mode of action.

With respect to other tumor sites, data are insufficient to conclude that any of the other hypothesized modes of action are operant. In the liver, a mutagenic mode of action mediated by CH, which has evidence for genotoxic effects, or some other oxidative metabolite of TCE cannot be ruled out, but data are insufficient to conclude it is operant. A second mode-of-action hypothesis for TCE-induced liver tumors involves activation of the PPARα receptor. Clearly, in vivo administration of TCE leads to activation of PPARα in rodents and likely does so in humans as well. However, the evidence as a whole does not support the view that PPARα is the sole operant mode of action mediating TCE hepatocarcinogenesis. Rather, there is evidential support for multiple TCE metabolites and multiple toxicity pathways contributing to TCE-induced liver tumors. Furthermore, recent experiments have demonstrated that PPARα activation and the sequence of key events in the hypothesized mode of action are not sufficient to induce hepatocarcinogenesis (Yang et al., 2007). Moreover, the demonstration that the PPARα agonist di(2-ethylhexyl) phthalate induces tumors in PPARα-null mice supports the view that the events comprising the hypothesized PPARα activation mode of action are not necessary for liver tumor induction in mice by this PPARα agonist (Ito et al., 2007). See Section 4.5.7 for additional discussion of the mode of action for TCE-induced liver tumors. For mouse lung tumors, as with the liver, a mutagenic mode of action involving CH has also been hypothesized, but there are insufficient data to conclude that it is operant. A second mode-of-action hypothesis for mouse lung tumors has been posited involving other effects of oxidative metabolites including cytotoxicity and regenerative cell proliferation, but experimental support remains limited, with no data on proposed key events in experiments of duration two weeks or longer. See Section 4.7.4 for additional discussion of the mode of action for TCE-induced lung tumors. A mode of action subsequent to in situ oxidative metabolism, whether involving mutagenicity, cytotoxicity, or other key events, may also be relevant to other tissues where TCE would undergo CYP metabolism. For instance, CYP2E1, oxidative metabolites, and protein adducts have been reported in the testes of rats exposed to TCE, and, in some rat bioassays, TCE exposure increased the incidence of rat testicular tumors. However, inadequate data exist to

adequately define a mode-of-action hypothesis for this tumor site (see Section 4.8.2.3 for additional discussion of the mode of action for TCE-induced testicular tumors).

6.1.5. Susceptibility (see Sections 4.10 and 4.11.3)

There is some evidence that certain populations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status, and lifestyle factors and nutrition status. Factors that affect early lifestage susceptibility include exposures such as transplacental transfer and breast milk ingestion, early lifestage-specific toxicokinetics, and differential outcomes in early lifestages such as developmental cardiac defects (see Section 4.10.1). Because the weight of evidence supports a mutagenic mode of action being operative for TCE carcinogenicity in the kidney (see Section 4.4.7), and there is an absence of chemical-specific data to evaluate differences in carcinogenic susceptibility, early-life susceptibility should be assumed and the ADAFs should be applied, in accordance with the Supplemental Guidance (see summary below in Section 6.2.2.5). Fewer data are available on later lifestages, although there is suggestive evidence to indicate that older adults may experience increased adverse effects than younger adults due to greater tissue distribution of TCE. In general, more studies specifically designed to evaluate effects in early and later lifestages are needed in order to more fully characterize potential lifestage-related TCE toxicity. Gender-specific (see Section 4.10.2.1) differences also exist in toxicokinetics (e.g., cardiac outputs, percent body fat, expression of metabolizing enzymes) and susceptibility to toxic endpoints (e.g., gender-specific effects on the reproductive system, gender differences in baseline risks to endpoints such as scleroderma or liver cancer). Genetic variation (see Section 4.10.2.2) likely has an effect on the toxicokinetics of TCE. Increased CYP2E1 activity and GST polymorphisms may influence susceptibility of TCE due to effects on production of toxic metabolites or may play a role in variability in toxic response. Differences in genetic polymorphisms related to the metabolism of TCE have also been observed among various race/ethnic groups (see Section 4.10.2.3). Preexisting diminished health status (see Section 4.10.2.4) may alter the response to TCE exposure. Individuals with increased body mass may have an altered toxicokinetic response due to the increased uptake of TCE into fat. Other conditions that may alter the response to TCE exposure include diabetes and hypertension, and lifestyle and nutrition factors (see Section 4.10.2.5) such as alcohol consumption, tobacco smoking, nutritional status, physical activity, and SES status. Alcohol intake has been associated with inhibition of TCE metabolism in both humans and experimental animals. In addition, such conditions have been associated with increased baseline risks for health effects also associated with TCE, such as kidney cancer and liver cancer. However, the interaction between TCE and

known risk factors for human diseases is not known, and further evaluation of the effects due to these factors is needed.

In sum, there is some evidence that certain populations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status, and lifestyle factors and nutrition status. However, except in the case of toxicokinetic variability characterized using the PBPK model described in Section 3.5, there are inadequate chemical-specific data to quantify the degree of differential susceptibility due to such factors.

6.2. DOSE-RESPONSE ASSESSMENT

This section summarizes the major conclusions of the dose-response analysis for TCE noncancer effects and carcinogenicity, with more detailed discussions in Chapter 5.

6.2.1. Noncancer Effects (see Section 5.1)

6.2.1.1. Background and Methods

As summarized above, based on the available human epidemiologic data and experimental and mechanistic studies, it is concluded that TCE poses a potential human health hazard for noncancer toxicity to the CNS, kidney, liver, immune system, male reproductive system, and developing fetus. The evidence is more limited for TCE toxicity to the respiratory tract and female reproductive system.

Dose-response analysis for a noncancer endpoint generally involves two steps: (1) the determination of a POD derived from a BMD,[61] a NOAEL, or a LOAEL, and (2) adjustment of the POD by endpoint/study-specific "uncertainty factors" (UFs), accounting for adjustments and uncertainties in the extrapolation from the study conditions to conditions of human exposure.

Because of the large number of noncancer health effects associated with TCE exposure and the large number of studies reporting on these effects, in contrast to toxicological reviews for chemicals with smaller databases of studies, a formal, quantitative screening process (see Section 5.1) was used to reduce the number of endpoints and studies to those that would best inform the selection of the *critical effects* for the inhalation RfC and oral RfD.[62] As described in Section 5.1, for all studies described in Chapter 4 which reported adverse noncancer health effects and provided quantitative dose-response data, PODs on the basis of applied dose,

[61]More precisely, it is the benchmark dose lower bound (BMDL), i.e., the (one-sided) 95% lower confidence bound on the dose corresponding to the benchmark response (BMR) for the effect, that is used as the POD.

[62]In EPA noncancer health assessments, the RfC [RfD] is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation [daily oral] exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark concentration [dose], with uncertainty factors generally applied to reflect limitations of the data used.

adjusted by endpoint/study-specific UFs, were used to develop candidate RfCs (cRfCs) and candidate RfDs (cRfDs) intended to be protective for each endpoint individually. Candidate critical effects − those with the lowest cRfCs and cRfDs taking into account the confidence in each estimate − were selected within each of the following health effect domains: (1) neurological, (2) kidney; (3) liver; (4) immunological; (5) reproductive; and (6) developmental. For each of these candidate critical effects, the PBPK model developed in Section 3.5 was used for interspecies, intraspecies, and route-to-route extrapolation on the basis of internal dose to develop PBPK model-based PODs. Plausible internal dose-metrics were selected based on what is understood about the role of different TCE metabolites in toxicity and the mode of action for toxicity. These PODs were then adjusted by endpoint/study-specific UFs, taking into account the use of the PBPK model, to develop PBPK model-based candidate RfCs (p-cRfCs) and candidate RfDs (p-cRfDs). The most sensitive cRfCs, p-cRfCs, cRfDs, and p-cRfDs were then evaluated, taking into account the confidence in each estimate, to arrive at overall candidate RfCs and RfDs for each health effect type. Then, the RfC and RfD for TCE were selected so as to be protective of the most sensitive effects. In contrast to the approach used in most previous assessments, in which the RfC and RfD are each based on a single critical effect, the final RfC and RfD for TCE were based on multiple critical effects that resulted in very similar candidate RfC and RfD values at the low end of the full range of values. This approach was taken here because it provides robust estimates of the RfC and RfD and because it highlights the multiple effects that are all yielding very similar candidate values.

6.2.1.2. Uncertainties and Application of UFs (see Sections 5.1.1 and 5.1.4)

An underlying assumption in deriving a reference value for a noncancer effect is that the dose-response relationship has a threshold. Thus, a fundamental uncertainty is the validity of that assumption. For some effects, in particular effects on very sensitive processes (e.g., developmental processes) or effects for which there is a nontrivial background level and even small exposures may contribute to background disease processes in more susceptible people, a practical threshold (i.e., a threshold within the range of environmental exposure levels of regulatory concern) may not exist.

Nonetheless, under the assumption of a threshold, the desired exposure level to have as a reference value is the maximum level at which there is no appreciable risk for an adverse effect in sensitive subgroups (of humans). However, because it is not possible to know what this level is, UFs are used to attempt to address quantitatively various aspects, depending on the data set, of qualitative uncertainty.

First there is uncertainty about the POD for the application of UFs. Conceptually, the POD should represent the maximum exposure level at which there is no appreciable risk for an

adverse effect in the study population under study conditions (i.e., the threshold in the dose-response relationship). Then, the application of the relevant UFs is intended to convey that exposure level to the corresponding exposure level for sensitive human subgroups exposed continuously for a lifetime. In fact, it is again not possible to know that exposure level even for a laboratory study because of experimental limitations (e.g., the power to detect an effect, dose spacing, measurement errors, etc.), and crude approximations like the NOAEL or a BMDL are used. If a LOAEL is used as the POD, then the LOAEL-to-NOAEL UF is applied as an adjustment factor to better approximate the desired exposure level (threshold), although the necessary extent of adjustment is unknown. The standard value for the LOAEL-to-NOAEL UF is 10, although sometimes a value of 3 is used if the effect is considered minimally adverse at the response level observed at the LOAEL or is an early marker for an adverse effect. For one POD in this assessment, a value of 30 was used for the LOAEL-to-NOAEL UF because the incidence rate for the adverse effect was ≥90% at the LOAEL.

If a BMDL is used as the POD, then there are uncertainties regarding the appropriate dose-response model to apply to the data, but these should be minimal if the modeling is in the observable range of the data. There are also uncertainties about what BMR to use to best approximate the desired exposure level (threshold, see above). For continuous endpoints, in particular, it is often difficult to identify the level of change that constitutes the "cut-point" for an adverse effect. Sometimes, to better approximate the desired exposure level, a BMR somewhat below the observable range of the data is selected. In such cases, the model uncertainty is increased, but this is a trade-off to reduce the uncertainty about the POD not being a good approximation for the desired exposure level.

For each of these types of PODs, there are additional uncertainties pertaining to adjustments to the administered exposures (doses). Typically, administered exposures (doses) are converted to equivalent continuous exposures (daily doses) over the study exposure period under the assumption that the effects are related to concentration × time, independent of the daily (or weekly) exposure regimen (i.e., a daily exposure of 6 hours to 4 ppm is considered equivalent to 24 hours of exposure to 1 ppm). However, the validity of this assumption is generally unknown, and, if there are dose-rate effects, the assumption of concentration times time ($C \times t$) equivalence would tend to bias the POD downwards. Where there is evidence that administered exposure better correlates to the effect than equivalent continuous exposure averaged over the study exposure period (e.g., visual effects), administered exposure was not adjusted. For the PBPK analyses in this assessment, the actual administered exposures are taken into account in the PBPK modeling, and equivalent daily values (averaged over the study exposure period) for the dose-metrics are obtained (see above, Section 5.1.3.2). Additional uncertainties about the PBPK-based estimates include uncertainties about the appropriate dose-metric for each effect,

although, for some effects, there was better information about relevant dose-metrics than for others, and uncertainties in the PBPK model predictions for the dose-metrics in humans, particularly for GSH conjugation (see Section 5.1.3.1).

There is also uncertainty about the other UFs. The human variability UF is, to some extent, an adjustment factor because, for more sensitive people, the dose-response relationship shifts to lower exposures. But there is uncertainty about the extent of the adjustment required (i.e., about the distribution of human susceptibility). Therefore, in the absence of data on a susceptible population(s) or on the distribution of susceptibility in the general population, an UF of 10 is generally used, which breaks down (approximately) to a factor of 3 for pharmacokinetic variability and a factor of 3 for pharmacodynamic variability. This standard value was used for all of the PODs based on applied dose in this assessment with the exception of the PODs for a few immunological effects that were based on data from a sensitive (autoimmune-prone) mouse strain. For those PODs, an UF of 3 (reflecting pharmacokinetics only) was used for human variability. The PBPK analyses in this assessment attempt to account for the pharmacokinetic portion of human variability using human data on pharmacokinetic variability. For PBPK model-based candidate reference values, the pharmacokinetic component of this UF was omitted. A quantitative uncertainty analysis of the PBPK derived dose-metrics used in the assessment is presented in Section 5.1.4.2. There is still uncertainty regarding the susceptible subgroups for TCE exposure and the extent of pharmacodynamic variability.

If the data used to determine a particular POD are from laboratory animals, an interspecies extrapolation UF is used. This UF is also, to some extent, an adjustment factor for the expected scaling for toxicologically equivalent doses across species (i.e., according to body weight to the ¾ power for oral exposures). However, there is also uncertainty about the true extent of interspecies differences for specific noncancer effects from specific chemical exposures. For oral exposures, the standard value for the interspecies UF is 10, which can be viewed as breaking down (approximately) to a factor of 3 for the "adjustment" (nominally pharmacokinetics) and a factor of 3 for the "uncertainty" (nominally pharmacodynamics). For inhalation exposures for systemic toxicants, such as TCE, for which the blood:air partition coefficient in laboratory animals is greater than that in humans, no adjustment across species is generally assumed for fixed air concentrations (ppm equivalence; U.S. EPA, 1994a), and the standard value for the interspecies UF is 3, reflecting only "uncertainty" (nominally pharmacodynamics). The PBPK analyses in this assessment attempt to account for the "adjustment" portion of interspecies extrapolation using rodent pharmacokinetic data to estimate internal doses for various dose-metrics. Equal doses of these dose-metrics, appropriately scaled, are then assumed to convey equivalent risk across species. For PBPK model-based candidate reference values, the "adjustment" component of this UF was omitted. With respect to the

—uncertainty" component, quantitative uncertainty analyses of the PBPK-derived dose-metrics used in the assessment are presented in Section 5.1.4.2. However, these only address the pharmacokinetic uncertainties in a particular dose-metric, and there is still uncertainty regarding the true dose-metrics. Nor do the PBPK analyses address the uncertainty in either cross-species pharmacodynamic differences (i.e., about the assumption that equal doses of the appropriate dose-metric convey equivalent risk across species for a particular endpoint from a specific chemical exposure) or in cross-species pharmacokinetic differences not accounted for by the PBPK model dose-metrics (e.g., departures from the assumed interspecies scaling of clearance of the active moiety, in the cases where only its production is estimated). A value of 3 is typically used for the —uncertainty" about cross-species differences, and this generally represents true uncertainty because it is usually unknown, even after adjustments have been made to account for the expected interspecies differences, whether humans have more or less susceptibility, and to what degree, than the laboratory species in question.

RfCs and RfDs apply to lifetime exposure, but sometimes the best (or only) available data come from less-than-lifetime studies. Lifetime exposure can induce effects that may not be apparent or as large in magnitude in a shorter study; consequently, a dose that elicits a specific level of response from a lifetime exposure may be less than the dose eliciting the same level of response from a shorter exposure period. If the effect becomes more severe with increasing exposure, then chronic exposure would shift the dose-response relationship to lower exposures, although the true extent of the shift is unknown. PODs based on subchronic exposure data are generally divided by a subchronic-to-chronic UF, which has a standard value of 10. If there is evidence suggesting that exposure for longer time periods does not increase the magnitude of an effect, a lower value of 3 or 1 might be used. For some reproductive and developmental effects, chronic exposure is that which covers a specific window of exposure that is relevant for eliciting the effect, and subchronic exposure would correspond to an exposure that is notably less than the full window of exposure.

Sometimes a database UF is also applied to address limitations or uncertainties in the database. The overall database for TCE is quite extensive, with studies for many different types of effects, including two-generation reproductive studies, as well as neurological and immunological studies. In addition, there were sufficient data to develop a reliable PBPK model to estimate route-to-route extrapolated doses for some candidate critical effects for which data were only available for one route of exposure. Thus, there is a high degree of confidence that the TCE database was sufficient to identify sensitive endpoints, and no database UF was used in this assessment.

6.2.1.2.1. Candidate Critical Effects and Reference Values (see Sections 5.1.2 and 5.1.3)

A large number of endpoints and studies were considered within each health effect domain. Chapter 5 contains a comprehensive discussion of all endpoints/studies that were considered for developing candidate reference values (cRfCs, cRfDs, p-cRfCs, and p-cRfDs), their PODs, and the UFs applied. The summary below reviews the selection of candidate critical effects for each health effect domain, the confidence in the reference values, the selection of PBPK model-based dose-metrics, and the impact of PBPK modeling on the candidate reference values.

6.2.1.2.2. Neurological effects

Candidate reference values were developed for several neurological domains for which there was evidence of hazard (see Tables 5-2 and 5-13). There is higher confidence in the candidate reference values for trigeminal nerve, auditory, or psychomotor effects, but the available data suggest that the more sensitive indicators of TCE neurotoxicity are changes in wakefulness, regeneration of the sciatic nerve, demyelination in the hippocampus, and degeneration of dopaminergic neurons. Therefore, these more sensitive effects are considered the candidate critical effects for neurotoxicity, albeit with more uncertainty in the corresponding candidate reference values. Of these more sensitive effects, there is greater confidence in the changes in wakefulness reported by Arito et al. (1994). In addition, trigeminal nerve effects are considered a candidate critical effect because this is the only type of neurological effect for which human data are available, and the POD for this effect is similar to that from the most sensitive rodent study (Arito et al., 1994, for changes in wakefulness). Between the two human studies of trigeminal nerve effects, Ruijten et al. (1991) is preferred for deriving noncancer reference values because its exposure characterization is considered more reliable.

Because of the lack of specific data as to the metabolites involved and the mode of action for the candidate critical neurologic effects, PBPK model predictions of total metabolism (scaled by body weight to the ¾ power) were selected as the preferred dose-metric based on the general observation that TCE toxicity is associated with metabolism. The AUC of TCE in blood was used as an alternative dose-metric. With these dose-metrics, the candidate reference values derived using the PBPK model were only modestly (~threefold or less) different than those derived on the basis of applied dose.

6.2.1.2.3. Kidney effects

Candidate reference values were developed for histopathological and weight changes in the kidney (see Tables 5-4 and 5-15), and these are considered to be candidate critical effects for several reasons. First, they appear to be the most sensitive indicators of toxicity that are

available for the kidney. In addition, as discussed in Sections 3.3 and 3.5, both in vitro and in vivo pharmacokinetic data indicate substantially more production of GSH-conjugates thought to mediate TCE kidney effects in humans relative to rats and mice. Several studies are considered reliable for developing candidate reference values for these endpoints. For histopathological changes, these were the only available inhalation study (the rat study of Maltoni et al., 1986), the NTP (1988) study in rats, and the NCI (NCI, 1976) study in mice. For kidney weight changes, both available studies (Woolhiser et al., 2006; Kjellstrand et al., 1983a) were chosen as candidate critical studies.

Due to the substantial evidence supporting the role of GSH conjugation metabolites in TCE-induced nephrotoxicity, the preferred PBPK model dose-metrics for kidney effects were the amount of DCVC bioactivated in the kidney for rat studies and the amount of GSH conjugation (both scaled by body weight to the ¾ power) for mouse studies (inadequate toxicokinetic data are available in mice for predicting the amount of DCVC bioactivation). With these dose-metrics, the candidate reference values derived using the PBPK model were 300–400-fold lower than those derived on the basis of applied dose. As discussed above and in Chapter 3, this is due to the available in vivo and in vitro data supporting not only substantially more GSH conjugation in humans than in rodents, but also substantial interindividual toxicokinetic variability. Overall, there is high confidence in the nephrotoxic hazard from TCE exposure and in the appropriateness of the dose-metrics discussed above; however, there is substantial uncertainty in the extrapolation of GSH conjugation from rodents to humans due to limitations in the available data (see Section 3.3.3.2).

6.2.1.2.4. Liver effects

Hepatomegaly appears to be the most sensitive indicator of toxicity that is available for the liver and is therefore considered a candidate critical effect. Several studies are considered reliable for developing high-confidence candidate reference values for this endpoint. Since they all indicated similar sensitivity but represented different species and/or routes of exposure, they were all considered candidate critical studies (see Tables 5-4 and 5-14).

Due to the substantial evidence supporting the role of oxidative metabolism in TCE-induced hepatomegaly (and evidence against TCA being the sole mediator of TCE-induced hepatomegaly (Evans et al., 2009)), the preferred PBPK model dose-metric for liver effects was the amount of hepatic oxidative metabolism (scaled by body weight to the ¾ power). Total (hepatic and extrahepatic) oxidative metabolism (scaled by body weight to the ¾ power) was used as an alternative dose-metric. With these dose-metrics, the candidate reference values derived using the PBPK model were only modestly (~threefold or less) different than those derived on the basis of applied dose.

6.2.1.2.5. Immunological effects

There is high qualitative confidence for TCE immunotoxicity and moderate confidence in the candidate reference values that can be derived from the available studies (see Tables 5-6 and 5-16). Decreased thymus weight reported at relatively low exposures in nonautoimmune-prone mice is a clear indicator of immunotoxicity (Keil et al., 2009), and is therefore considered a candidate critical effect. A number of studies have also reported changes in markers of immunotoxicity at relatively low exposures. Among markers for autoimmune effects, the more sensitive measures of autoimmune changes in liver and spleen (Kaneko et al., 2000) and increased anti-dsDNA and anti-ssDNA antibodies (early markers for autoimmune disease) (Keil et al., 2009) are considered the candidate critical effects. For markers of immunosuppression, the more sensitive measures of decreased PFC response (Woolhiser et al., 2006), decreased stem cell bone marrow recolonization, and decreased cell-mediated response to sRBC (both from Sanders et al., 1982b) are considered the candidate critical effects. Developmental immunological effects are discussed below as part of the summary of developmental effects.

Because of the lack of specific data as to the metabolites involved and the mode of action for the candidate critical immunologic effects, PBPK model predictions of total metabolism (scaled by body weight to the ¾ power) was selected as the preferred dose-metric based on the general observation that TCE toxicity is associated with metabolism. The AUC of TCE in blood was used as an alternative dose-metric. With these dose-metrics, the candidate reference values derived using the PBPK model were, with one exception, only modestly (~threefold or less) different than those derived on the basis of applied dose. For the Woolhiser et al. (2006) decreased PFC response, with the alternative dose-metric of AUC of TCE in blood, BMD modeling based on internal doses changed the candidate reference value by 17-fold higher than the cRfC based on applied dose. However, the dose-response model fit for this effect using this metric was substantially worse than the fit using the preferred metric of total oxidative metabolism, with which the change in candidate reference value was only 1.3-fold.

6.2.1.2.6. Reproductive effects

While there is high qualitative confidence in the male reproductive hazard posed by TCE, there is lower confidence in the reference values that can be derived from the available studies of these effects (see Tables 5-8 and 5-17). Relatively high PODs are derived from several studies reporting less sensitive endpoints (George et al., 1986; George et al., 1985; Land et al., 1981), and correspondingly higher cRfCs and cRfDs suggest that they are not likely to be critical effects. The studies reporting more sensitive endpoints also tend to have greater uncertainty. For the human study by Chia et al. (1996), there are uncertainties in the characterization of

exposure and the adversity of the effect measured in the study. For the Kumar et al. (2001b; 2000a; 2000b), Forkert et al. (2002), and Kan et al. (2007) studies, the severity of the sperm and testes effects appears to be continuing to increase with duration even at the end of the study, so it is plausible that a lower exposure for a longer duration may elicit similar effects. For the DuTeaux et al. (2004a) study, there is also duration- and low-dose extrapolation uncertainty due to the short duration of the study in comparison to the time period for sperm development as well as the lack of a NOAEL at the tested doses. Overall, even though there are limitations in the quantitative assessment, there remains sufficient evidence to consider these to be candidate critical effects.

There is moderate confidence both in the hazard and the candidate reference values for reproductive effects other than male reproductive effects. While there are multiple studies suggesting decreased maternal body weight with TCE exposure, this systemic change may not be indicative of more sensitive reproductive effects. None of the estimates developed from other reproductive effects is particularly uncertain or unreliable. Therefore, delayed parturition (Narotsky et al., 1995) and decreased mating (George et al., 1986), which yielded the lowest cRfDs, were considered candidate critical effects. These effects were also included so that candidate critical reproductive effects from oral studies would not include only that reported by DuTeaux et al. (2004a), from which deriving the cRfD entailed a higher degree of uncertainty.

Because of the general lack of specific data as to the metabolites involved and the mode of action for the candidate critical developmental effects, PBPK model predictions of total metabolism (scaled by body weight to the ¾ power) was selected as the preferred dose-metric based on the general observation that TCE toxicity is associated with metabolism. The AUC of TCE in blood was used as an alternative dose-metric. The only exception to this was for the DuTeaux et al. (2004a) study, which suggested that local oxidative metabolism of TCE in the male reproductive tract was involved in the effects reported. Therefore, in this case, AUC of TCE in blood was considered the preferred dose-metric, while total oxidative metabolism (scaled by body weight to the ¾ power) was considered the alternative metric. With these dose-metrics, the candidate reference values derived using the PBPK model were only modestly (~3.5-fold or less) different than those derived on the basis of applied dose.

6.2.1.2.7. Developmental effects

There is moderate-to-high confidence both in the hazard and the candidate reference values for developmental effects of TCE (see Tables 5-10 and 5-18). It is also noteworthy that the PODs for the more sensitive developmental effects were similar to or, in most cases, lower than the PODs for the more sensitive reproductive effects, suggesting that developmental effects are not a result of paternal or maternal toxicity. Among inhalation studies, candidate reference

values were only developed for effects in rats reported in Healy et al. (1982), of resorptions, decreased fetal weight, and delayed skeletal ossification. These were all considered candidate critical developmental effects. Because resorptions were also reported in oral studies, the most sensitive (rat) oral study for this effect (and most reliable for dose-response analysis) of Narotsky et al. (1995) was also selected as a candidate critical study. The confidence in the oral studies and candidate reference values developed for more sensitive endpoints is more moderate, but still sufficient for consideration as candidate critical effects. The most sensitive endpoints by far are the increased fetal heart malformations in rats reported by Johnson et al. (2003) and the developmental immunotoxicity in mice reported by Peden-Adams et al. (2006), and these are both considered candidate critical effects. Neurodevelopmental effects are a distinct type among developmental effects. Thus, the next most sensitive endpoints of decreased rearing postexposure in mice (Fredriksson et al., 1993), increased exploration postexposure in rats (Taylor et al., 1985), and decreased myelination in the hippocampus of rats (Isaacson and Taylor, 1989) are also considered candidate critical effects.

Because of the general lack of specific data as to the metabolites involved and the mode of action for the candidate critical developmental effects, PBPK model predictions of total metabolism (scaled by body weight to the ¾ power) was selected as the preferred dose-metric based on the general observation that TCE toxicity is associated with metabolism. The AUC of TCE in blood was used as an alternative dose-metric. The only exception to this was for the Johnson et al. (2003) study, which suggested that oxidative metabolites were involved in the effects reported based on similar effects being reported from TCA and DCA exposure. Therefore, in this case, total oxidative metabolism (scaled by body weight to the ¾ power) was considered the preferred dose-metric, while AUC of TCE in blood was considered the alternative metric. With these dose-metrics, the candidate reference values derived using the PBPK model were, with one exception, only modestly (~threefold or less) different than those derived on the basis of applied dose. For resorptions reported by Narotsky et al. (1995), BMD modeling based on internal doses changed the candidate reference value by seven to eightfold larger than the corresponding cRfD based on applied dose. However, there is substantial uncertainty in the low-dose curvature of the dose-response curve for modeling both with applied and internal dose, so the BMD remains somewhat uncertain for this endpoint/study. Finally, for two studies (Peden-Adams et al., 2006; Isaacson and Taylor, 1989), PBPK modeling of internal doses was not performed due to the inability to model the complicated exposure pattern (in utero, followed by lactational transfer, followed by drinking water postweaning).

6.2.1.2.8. Summary of most sensitive candidate reference values

As shown in Sections 5.1.3 and 5.1.5, the most sensitive candidate reference values are for the developmental effect of heart malformations in rats (candidate RfC of 0.0004 ppm and candidate RfD of 0.0005 mg/kg/day), developmental immunotoxicity in mice exposed pre- and postnatally (candidate RfD of 0.0004 mg/kg/day), immunological effects in mice (lowest candidate RfCs of 0.0003–0.003 ppm and lowest candidate RfDs of 0.0005–0.005 mg/kg/day), and kidney effects in rats and mice (candidate RfCs of 0.0006–0.002 ppm and candidate RfDs of 0.0003–0.001 mg/kg/day). The most sensitive candidate reference values also generally have low composite UFs (with the exception of some mouse immunological and kidney effects), so they are expected to be reflective of the most sensitive effects as well. Thus, the most sensitive candidate references values for multiple effects span about an order of magnitude for both inhalation (0.0003–0.003 ppm [0.002–0.02 mg/m^3]) and oral (0.0004–0.005 mg/kg/day) exposures. The most sensitive candidate references values for neurological and reproductive effects are about an order of magnitude higher (lowest candidate RfCs of 0.007–0.02 ppm [0.04–0.1 mg/m^3] and lowest candidate RfDs of 0.009–0.02 mg/kg/day). Lastly, the liver effects have candidate reference values that are another two orders of magnitude higher (candidate RfCs of 1–2 ppm [6–10 mg/m^3] and candidate RfDs of 0.9–2 mg/kg/day).

6.2.1.3. Noncancer Reference Values (see Section 5.1.5)

6.2.1.3.1. RfC

The goal is to select an overall RfC that is well supported by the available data (i.e., without excessive uncertainty given the extensive database) and protective for all of the candidate critical effects, recognizing that individual candidate RfC values are by nature somewhat imprecise. As discussed in Section 5.1, the lowest candidate RfC values within each health effect category span a 3,000-fold range from 0.0003 to 0.9 ppm (see Table 5-26). One approach to selecting an RfC would be to select the lowest calculated value of 0.0003 ppm for decreased thymus weight in mice. However, three candidate RfCs (cRfCs and p-cRfCs) are in the relatively narrow range of 0.0003–0.0006 ppm at the low end of the overall range (see Table 5-24). Given the somewhat imprecise nature of the individual candidate RfC values, and the fact that multiple effects/studies lead to similar candidate RfC values, the approach taken in this assessment is to select an RfC supported by multiple effects/studies. The advantages of this approach, which is only possible when there is a relatively large database of studies/effects and when multiple candidate values happen to fall within a narrow range at the low end of the overall range, are that it leads to a more robust RfC (less sensitive to limitations of individual studies) and that it provides the important characterization that the RfC exposure level is similar for multiple noncancer effects rather than being based on a sole explicit critical effect.

Therefore, two critical and one supporting studies/effects were chosen as the basis of the RfC for TCE noncancer effects (see Tables 5-28 and 5-29). These lowest candidate RfCs, ranging from 0.0003 to 0.0006 ppm for developmental, kidney, and immunologic effects, are values derived from route-to-route extrapolation using the PBPK model. The lowest candidate RfC estimate from an inhalation study is 0.001 ppm for kidney effects, which is higher than the route-to-route extrapolated candidate RfC estimate from the most sensitive oral study. For all of the candidate RfCs, the PBPK model was used for inter- and intraspecies extrapolation, based on the preferred dose-metric for each endpoint. There is moderate-to-high confidence in the lowest candidate RfC for immunological effects (see Section 5.1.2.5), and moderate confidence in the lowest candidate RfC for developmental effects (heart malformations) (see Section 5.1.2.8); these are considered the critical effects for deriving the RfC. For kidney effects (toxic nephropathy), there is high confidence in the nephrotoxic hazard from TCE exposure and in the appropriateness of the selected dose-metric; however, as discussed in Section 3.3.3.2, there remains substantial uncertainty in the extrapolation of GSH conjugation from rodents to humans due to limitations in the available data, and thus toxic nephropathy is considered a supporting effect.

As a whole, the estimates support an RfC of 0.0004 ppm (0.4 ppb or 2 $\mu g/m^3$). This value essentially reflects the midpoint between the similar candidate RfC estimates for the two critical effects (0.00033 ppm for decreased thymus weight in mice and 0.00037 ppm for heart malformations in rats), rounded to one significant figure. This value is also within a factor of 2 of the candidate RfC estimate of 0.0006 ppm for the supporting effect of toxic nephropathy in rats. Thus, this assessment does not rely on a single estimate alone; rather, each estimate is supported by estimates of similar magnitude from other effects. In other words, there is robust support for an RfC of 0.0004 ppm provided by estimates for multiple effects from multiple studies. The estimates are based on PBPK model-based estimates of internal dose for interspecies, intraspecies, and route-to-route extrapolation, and there is sufficient confidence in the PBPK model and support from mechanistic data for one of the dose-metrics (total oxidative metabolism for the heart malformations). There is high confidence that bioactivation of DCVC and total GSH metabolism would be appropriate dose-metrics for toxic nephropathy, but there is substantial uncertainty in the PBPK model predictions for these dose-metrics in humans (see Section 5.1.3.1). Note that there is some human evidence of developmental heart defects from TCE exposure in community studies (see Section 4.8.3.1.1) and of kidney toxicity in TCE-exposed workers (see Section 4.4.1).

In summary, the RfC is **0.0004 ppm** (0.4 ppb or 2 $\mu g/m^3$) based on route-to-route extrapolated results from oral studies for the critical effects of heart malformations (rats) and

immunotoxicity (mice). This RfC value is further supported by route-to-route extrapolated results from an oral study of toxic nephropathy (rats).

6.2.1.3.2. RfD

As with the RfC determination above, the goal is to select an overall RfD that is well-supported by the available data (i.e., without excessive uncertainty given the extensive database) and protective for all of the candidate critical effects, recognizing that individual candidate RfD values are by nature somewhat imprecise. As discussed in Section 5.1, the lowest candidate RfD values (cRfDs and p-cRfDs) within each health effect category span a nearly 3,000-fold range from 0.0003 to 0.8 mg/kg/day (see Table 5-26). However, multiple candidate RfDs are in the relatively narrow range of 0.0003–0.0008 mg/kg/day at the low end of the overall range. Given the somewhat imprecise nature of the individual candidate RfD values, and the fact that multiple effects/studies lead to similar candidate RfD values, the approach taken in this assessment is to select an RfD supported by multiple effects/studies. The advantages of this approach, which is only possible when there is a relatively large database of studies/effects and when multiple candidate values happen to fall within a narrow range at the low end of the overall range, are that it leads to a more robust RfD (less sensitive to limitations of individual studies) and that it provides the important characterization that the RfD exposure level is similar for multiple noncancer effects rather than being based on a sole explicit critical effect.

Therefore, three critical and two supporting studies/effects were chosen as the basis of the RfD for TCE noncancer effects (see Tables 5-30 and 5-31). All but one of the lowest candidate RfD values—0.0008 mg/kg/day for increased kidney weight in rats, 0.0005 mg/kg/day for both heart malformations in rats and decreased thymus weights in mice, and 0.0003 mg/kg/day for increased toxic nephropathy in rats—are derived using the PBPK model for inter- and intraspecies extrapolation, based on the preferred dose-metric for each endpoint, and the latter value is derived also using the PBPK model for route-to-route extrapolation from an inhalation study. The other of these lowest candidate RfDs—0.0004 mg/kg/day for developmental immunotoxicity (decreased PFC response and increased delayed-type hypersensitivity) in mice—is based on applied dose. There is moderate-to-high confidence in the candidate RfDs for decreased thymus weights (see Section 5.1.2.5) and developmental immunological effects, and moderate confidence in that for heart malformations (see Section 5.1.2.8); these are considered the critical effects for deriving the RfC. For kidney effects, there is high confidence in the nephrotoxic hazard from TCE exposure and in the appropriateness of the selected dose-metric; however, as discussed in Section 3.3.3.2, there remains substantial uncertainty in the extrapolation of GSH conjugation from rodents to humans due to limitations in the available data, and thus these effects are considered supporting effects.

As a whole, the estimates support an RfD of 0.0005 mg/kg/day. This value is within 20% of the estimates for the critical effects—0.0004 mg/kg/day for developmental immunotoxicity (decreased PFC and increased delayed-type hypersensitivity) in mice and 0.0005 mg/kg/day for both heart malformations in rats and decreased thymus weights in mice. This value is also within approximately a factor of 2 of the supporting effect estimates of 0.0003 mg/kg/day for toxic nephropathy in rats and 0.0008 mg/kg/day for increased kidney weight in rats. Thus, this assessment does not rely on any single estimate alone; rather, each estimate is supported by estimates of similar magnitude from other effects. In other words, there is strong, robust support for an RfD of 0.0005 mg/kg/day provided by the concordance of estimates derived from multiple effects from multiple studies. The estimates for kidney effects, thymus effects, and developmental heart malformations are based on PBPK model-based estimates of internal dose for interspecies and intraspecies extrapolation, and there is sufficient confidence in the PBPK model and support from mechanistic data for one of the dose-metrics (total oxidative metabolism for the heart malformations). There is high confidence that bioactivation of DCVC would be an appropriate dose-metric for toxic nephropathy, but there is substantial uncertainty in the PBPK model predictions for this dose-metric in humans (see Section 5.1.3.1). Note that there is some human evidence of developmental heart defects from TCE exposure in community studies (see Section 4.8.3.1.1) and of kidney toxicity in TCE-exposed workers (see Section 4.4.1).

In summary, the RfD is **0.0005 mg/kg/day** based on the critical effects of heart malformations (rats), adult immunological effects (mice), and developmental immunotoxicity (mice), and toxic nephropathy (rats), all from oral studies. This RfD value is further supported by results from an oral study for the effect of toxic nephropathy (rats) and route-to-route extrapolated results from an inhalation study for the effect of increased kidney weight (rats).

6.2.2. Cancer (see Section 5.2)

6.2.2.1. Background and Methods (rodent: see Section 5.2.1.1; human: see Section 5.2.2.1)

As summarized above, following EPA (2005b) *Guidelines for Carcinogen Risk Assessment*, TCE is characterized as "carcinogenic to humans" by all routes of exposure, based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer, but there is also human evidence of TCE carcinogenicity in the liver and lymphoid tissues. This conclusion is further supported by rodent bioassay data indicating carcinogenicity of TCE in rats and mice at tumor sites that include those identified in human epidemiologic studies. Therefore, both human epidemiologic studies as well as rodent bioassays were considered for deriving PODs for dose-response assessment of cancer endpoints. For PODs derived from rodent bioassays, default dosimetry procedures were applied to convert applied

rodent doses to HEDs. Essentially, for inhalation exposures, "ppm equivalence" across species was assumed, as recommended by U.S. EPA (1994a) for Category 3 gases for which the blood:air partition coefficient in laboratory animals is greater than that in humans. For oral doses, ¾-power body-weight scaling was used, with a default average human body weight of 70 kg. In addition to applied doses, several internal dose-metrics estimated using a PBPK model for TCE and its metabolites were used in the dose-response modeling for each tumor type. In general, an attempt was made to use tissue-specific dose-metrics representing particular pathways or metabolites identified from available data as having a likely role in the induction of a tissue-specific cancer. Where insufficient information was available to establish particular metabolites or pathways of likely relevance to a tissue-specific cancer, more general "upstream" metrics had to be used. In addition, the selection of dose-metrics was limited to metrics that could be adequately estimated by the PBPK model.

Regarding low-dose extrapolation, a key consideration in determining what extrapolation approach to use is the mode(s) of action. However, mode-of-action data are lacking or limited for each of the cancer responses associated with TCE exposure, with the exception of the kidney tumors. For the kidney tumors, the weight of the available evidence supports the conclusion that a mutagenic mode of action is operative; this mode of action supports linear low-dose extrapolation. The weight of evidence also supports involvement of processes of cytotoxicity and regenerative proliferation in the carcinogenicity of TCE, although not with the extent of support as for a mutagenic mode of action. In particular, data linking TCE-induced proliferation to increased mutation or clonal expansion are lacking, as are data informing the quantitative contribution of cytotoxicity. Moreover, it is unlikely that any contribution from cytotoxicity leads to a non-linear dose-response relationship near the PODs. In the case of the rodent bioassays, maximal levels of toxicity are reached before the onset of tumors. Finally, because any possible involvement of a cytotoxicity mode of action would be additional to mutagenicity, the dose-response relationship would nonetheless be expected to be linear at low doses. Therefore, the additional involvement of a cytotoxicity mode of action does not provide evidence against the use of linear extrapolation from the POD. For the other TCE-induced cancers, the mode(s) of action is unknown. When the mode(s) of action cannot be clearly defined, EPA generally uses a linear approach to estimate low-dose risk (2005b), based on the following general principles:

- A chemical's carcinogenic effects may act additively to ongoing biological processes, given that diverse human populations are already exposed to other agents and have substantial background incidences of various cancers.
- A broadening of the dose-response curve (i.e., less rapid fall-off of response with decreasing dose) in diverse human populations and, accordingly, a greater potential for

risks from low-dose exposures (Lutz et al., 2005; Zeise et al., 1987) is expected for two reasons. First, even if there is a "threshold" concentration for effects at the cellular level, that threshold is expected to differ across individuals. Second, greater variability in response to exposures would be anticipated in heterogeneous populations than in inbred laboratory species under controlled conditions (due to, e.g., genetic variability, disease status, age, nutrition, and smoking status).

- The general use of linear extrapolation provides reasonable upper-bound estimates that are believed to be health-protective (U.S. EPA, 2005b) and also provides consistency across assessments.

6.2.2.2. Inhalation Unit Risk Estimate (rodent: see Section 5.2.1.3; human: see Sections 5.2.2.1 and 5.2.2.2)

The inhalation unit risk for TCE is defined as a plausible upper bound lifetime extra risk of cancer from chronic inhalation of TCE per unit of air concentration. The inhalation unit risk for TCE is 2.20×10^{-2} per ppm (**2×10^{-2} per ppm [4×10^{-6} per µg/m^3]** rounded to one significant figure), based on human kidney cancer risks reported by Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer. This estimate is based on good-quality human data, thus avoiding the uncertainties inherent in interspecies extrapolation. The Charbotel et al. (2006) case-control study of 86 incident RCC cases and 316 age- and sex-matched controls, with individual cumulative exposure estimates for TCE inhalation for each subject, provides a sufficient human data set for deriving quantitative cancer risk estimates for RCC in humans. The study is a high-quality study that used a detailed exposure assessment (Fevotte et al., 2006) and took numerous potential confounding factors, including exposure to other chemicals, into account. A significant dose-response relationship was reported for cumulative TCE exposure and RCC (Charbotel et al., 2006). Human data on TCE exposure and cancer risk sufficient for dose-response modeling are only available for RCC, yet human and rodent data suggest that TCE exposure increases the risk of other cancers as well. In particular, there is evidence from human (and rodent) studies for increased risks of lymphoma and liver cancer. Therefore, the inhalation unit risk estimate derived from human data for RCC incidence was adjusted to account for potential increased risk of those cancer types. To make this adjustment, a factor accounting for the relative contributions to the extra risk for cancer incidence from TCE exposure for these three cancer types combined versus the extra risk for RCC alone was estimated, and this factor was applied to the unit risk estimate for RCC to obtain a unit risk estimate for the three cancer types combined (i.e., lifetime extra risk for developing *any* of the three types of cancer). This estimate is considered a better estimate of total cancer risk from TCE exposure than the estimate for RCC alone. Although only the Charbotel et al. (2006) study was found adequate for direct estimation of inhalation unit risks, the available epidemiologic data provide sufficient

information for estimating the *relative* potency of TCE across tumor sites. In particular, the relative contributions to extra risk (for cancer incidence) were calculated from two different data sets to derive the adjustment factor for adjusting the unit risk estimate for RCC to a unit risk estimate for the three types of cancers (RCC, NHL, and liver) combined. The first calculation is based on the results of the meta-analyses of human epidemiologic data for the three cancer types; the second calculation is based on the results of the Raaschou-Nielsen et al. (2003) study, the largest single human epidemiologic study by far with RR estimates for all three cancer types. These calculations support an adjustment factor of 4.

The inhalation unit risk based on human epidemiologic data is supported by inhalation unit risk estimates from multiple rodent bioassays, the most sensitive of which range from **1×10^{-2} to 2×10^{-1} per ppm [2×10^{-6} to 3×10^{-5} per µg/m^3]**. From the inhalation bioassays selected for analysis in Section 5.2.1.1, and using the preferred PBPK model-based dose-metrics, the inhalation unit risk estimate for the most sensitive sex/species is 8×10^{-2} per ppm [2×10^{-5} per µg/m^3], based on kidney adenomas and carcinomas reported by Maltoni et al. (1986) for male Sprague-Dawley rats. Leukemias and Leydig cell tumors were also increased in these rats, and, although a combined analysis for these cancer types which incorporated the different site-specific preferred dose-metrics was not performed, the result of such an analysis is expected to be similar, about 9×10^{-2} per ppm [2×10^{-5} per µg/m^3]. The next most sensitive sex/species from the inhalation bioassays is the female mouse, for which lymphomas were reported by Henschler et al. (1980); these data yield a unit risk estimate of 1.0×10^{-2} per ppm [2×10^{-6} per µg/m^3]. In addition, the 90% CIs (i.e., 5–95% bounds) reported in Table 5-41 for male rat kidney tumors from Maltoni et al. (1986) and female mouse lymphomas from Henschler et al. (1980), derived from the quantitative analysis of PBPK model uncertainty, both included the estimate based on human data of 2×10^{-2} per ppm. Furthermore, PBPK model-based route-to-route extrapolation of the results for the most sensitive sex/species from the oral bioassays, kidney tumors in male Osborne-Mendel rats and testicular tumors in Marshall rats (NTP, 1988), leads to inhalation unit risk estimates of 2×10^{-1} per ppm [3×10^{-5} per µg/m^3] and 4×10^{-2} per ppm [8×10^{-6} per µg/m^3], respectively, with the preferred estimate based on human data falling within the route-to-route extrapolation of the 90% CIs reported in Table 5-42. Finally, for all of these estimates, the ratios of BMDs to the BMDLs did not exceed a value of 3, indicating that the uncertainties in the dose-response modeling for determining the POD in the observable range are small.

Although there are uncertainties in these various estimates, confidence in the proposed inhalation unit risk estimate of 2×10^{-2} per ppm [4×10^{-6} per µg/m^3], based on human kidney cancer risks reported by Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer (as summarized in Section 6.1.4), is further increased by the similarity of this estimate to

estimates based on multiple rodent data sets. Application of the ADAFs for the kidney cancer risks, due to the weight of evidence supporting a mutagenic mode of action for this endpoint, is summarized in Section 6.2.2.5.

6.2.2.3. Oral Slope Factor Estimate (rodent: see Section 5.2.1.3; human: see Section 5.2.2.3)

The oral slope factor for TCE is defined as a plausible upper bound lifetime extra risk of cancer from chronic ingestion of TCE per mg/kg/day oral dose. The oral slope factor is 4.64×10^{-2} per mg/kg/day (**5×10^{-2} per mg/kg/day** rounded to one significant figure), resulting from PBPK model-based route-to-route extrapolation of the inhalation unit risk estimate based on the human kidney cancer risks reported in Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer. This estimate is based on good-quality human data, thus avoiding uncertainties inherent in interspecies extrapolation. In addition, uncertainty in the PBPK model-based route-to-route extrapolation is relatively low (Chiu, 2006; Chiu and White, 2006). In this particular case, extrapolation using different dose-metrics yielded expected population mean risks within about a twofold range, and, for any particular dose-metric, the 95% CI for the extrapolated population mean risks for each site spanned a range of no more than about threefold.

This value is supported by oral slope factor estimates from multiple rodent bioassays, the most sensitive of which range from **3×10^{-2} to 3×10^{-1} per mg/kg/day**. From the oral bioassays selected for analysis in Section 5.2.1.1, and using the preferred PBPK model-based dose-metrics, the oral slope factor estimate for the most sensitive sex/species is 3×10^{-1} per mg/kg/day, based on kidney tumors in male Osborne-Mendel rats (NTP, 1988). The oral slope factor estimate for testicular tumors in male Marshall rats (NTP, 1988) is somewhat lower at 7×10^{-2} per mg/kg/day. The next most sensitive sex/species result from the oral studies is for male mouse liver tumors (NCI, 1976), with an oral slope factor estimate of 3×10^{-2} per mg/kg/day. In addition, the 90% CIs reported in Table 5-42 for male Osborne-Mendel rat kidney tumors (NTP, 1988), male F344 rat kidney tumors (NTP, 1990), and male Marshall rat testicular tumors (NTP, 1988), derived from the quantitative analysis of PBPK model uncertainty, all included the estimate based on human data of 5×10^{-2} per mg/kg/day, while the upper 95% confidence bound for male mouse liver tumors from NCI (1976) was slightly below this value at 4×10^{-2} per mg/kg/day. Furthermore, PBPK model-based route-to-route extrapolation of the most sensitive endpoint from the inhalation bioassays, male rat kidney tumors from Maltoni et al. (1986), leads to an oral slope factor estimate of 1×10^{-1} per mg/kg/day, with the preferred estimate based on human data falling within the route-to-route extrapolation of the 90% CI reported in Table 5-41. Finally, for all of these estimates, the ratios of BMDs to the BMDLs did not exceed a value of 3,

indicating that the uncertainties in the dose-response modeling for determining the POD in the observable range are small.

Although there are uncertainties in these various estimates, confidence in the proposed oral slope factor estimate of 5×10^{-2} per mg/kg/day, resulting from PBPK model-based route-to-route extrapolation of the inhalation unit risk estimate based on the human kidney cancer risks reported in Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer (as summarized above for the inhalation unit risk estimate, but with an adjustment factor of 5 for oral exposure because of the differences in the relative values of the dose-metrics), is further increased by the similarity of this estimate to estimates based on multiple rodent data sets. Application of the ADAFs for the kidney cancer risks, due to the weight of evidence supporting a mutagenic mode of action for this endpoint, is summarized below in Section 6.2.2.5.

6.2.2.4. Uncertainties in Cancer Dose-Response Assessment

6.2.2.4.1. Uncertainties in estimates based on human epidemiologic data (see Section 5.2.2.1.3)

All risk assessments involve uncertainty, as study data are extrapolated to make general inferences about potential effects in humans from environmental exposure. The values for the slope factor and unit risk estimates are based on good quality human data, which avoids interspecies extrapolation, one of the major sources of uncertainty in quantitative cancer risk estimates.

A remaining major uncertainty in the unit risk estimate for RCC incidence derived from the Charbotel et al. (2006) study is the extrapolation from occupational exposures to lower environmental exposures. There was some evidence of a contribution to increased RCC risk from peak exposures; however, there remained an apparent dose-response relationship for RCC risk with increasing cumulative exposure without peaks, and the OR for exposure with peaks compared to exposure without peaks was not significantly elevated (Charbotel et al., 2006). Although the actual exposure-response relationship at low exposure levels is unknown, the conclusion that a mutagenic mode of action is operative for TCE-induced kidney tumors supports the linear low-dose extrapolation that was used (U.S. EPA, 2005b). Additional support for use of linear extrapolation is discussed above in Section 6.2.2.1.

Another source of uncertainty is the dose-response model used to model the study data to estimate the POD. A weighted linear regression across the categorical ORs was used to obtain a slope estimate; use of a linear model in the observable range of the data is often a good general approach for human data because epidemiological data are frequently too limited (the Charbotel et al. [(2006)] study had 86 RCC cases, 37 of which had TCE exposure) to clearly identify an alternate model (U.S. EPA, 2005b). The ratio of the maximum likelihood estimate of the

effective concentration for a 1% response (EC_{01}) to the LEC_{01}, which gives some indication of the statistical uncertainties in the dose-response modeling, was about a factor of 2.

A further source of uncertainty is the retrospective estimation of TCE exposures in the Charbotel et al. (2006) study. This case-control study was conducted in the Arve Valley in France, a region with a high concentration of screw cutting workshops using TCE and other degreasing agents. Since the 1960s, occupational physicians of the region have collected a large quantity of well-documented measurements, including TCE air concentrations and urinary metabolite levels (Fevotte et al., 2006). The study investigators conducted a comprehensive exposure assessment to estimate cumulative TCE exposures for the individual study subjects, using a detailed occupational questionnaire with a customized task-exposure matrix for the screw-cutting workers and a more general occupational questionnaire for workers exposed to TCE in other industries (Fevotte et al., 2006). The exposure assessment also attempted to take dermal exposure from hand-dipping practices into account by equating it with an equivalent airborne concentration based on biological monitoring data. Despite the appreciable effort of the investigators, considerable uncertainty associated with any retrospective exposure assessment is inevitable, and some exposure misclassification is unavoidable. Such exposure misclassification was most likely for the 19 deceased cases and their matched controls, for which proxy respondents were used, and for exposures outside the screw-cutting industry. The exposure estimates from the RCC study of Moore et al. (2010) were not considered to be as quantitatively accurate as those of Charbotel et al. (2006) and so were not used for derivation of a unit risk estimate (see Section 5.2.2); nonetheless, it should be noted that these exposure estimates are substantially lower than those of Charbotel et al. (2006) for comparable OR estimates. If the exposure estimates for Charbotel et al. (2006) are overestimated, as suggested by the exposure estimates from Moore et al. (2010), the slope of the linear regression model, and hence the unit risk estimate, would be correspondingly underestimated.

Another source of uncertainty in the Charbotel et al. (2006) study is the possible influence of potential confounding or modifying factors. This study population, with a high prevalence of metal-working, also had relatively high prevalences of exposure to petroleum oils, cadmium, petroleum solvents, welding fumes, and asbestos (Fevotte et al., 2006). Other exposures assessed included other solvents (including other chlorinated solvents), lead, and ionizing radiation. None of these exposures was found to be significantly associated with RCC at a $p = 0.05$ significance level. Cutting fluids and other petroleum oils were associated with RCC at a $p = 0.1$ significance level; however, further modeling suggested no association with RCC when other significant factors were taken into account (Charbotel et al., 2006). Moreover, a review of other studies suggested that potential confounding from cutting fluids and other petroleum oils is of minimal concern (see Section 4.4.2.3). Nonetheless, a sensitivity analysis

was conducted using the OR estimates further adjusted for cutting fluids and other petroleum oils from the unpublished report by Charbotel et al. (2005), and an essentially identical unit risk estimate of 5.46×10^{-3} per ppm was obtained. In addition, the medical questionnaire included familial kidney disease and medical history, such as kidney stones, infection, chronic dialysis, hypertension, and use of antihypertensive drugs, diuretics, and analgesics. BMI was also calculated, and lifestyle information such as smoking habits and coffee consumption was collected. Univariate analyses found high levels of smoking and BMI to be associated with increased odds of RCC, and these two variables were included in the conditional logistic regressions. Thus, although impacts of other factors are possible, this study took great pains to attempt to account for potential confounding or modifying factors.

Some other sources of uncertainty associated with the epidemiological data are the dose-metric and lag period. As discussed above, there was some evidence of a contribution to increased RCC risk from peak TCE exposures; however, there appeared to be an independent effect of cumulative exposure without peaks. Cumulative exposure is considered a good measure of total exposure because it integrates exposure (levels) over time. If there is a contributing effect of peak exposures, not already taken into account in the cumulative exposure metric, the linear slope may be overestimated to some extent. Sometimes, cancer data are modeled with the inclusion of a lag period to discount more recent exposures not likely to have contributed to the onset of cancer. In an unpublished report, Charbotel et al. (2005) also presented the results of a conditional logistic regression with a 10-year lag period, and these results are very similar to the unlagged results reported in their published paper, suggesting that the lag period might not be an important factor in this study.

Some additional sources of uncertainty are not so much inherent in the exposure-response modeling or in the epidemiologic data themselves but, rather, arise in the process of obtaining more general Agency risk estimates from the epidemiologic results. EPA cancer risk estimates are typically derived to represent an upper bound on increased risk of cancer incidence for all sites affected by an agent for the general population. From experimental animal studies, this is accomplished by using tumor incidence data and summing across all of the tumor sites that demonstrate significantly increased incidences, customarily for the most sensitive sex and species, to attempt to be protective of the general human population. However, in estimating comparable risks from the Charbotel et al. (2006) epidemiologic data, certain limitations are encountered. For one thing, these epidemiology data represent a geographically limited (Arve Valley, France) and likely not very diverse population of working adults. Thus, there is uncertainty about the applicability of the results to a more diverse general population.

Additionally, the Charbotel et al. (2006) study was a study of RCC only, and so the risk estimate derived from it does not represent all of the tumor sites that may be affected by TCE.

This uncertainty was addressed by adjusting the RCC estimate to multiple sites, but there are also uncertainties related to the assumptions inherent in the calculations for this adjustment. As discussed in Section 5.2.2.2, adequate quantitative dose-response data were only available for one cancer type in humans, so other human data were used to adjust the estimate derived for RCC to include risk for other cancers with substantial human evidence of hazard (NHL and liver cancer). The relative contributions to extra risk (for cancer incidence) were calculated from two different data sets to derive an adjustment factor. The first calculation is based on the results of the meta-analyses for the three cancer types; the second calculation is based on the results of the Raaschou-Nielsen et al. (2003) study, the largest single study by far with RR estimates for all three cancer types. The fact that the calculations based on two different data sets yielded comparable values for the adjustment factor (both within 25% of the selected factor of 4) provides more robust support for the use of the factor of 4. Additional uncertainties pertain to the weight of evidence supporting the association of TCE exposure with increased risk of cancer for the three cancer types. As discussed in Section 4.11.2, it is concluded that the weight of evidence for kidney cancer is sufficient to classify TCE as "carcinogenic to humans." It is also concluded that there is strong evidence that TCE causes NHL as well, although the evidence for liver cancer was more limited. In addition, the rodent studies demonstrate clear evidence of multisite carcinogenicity, with cancer types including those for which associations with TCE exposure are observed in human studies (i.e., liver and kidney cancers and lymphomas). Overall, the evidence is sufficiently persuasive to support the use of the adjustment factor of 4 based on these three cancer types. Alternatively, if one were to use the factor based only on the two cancer types with the strongest human evidence, the cancer inhalation unit risk estimate would be only slightly reduced (25%).

Finally, the value for the oral slope factor estimate was based on route-to-route extrapolation of the inhalation unit risk based on human data using predictions from the PBPK model. Because different internal dose-metrics are preferred for each target tissue site, a separate route-to-route extrapolation was performed for each site-specific slope factor estimate. As discussed above, uncertainty in the PBPK model-based route-to-route extrapolation is relatively low (Chiu, 2006; Chiu and White, 2006). In this particular case, extrapolation using different dose-metrics yielded expected population mean risks within about a twofold range, and, for any particular dose-metric, the 95% CI for the extrapolated population mean risks for each site spanned a range of no more than about threefold.

6.2.2.4.2. Uncertainties in estimates based on rodent bioassays (see Section 5.2.1.4)

With respect to rodent-based cancer risk estimates, the cancer risk is typically estimated from the total cancer burden from all sites that demonstrate an increased tumor incidence for the

most sensitive experimental species and sex. It is expected that this approach is protective of the human population, which is more diverse but is exposed to lower exposure levels. In the case of TCE, the impact of selection of the bioassay is limited, since, as discussed in Sections 5.2.1.3 and 5.2.3, estimates based on the two or three most sensitive bioassays are within an order of magnitude of each other, and are consistent across routes of exposure when extrapolated using the PBPK model.

Another source of uncertainty in the TCE rodent-based cancer risk estimates is interspecies extrapolation. Several plausible PBPK model-based dose-metrics were used for extrapolation of toxicokinetics, but the cancer slope factor and unit risk estimates obtained using the preferred dose-metrics were generally similar (within about threefold) to those derived using default dosimetry assumptions, with the exception of the bioactivated DCVC dose-metric for rat kidney tumors and the metric for the amount of TCE oxidized in the respiratory tract for mouse lung tumors occurring from oral exposure. However, there is greater biological support for these selected dose-metrics. The uncertainty in the PBPK model predictions themselves was analyzed quantitatively through an analysis of the impact of parameter uncertainties in the PBPK model. The 95% lower bounds on the BMD including parameter uncertainties in the PBPK model were no more than fourfold lower than those based on central estimates of the PBPK model predictions. The greatest uncertainty was for slope factors and unit risks derived from rat kidney tumors, primarily reflecting the substantial uncertainty in the rat internal dose and in the extrapolation of GSH conjugation from rodents to humans.

Regarding low-dose extrapolation, a key consideration in determining what extrapolation approach to use is the mode(s) of action. However, mode-of-action data are lacking or limited for each of the cancer responses associated with TCE exposure, with the exception of the kidney tumors. For the kidney tumors, the weight of the available evidence supports the conclusion that a mutagenic mode of action is operative; this mode of action supports linear low-dose extrapolation. For the other TCE-induced cancers, the data either support a complex mode of action or are inadequate to specify the key events and modes of action involved. When the mode(s) of action cannot be clearly defined, EPA generally uses a linear approach to estimate low-dose risk (U.S. EPA, 2005b), based on the general principles discussed above.

With respect to uncertainties in the dose-response modeling, the two-step approach of modeling only in the observable range, as put forth in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), is designed in part to minimize model dependence. The ratios of the BMDs to the BMDLs, which give some indication of the statistical uncertainties in the dose-response modeling, did not exceed a value of 2.5 for all of the primary analyses used in this assessment. Thus, overall, modeling uncertainties in the observable range are considered to be minimal. Some additional uncertainty is conveyed by uncertainties in the survival adjustments

made to some of the bioassay data; however, a comparison of the results of two different survival adjustment methods suggest that their impact is minimal relative to the uncertainties already discussed.

6.2.2.5. Application of ADAFs (see Section 5.2.3.3)

When there is sufficient weight of evidence to conclude that a carcinogen operates through a mutagenic mode of action, and in the absence of chemical-specific data on age-specific susceptibility, EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) recommends the application of default ADAFs to adjust for potential increased susceptibility from early-life exposure. See the *Supplemental Guidance* for detailed information on the general application of these adjustment factors. In brief, the *Supplemental Guidance* establishes ADAFs for three specific age groups. The current ADAFs and their age groupings are 10 for <2 years, 3 for 2–<16 years, and 1 for ≥16 years (U.S. EPA, 2005b). For risk assessments based on specific exposure assessments, the 10- and 3-fold adjustments to the slope factor or unit risk estimates are to be combined with age-specific exposure estimates when estimating cancer risks from early-life (<16 years age) exposure.

In the case of TCE, the inhalation unit risk and oral slope factor estimates reflect lifetime risk for cancer at multiple sites, and a mutagenic mode of action has been established for one of these sites, the kidney. The weight of evidence also supports involvement of processes of cytotoxicity and regenerative proliferation in the carcinogenicity of TCE, although not with the extent of support as for a mutagenic mode of action. In particular, data linking TCE-induced proliferation to increased mutation or clonal expansion are lacking, as are data informing the quantitative contribution of cytotoxicity. Because any possible involvement of a cytotoxicity mode of action would be additional to mutagenicity, the mutagenic mode of action would be expected to dominate at low doses. Therefore, the additional involvement of a cytotoxicity mode of action does not provide evidence against application of ADAFs. In addition, as discussed in Section 4.10, inadequate TCE-specific data exists to quantify early-life susceptibility to TCE carcinogenicity; therefore, as recommended in the *Supplemental Guidance,* the default ADAFs are used. As illustrated in the example calculations in Sections 5.2.3.3.1 and 5.2.3.3.2, application of the default ADAFs to the kidney cancer inhalation unit risk and oral slope factor estimates for TCE is likely to have minimal impact on the total cancer risk except when exposure is primarily during early life.

In addition to the uncertainties discussed above for the inhalation and oral total cancer unit risk and slope factor estimates, there are uncertainties in the application of ADAFs to adjust for potential increased early-life susceptibility. The adjustment is made only for the kidney cancer component of total cancer risk because that is the tumor type for which the weight of

evidence was sufficient to conclude that TCE-induced carcinogenesis operates through a mutagenic mode of action. However, it may be that TCE operates through a mutagenic mode of action for other cancer types as well or that it operates through other modes of action that might also convey increased early-life susceptibility. Additionally, the ADAFs from the 2005 Supplemental Guidance are not specific to TCE, and it is uncertain to what extent they reflect increased early-life susceptibility to kidney cancer from exposure to TCE, if increased early-life susceptibility occurs.

6.3. OVERALL CHARACTERIZATION OF TCE HAZARD AND DOSE RESPONSE

There is substantial potential for human exposure to TCE, as it has a widespread presence in ambient air, indoor air, soil, and groundwater. At the same time, humans are likely to be exposed to a variety of compounds that are either metabolites of TCE or have common metabolites or targets of toxicity. Once exposed, humans, as well as laboratory animal species, rapidly absorb TCE, which is then distributed to tissues via systemic circulation, extensively metabolized, and then excreted primarily in breath as unchanged TCE or CO_2, or in urine as metabolites.

Based on the available human epidemiologic data and experimental and mechanistic studies, it is concluded that TCE poses a potential human health hazard for noncancer toxicity to the CNS, the kidney, the liver, the immune system, the male reproductive system, and the developing fetus. The evidence is more limited for TCE toxicity to the respiratory tract and female reproductive system. Following EPA (2005b) *Guidelines for Carcinogen Risk Assessment*, TCE is characterized as "carcinogenic to humans" by all routes of exposure. This conclusion is based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer. The human evidence of carcinogenicity from epidemiologic studies of TCE exposure is strong for NHL, but less convincing than for kidney cancer, and more limited for liver and biliary tract cancer. Less human evidence is found for an association between TCE exposure and other types of cancer, including bladder, esophageal, prostate, cervical, breast, and childhood leukemia. Further support for the characterization of TCE as "carcinogenic to humans" by all routes of exposure is derived from positive results in multiple rodent cancer bioassays in rats and mice of both sexes, similar toxicokinetics between rodents and humans, mechanistic data supporting a mutagenic mode of action for kidney tumors, and the lack of mechanistic data supporting the conclusion that any of the mode(s) of action for TCE-induced rodent tumors are irrelevant to humans.

As TCE toxicity and carcinogenicity are generally associated with TCE metabolism, susceptibility to TCE health effects may be modulated by factors affecting toxicokinetics, including lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status,

lifestyle, and nutrition status. In addition, while some of these factors are known risk factors for effects associated with TCE exposure, it is not known how TCE interacts with known risk factors for human diseases.

For noncancer effects, the most sensitive types of effects, based either on HECs/HEDs or on candidate RfCs/RfDs, appear to be developmental, kidney, and immunological (adult and developmental) effects. The neurological and reproductive effects appear to be about an order of magnitude less sensitive, with liver effects another 2 orders of magnitude less sensitive. The RfC of **0.0004 ppm** (0.4 ppb or 2 $\mu g/m^3$) is based on route-to-route extrapolated results from oral studies for the critical effects of heart malformations (rats) and immunotoxicity (mice). This RfC value is further supported by route-to-route extrapolated results from an oral study of toxic nephropathy (rats). Similarly, the RfD for noncancer effects of **0.0005 mg/kg/day** is based on the critical effects of heart malformations (rats), adult immunological effects (mice), and developmental immunotoxicity (mice), all from oral studies. This RfD value is further supported by results from an oral study for the effect of toxic nephropathy (rats) and route-to-route extrapolated results from an inhalation study for the effect of increased kidney weight (rats). There is high confidence in these noncancer reference values, as they are supported by moderate-to-high confidence estimates for multiple effects from multiple studies.

For cancer, the inhalation unit risk is **2×10^{-2} per ppm [4×10^{-6} per $\mu g/m^3$]**, based on human kidney cancer risks reported by Charbotel et al. (2006) and adjusted, using human epidemiologic data, for potential risk for NHL and liver cancer. The oral slope factor for cancer is **5×10^{-2} per mg/kg/day**, resulting from PBPK model-based route-to-route extrapolation of the inhalation unit risk estimate based on the human kidney cancer risks reported in Charbotel et al. (2006) and adjusted, using human epidemiologic data, for potential risk for NHL and liver cancer. There is high confidence in these unit risks for cancer, as they are based on good-quality human data, as well as being similar to unit risk estimates based on multiple rodent bioassays. There is both sufficient weight of evidence to conclude that TCE operates through a mutagenic mode of action for kidney tumors and a lack of TCE-specific quantitative data on early-life susceptibility. Generally, the application of ADAFs is recommended when assessing cancer risks for a carcinogen with a mutagenic mode of action. However, because the ADAF adjustment applies only to the kidney cancer component of the total risk estimate, it is likely to have a minimal impact on the total cancer risk except when exposures are primarily during early life.

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TOXICOLOGICAL REVIEW

OF

TRICHLOROETHYLENE

APPENDICES

(CAS No. 79-01-6)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2011

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Appendices
(CAS No. 79-01-6)

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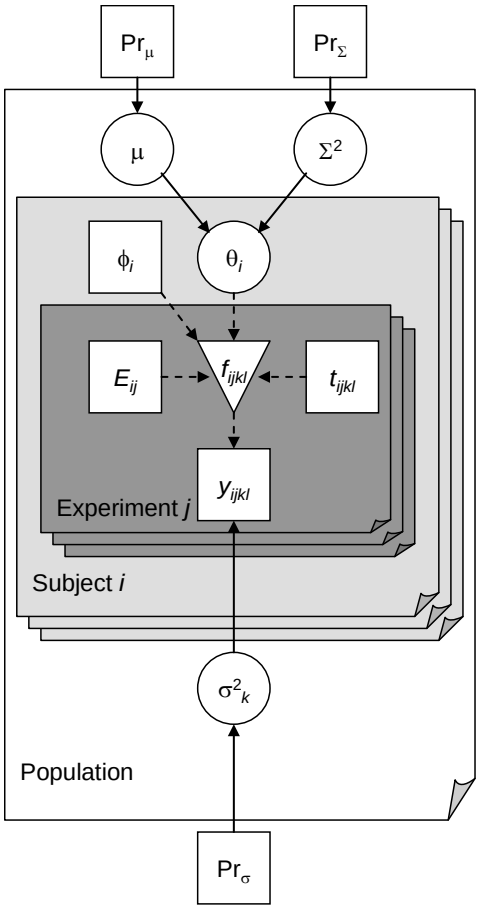
A. PBPK MODELING OF TCE AND METABOLITES—DETAILED METHODS AND RESULTS

A.1. THE HIERARCHICAL BAYESIAN APPROACH TO CHARACTERIZING PBPK MODEL UNCERTAINTY AND VARIABILITY

The Bayesian approach for characterizing uncertainty and variability in PBPK model parameters, used previously for TCE in Bois (2000a, b) and Hack et al. (2006), is briefly described here as background. Once a PBPK model structure is specified, characterizing the model reduces to calibrating and making inferences about model parameters. The use of least-squares point estimators is limited by the large number of parameters and small amounts of data. The use of least-squares estimation is reported after imposing constraints for several parameters (Clewell et al., 2000; Fisher, 2000). This is reasonable for a first estimate, but it is important to follow-up with a more refined treatment. This is implemented by a Bayesian approach to estimate posterior distributions on the unknown parameters, a natural choice, and almost a compulsory consequence given the large number of parameters and relatively small amount of data, and given the difficulties of frequentist estimation in this setting.

As described by Gelman et al. (1996), the Bayesian approach to population PBPK modeling involves setting up the overall model in several stages. A nonlinear PBPK model, with predictions denoted *f*, describes the absorption, distribution, metabolism, and excretion of a compound and its metabolites in the body. This model depends on several, usually known, parameters such as measurement times *t*, exposure *E*, and measured covariates φ. Additionally, each subject *i* in a population has a set of unmeasured parameters θ_i. A random effects model describes their population variability $P(\theta_i \mid \mu, \Sigma^2)$, and a prior distribution $P(\mu, \Sigma^2)$ on the population mean μ and covariance Σ^2 (often assumed to be diagonal) incorporates existing scientific knowledge about them. Finally, a "measurement error" model $P(y \mid f[\theta, \varphi, E, t], \sigma^2)$ describes deviations (with variance σ^2) between the data *y* and model predictions *f* (which of course depends on the unmeasured parameters θ_i and the measured parameters *t*, *E*, and φ). This "measurement error" level of the hierarchical model typically also encompasses intrasubject variability as well as model misspecification, but for notational convenience we refer to it here as "measurement error." Because these other sources of variance are lumped into a single "measurement error," a prior distribution of its variance σ^2 must be specified even if the actual analytic measurement error is known. All of these components are illustrated graphically in Figure A-1.



Square nodes denote fixed or observed quantities; circle notes represent uncertain or unobserved quantities, and the nonlinear model outputs are denoted by the inverted triangle. Solid arrows denote a stochastic relationship represented by a conditional distribution [$A \rightarrow B$ means $B \sim P(B|A)$], while dashed arrows represent a function relationship [$B = f(A)$]. The population consists of subjects i, each of which undergoes one or more experiments j with exposure parameters E_{ij} with data y_{ijkl} collected at times t_{ijkl}, where k denotes different types of outputs and l denotes the different time points. The PBPK model produces outputs f_{ijkl} for comparison with the data y_{ijkl}. The difference between them ("measurement error") has variance σ^2_k, with a fixed prior distribution Pr, which in this case is the same for the entire population. The PBPK model also depends on measured covariates ϕ_i (e.g., body weight) and unobserved model parameters θ_i (e.g., V_{MAX}). The parameters θ_i are drawn from a population with mean μ and variance Σ^2, each of which is uncertain and has a prior distribution assigned to it.

Source: Gelman et al. (1996).

Figure A-1. Hierarchical population statistical model for PBPK model parameter uncertainty and variability.

The posterior distribution for the unknown parameters is obtained in the usual manner by multiplying: (1) the prior distribution for the population mean and variance and the "measurement" error $P(\mu, \Sigma^2)\, P(\sigma^2)$; (2) the population distribution for the subject parameters $P(\theta \mid \mu, \Sigma^2)$; and (3) the likelihood $P(y \mid \theta, \sigma^2)$, where for notational convenience, the dependence on *f*, φ, *E*, and *t* (which are taken as fixed for a given data set) is dropped:

$$P(\theta, \mu, \Sigma^2, \sigma^2 \mid y) \propto P(\mu, \Sigma^2)\, P(\sigma^2)\, P(\theta \mid \mu, \Sigma^2)\, P(y \mid \theta, \sigma^2) \qquad \text{(Eq. A-1)}$$

Here, each subject's parameters θ_i have the same sampling distribution (i.e., they are independently and identically distributed), so their joint prior distribution is:

$$P(\theta \mid \mu, \Sigma^2) = \prod_{i=1\ldots n} P(\theta_i \mid \mu, \Sigma^2) \qquad \text{(Eq. A-2)}$$

Different experiments $j = 1\ldots n_j$ may have different exposure and different data collected and different time points. In addition, different types of measurements $k = 1\ldots n_k$ (e.g., TCE blood, TCE breath, TCA blood, etc.) may have different errors, but errors are otherwise assumed to be iid. Since the subjects are treated as independent given $\theta_{1\ldots n}$, the total likelihood function is simply

$$P(y \mid \theta, \sigma^2) = \prod_{I = 1\ldots n} \prod_{j = 1\ldots nij} \prod_{k = 1\ldots m} \prod_{l = 1\ldots Nijk} P(y_{ijkl} \mid \theta_i, \sigma_k^2, t_{ijkl}) \qquad \text{(Eq. A-3)}$$

where n is the number of subjects, n_{ij} is the number of experiments in that subject, *m* is the number of different types of measurements, N_{ijk} is the number (possibly 0) of measurements (e.g., time points) for subject *i* of type *k* in experiment *j*, and t_{ijkl} are the times at which measurements for subject *i* of type *k* were made in experiment *j*.

Given the large number of parameters, complex likelihood functions, and nonlinear PBPK model, Markov chain Monte Carlo (MCMC) simulation was used to generate samples from the posterior distribution. An important practical advantage of MCMC sampling is the ability to implement inference in nearly any probability model and the possibility to report inference on any event of interest. MCMC simulation was introduced by Gelfand and Smith (1990) as a generic tool for posterior inference. See Gilks et al. (1995) for a review. In addition, because many parameters are allowed to vary simultaneously, the local parameter sensitivity analyses often performed with PBPK models (in which the changes in model predictions are assessed with each parameter varied by a small amount) are unnecessary.[1] In the context of PBPK models, the MCMC simulation can be carried out as described by Hack et al. (2006). The

[1]In particular, local sensitivity analyses are typically used to assess the impact of alternative parameter estimates on model predictions, inform experimental design, or assist prioritizing risk assessment research. Only the first purpose is relevant here; however, the full uncertainty and variability analysis allows for a more comprehensive assessment than can be done with sensitivity analyses. Separately, such analyses could be done to design experiments and prioritize research that would be most likely to help reduce the remaining uncertainties in TCE toxicokinetics, but that is beyond the scope of this assessment.

simulation program MCSim (version 5.0.0) was used to implement MCMC posterior simulation, with analysis of the results performed using the *R* statistical package. Simulation-based parameter estimation with MCMC posterior simulation gives rise to an additional source of uncertainty. For instance, averages computed from the MCMC simulation output represent the desired posterior means only asymptotically, in the limit as the number of iterations goes to infinity. Any implementation needs to include a convergence diagnostic to judge practical convergence. The potential scale-reduction-factor convergence diagnostic *R* of Gelman et al. (1996) was used here, as it was in Hack et al. (2006).

A.2. EVALUATION OF THE HACK ET AL. (2006) PBPK MODEL

U.S. EPA obtained the original model code for the version of the TCE PBPK model published in Hack et al. (2006) and conducted a detailed evaluation of the model, focusing on the following areas: convergence, posterior estimates for model parameters, and comparison of model predictions with in vivo data.

A.2.1. Convergence

As noted in Hack et al. (2006), the diagnostics for the MCMC simulations (three chains of length 20,000–25,000 for each species) indicated that additional samples might further improve convergence. A recent analysis of tetrachloroethylene pharmacokinetics indicated the need to be especially careful in ensuring convergence (Chiu and Bois, 2007). Therefore, the number of MCMC samples per chain was increased to 75,000 for rats (first 25,000 discarded) and 175,000 for mice and humans (first 75,000 discarded). Using these chain lengths, the vast majority of the parameters had potential scale reduction factors $R \leq 1.01$, and all population parameters had $R \leq 1.05$, indicating that longer chains would be expected to reduce the SD (or other measure of scale, such as a CI) of the posterior distribution by less than this factor (Gelman et al., 2003).

In addition, analysis of autocorrelation within chains using the R-CODA package (Plummer et al., 2006) indicated that there was significant serial correlation, so additional "thinning" of the chains was performed in order to reduce serial correlations. In particular, for rats, for each of three chains, every 100^{th} sample from the last 50,000 samples was used; and for mice and humans, for each of three chains, every 200^{th} sample from the last 100,000 samples was used. This thinning resulted in a total of 1,500 samples for each species available for use for posterior inference.

Finally, an evaluation was made of the "convergence" of dose-metric predictions—that is, the extent to which the SD or CIs for these predictions would be reduced with additional samples. This is analogous to a "sensitivity analysis" performed so that most effort is spent on parameters that are most influential in the result. In this case, the purpose is to evaluate whether one can sample chains only long enough to ensure convergence of predictions of interest, even if

certain more poorly identified parameters take longer chains to converge. The motivation for this analysis is that for a more complex model, running chains until all parameters have $R \leq 1.01$ or 1.05 may be infeasible given the available time and resource. In addition, as some of the model parameters had prior distributions derived from "visual fitting" to the same data, replacing those distributions with less informative distributions (in order to reduce bias from "using the same data twice") may require even longer chains for convergence.

Indeed, it was found that *R*-values for dose-metric predictions approached one more quickly than PBPK model input parameters. The most informative simulations were for mice, which converged the slowest and, thus, had the most potential for convergence-related error. Results for rats could not be assessed because the model converged so rapidly, and results for humans were similar to those in mice, though the deviations were all less because of the more rapid convergence. In the mouse model, after 25,000 iterations, many PBPK model parameters had *R*-values >2, with >25% >1.2. However, all dose-metric predictions had $R < 1.4$, with the >96% of them <1.2 and the majority of them <1.01. In addition, when compared to the results of the last 100,000 iterations (after the total of 175,000 iterations), >90% of the medians estimates shifted by <20%, with the largest shifts <40% (for GSH metabolism dose-metrics, which had no relevant calibration data). Tail quantiles had somewhat larger shifts, which was expected given the limited number of samples in the tail, but still >90% of the 2.5 and 97.5 percentile quantiles had shifts of <40%. Again, the largest shifts, on the order of twofold, were for GSH-related dose-metrics that had high uncertainty, so the relative impact of limited sample size is small.

Therefore, the additional simulations performed in this evaluation, with three- to sevenfold longer chains, did not result in much change in risk assessment predictions from the original Hack et al. (2006) results. Thus, assessing prediction convergence appears sufficient for assessing convergence of the TCE PBPK model for the purposes of risk assessment prediction.

A.2.2. Evaluation of Posterior Distributions for Population Parameters

Posterior distributions for the population parameters were first checked for whether they appeared reasonable given the prior distributions. Inconsistency between the prior and posterior distributions may indicate an insufficiently broad prior distribution (i.e., overconfidence in their specification), a mis-specification of the model structure, or an error in the data. Parameters that were flagged for further investigation were those for which the interquartile ranges (intervals bounded by the 25^{th} and 75^{th} percentiles) of the prior and posterior distributions did not overlap. In addition, lumped metabolism and clearance parameters for TCA, TCOH, and TCOG were checked to make sure that they remained physiological—e.g., metabolic clearance was not more than hepatic blood flow and urinary clearance not more than kidney blood flow (constraints that were not present in the Hack et al. (2006) priors).

In mice, population mean parameters that had lack of overlap between priors and posteriors included the affinity of oxidative metabolism ($\ln K_M$), the TCA plasma-blood

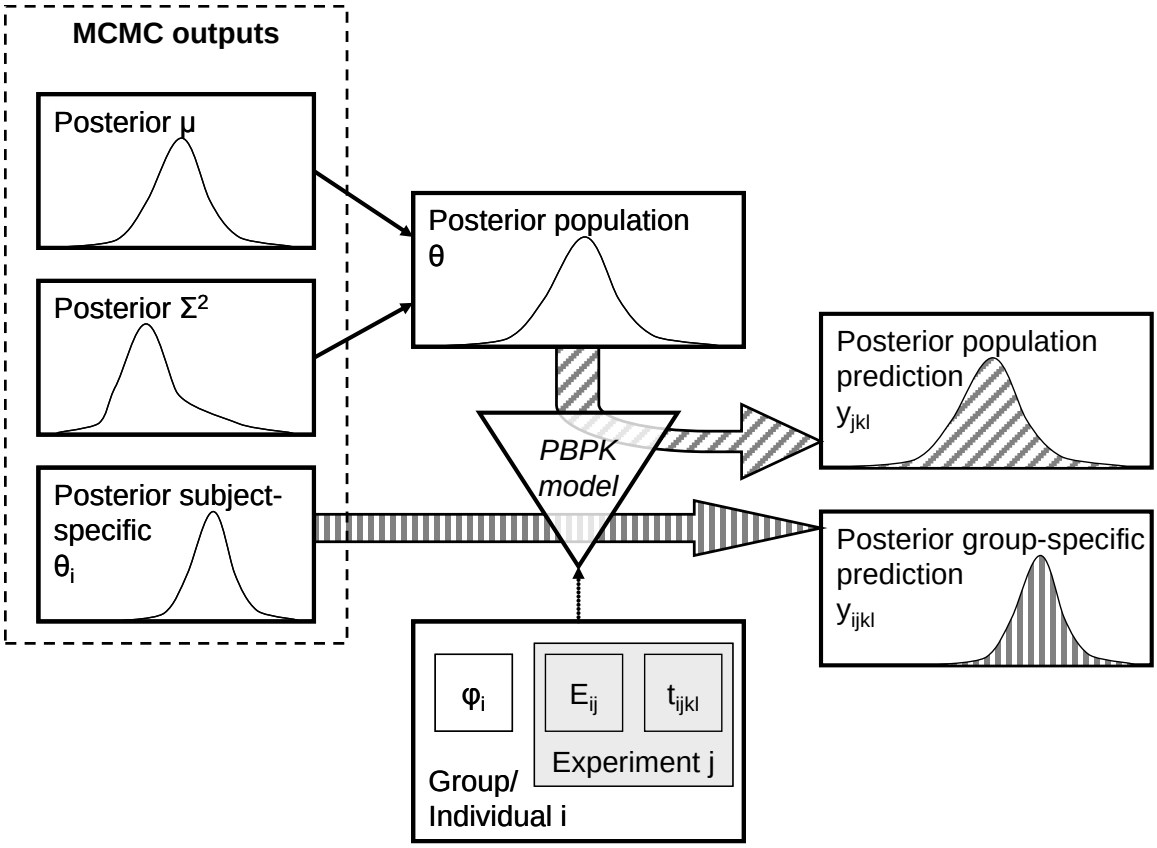
concentration ratio (TCAPlas), the TCE stomach to duodenum transfer coefficient (lnKTSD), and the urinary excretion rates of TCA and TCOG (lnkUrnTCAC and lnkUrnTCOGC). For K_M, this is not unexpected, as previous investigators have noted inconsistency in the K_M values between in vitro values (upon which the prior distribution was based) and in vivo values derived from oral and inhalation exposures in mice (Greenberg et al., 1999; Abbas and Fisher, 1997). For the other mean parameters, the central estimates were based on visual fits, without any other a priori data, so it is reasonable to assume that the inconsistency is due to insufficiently broad prior distributions. In addition, the population variance for the TCE absorption coefficient from the duodenum (kAD) was rather large compared to the prior distribution, likely due to the fact that oral studies included TCE in both oil and aqueous solutions, which are known to have very different absorption properties. Thus, the larger population variance was required to accommodate both of them. Finally, the estimated clearance rate for glucuronidation of TCOH was substantially greater than hepatic blood flow. This is an artifact of the one-compartment model used for TCOH and TCOG, and suggests that first-pass effects are important for TCOH glucuronidation. Therefore, the model would benefit from the addition of a separate liver compartment so that first-pass effects can be accounted for, particularly when comparing across dose-routes.

In rats, the only population mean or variance parameter for which the posterior distribution was somewhat inconsistent with the prior distribution was the population mean for the lnK_M. While the interquartile regions did not overlap, the 95^{th} percentile regions did, so the discordance was relatively minor. However, as with mice, the estimated clearance rate for glucuronidation of TCOH was substantially greater than hepatic blood flow.

In humans, some of the chemical-specific parameters for which priors were established using visual fits had posterior distributions that were somewhat inconsistent, including the oxidative split between TCA and TCOH, biliary excretion of TCOG (lnkBileC), and the TCOH distribution volume (VBodC). More concerning was the fact that the posterior distributions for several physiological volumes and flows were rather strongly discordant with the priors and/or near their truncation limits, including gut, liver, and slowly perfused blood flow, the volumes of the liver and rapidly perfused compartments. In addition, a number of tissue partition coefficients were somewhat inconsistent with their priors, including those for TCE in the gut, rapidly perfused, and slowly perfused tissues, and TCA in the body and liver. Finally, a number of population variances (for TCOH clearance [lnClTCOHC], urinary excretion of TCOG [lnkUrnTCOGC], ventilation-perfusion ratio [lnVPRC], cardiac output [lnQCC], fat blood flow and volume [QFatC and VFatC], and TCE blood-air partition coefficient [PBC]) were somewhat high compared to their prior distributions, indicating much greater population variability than expected.

A.2.3. Comparison of Model Predictions With Data

A schematic of the comparisons between model predictions and data are shown in Figure A-2. In the hierarchical population model, subject-specific parameters were estimated for each data set used in calibrating the model (posterior subject-specific θ_i in Figure A-2). Because these parameters are in a sense "optimized" to the experimental data themselves, the subject-specific predictions (posterior subject-specific y_{ij} in Figure A-2) using these parameters should be accurate by design. Poor fits to the data using these subject-parameters may indicate a misspecification of the model structure, prior parameter distributions, or an error in the data. In addition, it is useful to generate "population-based" parameters (posterior population θ) using only the posterior distributions for the population means (μ) and variances (Σ^2), instead of the estimated subject-specific parameters. These population predictions provide a sense as to whether the model and the predicted degree of population uncertainty and variability adequately account for the range of heterogeneity in the experimental data. Furthermore, assuming the subject-specific predictions are accurate, the population-based predictions are useful to identify whether one or more if the data sets are "outliers" with respect to the predicted population. In addition, a substantial number of in vivo data sets was available in all three species that were not previously used for calibration. Thus, it is informative to compare the population-based model predictions, discussed above, to these additional "validation" data in order to assess the predictive power of the PBPK model.



Two sets of posterior predictions were generated: population predictions (diagonal hashing) and subject-specific predictions (vertical hashing).

Figure A-2. Schematic of how posterior predictions were generated for comparison with experimental data.

A.2.3.1. Mouse Model

A.2.3.1.1. Subject-specific and population-based predictions

Initially, the sampled subject-specific parameters were used to generate predictions for comparison to the calibration data. Because these parameters were "optimized" for each subject, these "subject-specific" predictions should be accurate by design. However, unlike for the rat (see below), this was not the case for some experiments (this is partially responsible for the slower convergence). In particular, the predictions for TCE and TCOH concentrations for the Abbas and Fisher (1997) data were poor. In addition, TCE blood concentrations for the Greenberg et al. (1999) data were consistently overpredicted. These data are discussed further in Table A-1.

Table A-1. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in mice

Reference	Simulation #	Calibration data	Discussion
Abbas et al. (1997)	41–42		These data are only published as an abstract. They consist of TCA and TCOH blood and urine data from TCA and TCOH i.v. dosing. Blood levels of TCA and TCOH are fairly accurately predicted. From TCOH dosing, urinary TCOG excretion is substantially overpredicted, and from TCA dosing, urinary TCA excretion is substantially overpredicted.
Abbas and Fisher (1997)	3–6	√	Results for these data were mixed. TCA levels were the best fit. The calibration data included TCA blood and liver data, which were well predicted except at the earliest time-point. In addition, TCA concentrations in the kidney were fairly consistent with the surrogate TCA body concentrations predicted by the model. Urinary TCA was well predicted at the lower two and highest doses, but somewhat underpredicted (though still in the 95% confidence region) at 1,200 mg/kg. TCE levels were in general not well fit. Calibration data included blood, fat, and liver concentrations, which were predicted poorly particularly at early and late times. One reason for this is probably the representation of oral uptake. Although both the current model and the original Abbas and Fisher (1997) model had two-compartments representing oral absorption, in the current model uptake can only occur from the second compartment. By contrast, the Abbas and Fisher (1997) model had uptake from both compartments, with the majority occurring from the first compartment. Thus, the explanation for the poor fit, particularly of blood and liver concentrations, at early times is probably simply due to differences in modeling oral uptake. This is also supported by the fact that the oral uptake parameters tended to be among those that took the longest to converge. Subject-specific blood TCOH predictions were poor, with underprediction at early times and overprediction at late times. Population-based blood TCOH predictions tended to be underpredicted, though generally within the 95% confidence region. Subject-specific urinary TCOG predictions were fairly accurate except at the highest dose. These predictions are also probably affected by the apparent misrepresentation of oral uptake. In addition, a problem as found in the calibration data in that data on free TCOH was calibrated against predictions of total TCOH (TCOH+TCOG). A number of TCOH and TCOG measurements were not included in the calibration—among them tissue concentrations of TCOH and tissue and blood concentrations of TCOG. Blood concentrations (the only available surrogate) were poor predictors of tissue concentrations of TCOH and TCOG (model generally underpredicted). For TCOG, this may be due in part to the model assumption that the distribution volume of TCOG is equal to that of TCOH.

Table A-1. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in mice (continued)

Reference	Simulation #	Calibration data	Discussion
Fisher et al. (1991)	1–2 (open-chamber)	√	Venous blood TCE concentrations were somewhat underpredicted (a common issue with inhalation exposures in mice below) (Greenberg et al., 1999), but within the 95% confidence region of both subject-specific and population-based predictions. Plasma TCA levels were well predicted, with most of the data near the interquartile region of both subject-specific and population-based predictions (but with substantial scatter in the male mice). However, it should be noted that only a single exposure concentration for each sex was used in calibration, with six additional exposures (three for each sex) not included (see simulations 21–26, below).
	7–16 (closed-chamber)	√	Good posterior fits were obtained for these data—closed-chamber data with initial concentrations from 300 to 10,000 ppm. Some variability in V_{MAX}, however, was noted in the posterior distributions for that parameter. Using subject-specific V_{MAX} values resulted in better fits to these data. However, there appears to be a systematic trend of lower estimated apparent V_{MAX} at higher exposures. Similarly, posterior estimates of cardiac output and the ventilation-perfusion ratio declined (slightly) with higher exposures. These could be related to documented physiological changes (e.g., reduced ventilation rate and body temperature) in mice when exposed to some volatile organics.
	21–26 (open-chamber, additional exposures)		Data from three additional exposures for each sex were available for comparison to model predictions. Plasma TCA levels were generally well predicted, though the predictions for female mice data showed some systematic overprediction, particularly at late times (i.e., data showed shorter apparent half-life). Blood TCE concentrations were consistently overpredicted, sometimes by almost an order of magnitude, except in the case of female mice at 236 ppm, for which predictions were fairly accurate.
Fisher and Allen (1993)	31–36		Predictions for these gavage data were generally fairly accurate. There was a slight tendency to overpredict TCA plasma concentrations, with predictions tending to be worse in the female mice. Blood levels of TCE were adequately predicted, though there was some systematic underprediction at 2–6 hrs after dosing.
Green and Prout (1985)	40		This datum consists of a single measurement of urinary excretion of TCA at 24 hrs as a fraction of dose, from TCA i.v. dosing. The model substantially overpredicts the amount excreted. Whereas Green and Prout (1985) measured 35% excreted at 24 hrs, the model predicts virtually complete excretion at 24 hrs.
Greenberg et al. (1999)	17–18	√	The calibration data included blood TCE, TCOH, and TCA data. Fits to blood TCA and TCOH were adequate, but as with the Fisher et al. (1991) inhalation data, TCE levels were overpredicted (outside the 95% confidence region during and shortly after exposure). As with Abbas and Fisher (1997), there were additional data in the study that was not used in calibration, including blood levels of TCOG and tissue levels of TCE, TCA, TCOH, and TCOG. Tissue levels of TCE were somewhat overpredicted, but generally within the 95% confidence region. TCA levels were adequately predicted, and mostly in or near the interquartile region. TCOH levels were somewhat underpredicted, though within the 95% confidence region. TCOG levels, for which blood served as a surrogate for all tissues, were well predicted in blood and the lung, generally within the interquartile region. However, blood TCOG predictions underpredicted liver and kidney concentrations.

Table A-1. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in mice (continued)

Reference	Simulation #	Calibration data	Discussion
Larson and Bull (1992a)	37–39		Blood TCA predictions were fairly accurate for these data. However, TCE and TCOH blood concentrations were underpredicted by up to an order of magnitude (outside the 95% confidence region). Part of this may be due to uncertain oral dosing parameters. Urinary TCA and TCOG were also generally underpredicted, in some cases outside of the 95% confidence region.
Prout et al. (1985)	19	√	Fits to these data were generally adequate—within or near the interquartile region.
	27–30 (urinary excretion at different doses)		These data consisted of mass balance studies of the amount excreted in urine and exhaled unchanged at doses from 10 to 2,000 mg/kg. TCA excretion was consistently overpredicted, except at the highest dose. TCOG excretion was generally well predicted—within the interquartile range. The amount exhaled was somewhat overpredicted, with a fourfold difference (but still within 95% confidence) at the highest dose.
Templin et al. (1993)	20	√	Blood TCA levels from these data were well predicted by the model. Blood TCE and TCOH levels were well predicted using subject-specific parameters, but did not appear representative using population-derived parameters. However, this is probably a result of the subject-specific oral absorption parameter, which was substantially different than the population mean.

Next, only samples of the population parameters (means and variances) were used, and "new subjects" were sampled from appropriate distributions using these population means and variances. These "new subjects" then represent the predicted population distribution, incorporating both variability in the population as well as uncertainty in the population means and variances. These "population-based" predictions were then compared to both the data used in calibration, as well as the additional data identified that was not used in calibration. The PBPK model was modified to accommodate some of the different outputs (e.g., tissue concentrations) and exposure routes (TCE, TCA, and TCOH i.v.) used in the "noncalibration" data, but otherwise it is unchanged.

A.2.3.1.1.1. Subject-specific predictions and calibration data

(See "Supplementary data for TCE assessment: Hack mouse subject calibration," 2011)

A.2.3.1.1.2. Population-based predictions and calibration and additional evaluation data

(See "Supplementary data for TCE assessment: Hack mouse population calibration evaluation," 2011)

A.2.3.1.2. Conclusions regarding mouse model

A.2.3.1.2.1. TCE concentrations in blood and tissues not well-predicted

The PBPK model for the parent compound does not appear to be robust. Even subject-specific fits to data sets used for calibration were not always accurate. For oral dosing data, there is clearly high variability in oral uptake parameters, and the addition of uptake through the first (stomach) compartment should improve the fit. Unfortunately, inaccurate TCE uptake parameters may lead to inaccurately estimated kinetic parameters for metabolites, TCA and TCOH, even if current fits are adequate.

The TCE data from inhalation experiments also are not well estimated, particularly blood levels of TCE. While fractional uptake has been hypothesized, direct evidence for this is lacking. In addition, physiologic responses to TCE vapors (reduced ventilation rates, lowered body temperature) are a possibility. These are weakly supported by the closed-chamber data, but the amount of the changes is not sufficient to account for the low blood levels of TCE observed in the open-chamber experiments. It is also not clear what role presystemic elimination due to local metabolism in the lung may play. It is known that the mouse lung has a high capacity to metabolize TCE (Green et al., 1997b). However, in the Hack et al. (2006) model, lung metabolism is limited by flow to the tracheobronchial region. An alternative formulation for lung metabolism in which TCE is available for metabolism directly from inhaled air (similar to that used for styrene) (Sarangapani et al., 2003), may allow for greater presystemic elimination of TCE, as well as for evaluating the possibility of wash-in/wash-out effects. Furthermore, the potential impact of other extrahepatic metabolism has not been evaluated. Curiously, predictions

for the tissue concentrations of TCE observed by Greenberg et al. (1999) were not as discrepant as those for blood. A number of these hypotheses could be tested; however, the existing data may not be sufficient to distinguish them. The Merdink et al. (1998) study, in which TCE was given by i.v. (thereby avoiding both first-pass in the liver and any fractional uptake issue in the lung), may be somewhat helpful, but unfortunately only oxidative metabolite concentrations were reported, not TCE concentrations.

A.2.3.1.2.2. **TCA blood concentrations well predicted following TCE exposures, but TCA flux and disposition may not be accurate**

TCA blood and plasma concentrations following TCE exposure are consistently well predicted. However, the total flux of TCA may not be correct, as evidenced by the varying degrees of consistency with urinary excretion data. Of particular importance are TCA dosing studies, none of which were included in the calibration. In these studies, total recovery of urinary TCA was found to be substantially less than the administered dose. However, the current model assumes that urinary excretion is the only source of clearance of TCA, leading to overestimation of urinary excretion. This fact, combined with the observation that under TCE dosing, the model appears to give accurate predictions of TCA urinary excretion for several data sets, strongly suggests a discrepancy in the amount of TCA formed from TCE. That is, since the model appears to overpredict the fraction of TCA that appears in urine, it may be reducing TCA production to compensate. Inclusion of the TCA dosing studies (including some oral dosing studies), along with inclusion of a nonrenal clearance pathway, would probably be helpful in reducing these discrepancies. Finally, improvements in the TCOH/TCOG submodel, below, should also help to ensure accurate estimates of TCA kinetics.

A.2.3.1.2.3. **TCOH/TCOG submodel requires revision and recalibration**

Blood levels of TCOH and TCOG were inconsistently predicted. Part of this is due to the problems with oral uptake, as discussed above. In addition, the problems identified with the use of the Abbas and Fisher (1997) data (i.e., free TCOH vs. total TCOH), mean that this submodel is not likely to be robust.

An additional concern is the overprediction of urinary TCOG from the Abbas et al. (1997) TCOH i.v. data. Like the case of TCA, this indicates that some other source of TCOH clearance (not to TCA or urine—e.g., to DCA or some other untracked metabolite) is possible. This pathway can be considered for inclusion, and limits can be placed on it using the available data.

Also, like for TCA, the fact that blood and urine are relatively well predicted from TCE dosing strongly suggests a discrepancy in the amount of TCOH formed from TCE. That is, since the model appears to overpredict the fraction of TCOH that appears in urine, it may be reducing

TCOH production to compensate. Including the TCOH dosing data would likely be helpful in reducing these discrepancies.

Finally, as with the rat, the model needs to ensure that any first-pass effect is accounted for appropriately. Importantly, the estimated clearance rate for glucuronidation of TCOH is substantially greater than hepatic blood flow. As was shown in Okino et al. (2005), in such a situation, the use of a single compartment model across dose routes will be misleading because it implies a substantial first-pass effect in the liver that cannot be modeled in a single compartment model. That is, since TCOH is formed in the liver from TCE, and TCOH is also glucuronidated in the liver to TCOG, a substantial portion of the TCOH may be glucuronidated before reaching systemic circulation. This suggests that a liver compartment for TCOH is necessary. Furthermore, because substantial TCOG can be excreted in bile from the liver prior to systemic circulation, a liver compartment for TCOG may also be necessary to address that first-pass effect.

The addition of the liver compartment will necessitate several changes to model parameters. The distribution volume for TCOH will be replaced by two parameters: the liver:blood and body:blood partition coefficients. Similarly for TCOG, liver:blood and body:blood partition coefficients will need to be added. Clearance of TCOH to TCA and TCOG can be redefined as occurring in the liver, and urinary clearance can be redefined as coming from the rest of the body. Fortunately, there are substantial data on circulating TCOG that has not been included in the calibration. These data should be extremely informative in better estimating the TCOH/TCOG submodel parameters.

A.2.3.1.2.4. **Uncertainty in estimates of total metabolism**

Closed-chamber data are generally thought to provide a good indicator of total metabolism. Both subject-specific and population-based predictions of the only available closed-chamber data (Fisher et al., 1991) were fairly accurate. Unfortunately, no additional closed-chamber data were available. In addition, the discrepancies in observed and predicted TCE blood concentrations following inhalation exposures remain unresolved. Hypothesized explanations such as fractional uptake or presystemic elimination could have a substantial impact on estimates of total metabolism.

In addition, no data are directly informative as to the fraction of total metabolism in the lung, the amount of "untracked" hepatic oxidative metabolism (parameterized as "FracDCA"), or any other extrahepatic metabolism. The lung metabolism as currently modeled could just as well be located in other extrahepatic tissues, with little change in calibration. In addition, it is difficult to distinguish between untracked hepatic oxidative metabolism and GSH conjugation, particularly at low doses.

A.2.3.2. Rat Model

A.2.3.2.1. Subject-specific and population-based predictions

As with the mouse mode, initially, the sampled subject-specific parameters were used to generate predictions for comparison to the calibration data. Because these parameters were "optimized" for each subject, these "subject-specific" predictions should be accurate by design, and indeed they were, as discussed in more detail in Table A-2.

Next, as with the mouse, only samples of the population parameters (means and variances) were used, and "new subjects" were sampled from appropriate distribution using these population means and variances. These "new subjects" then represent the predicted population distribution, incorporating both variability in the population as well as uncertainty in the population means and variances. These "population-based" predictions were then compared to both the data used in calibration, as well as the additional data identified that were not used in calibration. The Hack et al. (2006) PBPK model used for prediction was modified to accommodate some of the different outputs (e.g., tissue concentrations) and exposure routes (i.v., i.a., and p.v.) used in the "noncalibration" data, but otherwise unchanged.

Table A-2. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in rats

Reference	Simulation #	Calibration data	Discussion
Andersen et al. (1987b)	7–11	√	Good posterior fits were obtained for these data—closed-chamber data with initial concentrations of 100–4,640 ppm.
Barton et al. (1995)	17–20		It was assumed that the closed-chamber volume was the same as for Andersen et al. (1987b). However, the initial chamber concentrations are not clear in the paper. The values that were used in the simulations do not appear to be correct, since in many cases the time-course is inaccurately predicted even at the earliest time-points. Conclusions as to these data need to await definitive values for the initial chamber concentrations, which were not available.
Bernauer et al. (1996)	1–3	√	Urinary time-course data (see Figure 6-7) for TCA, TCOG, and NAcDCVC was given in concentration units (mg/mg creat-hr), whereas total excretion at 48 hrs (see Table 2) was given in molar units (mmol excreted). In the original calibration files, the conversion from concentration to cumulative excretion was not consistent (i.e., the amount excreted at 48 hrs was different). The data were revised using a conversion that forced consistency. One concern, however, is that this conversion amounts to 6.2 mg creatinine over 48 hrs, or 1.14 micromol/hr. This seems very low for rats; Trevisan et al. (2001), in samples from 195 male control rats, found a median value of 4.95 micromol/hr, a mean of 5.39 micromol/hr, and a 1–99th percentile range of 2.56–10.46 micromol/hr. In addition, the NAcDCVC data were revised in include both 1,2- and 2,2-isomers, since the goal of the GSH pathway is primarily to constrain the total flux. Furthermore, because of the extensive interorgan processing of GSH conjugates, and the fact that excretion was still ongoing at the end of the study (48 hrs), the amount of NAcDCVC recovered can only be a lower bound on the amount ultimately excreted in urine. However, the model does not attempt to represent the excretion time-course of GSH conjugates—it merely models the total flux. This is evinced by the fact that the model predicts complete excretion by the first time point of 12 hrs, whereas in the data, there is still substantial excretion occurring at 48 hrs. Posterior fits to these data were poor in all cases except urinary TCA at the highest dose. In all other cases, TCOH/TCOG and TCA excretion was substantially overpredicted, though this is due to the revision of the data (i.e., the different assumptions about creatinine excretion). Unfortunately, of the original calibration data, this is the only one with TCA and TCOH/TCOG urinary excretion. Therefore, that part of the model is poorly calibrated. On the other hand, NAcDCVC was underpredicted for a number of reasons, as noted above. Because of the incomplete capture of NAcDCVC in urine, unless the model can accurately portray the time-course of NAcDCVC in urine, it should probably not be used for calibration of the GSH pathway, except perhaps as a lower bound.

Table A-2. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in rats (continued)

Reference	Simulation #	Calibration data	Discussion
Birner et al. (1993)	21–22		These data only showed urine concentrations, so a conversion was made to cumulative excretion based on an assumed urine flow rate of 22.5 mL/d. Based on this, urinary NAcDCVC was underestimated by 100- to 1,000-fold. Urinary TCA was underestimated by about twofold in females (barely within the 95% CI), and was accurately estimated in males. Note that data on urinary flow rate from Trevisan et al. (2001) in samples from 195 male control rats showed high variability, with a GSD of 1.75, so this may explain the discrepancy in urinary TCA. However, the underestimation of urinary NAcDCVC cannot be explained this way.
Dallas et al. (1991)	23–24		At the lower (50 ppm) exposure, arterial blood concentrations were consistently overpredicted by about 2.5-fold, while at the higher (500 ppm) exposure, arterial blood was overpredicted by 1.5–2-fold, but within the range of variability. Exhaled breath concentrations were in the middle of the predicted range of variability at both exposure levels. The ratio of exhaled breath and arterial blood should depend largely on the blood-air partition coefficient, with minor dependence on the assumed dead space. This suggests the possibility of some unaccounted-for variability in the partition coefficient (e.g., posterior mean estimated to be 15.7; in vitro measured values from the literature are as follows: 25.82 (Sato et al., 1977), 21.9 (Gargas et al., 1989), 25.8 (Koizumi, 1989), 13.2 (Fisher et al., 1989), posterior). Alternatively, there may be a systematic error in these data, since, as discussed below, the fit of the model to the arterial blood data of Keys et al. (2003) was highly accurate.
Fisher et al. (1989)	25–28		Good posterior fits were obtained for these data (in females)—closed-chamber data with initial concentrations from 300 to 5,100 ppm. There was some slight overprediction of chamber concentrations (i.e., data showed more uptake/metabolism) at the lower doses, but still within the 95% CI.
Fisher et al. (1991)	4–6	√	Good posterior fits were obtained from these data—plasma levels of TCA and venous blood levels of TCE.
Green and Prout (1985)	29–30		In naive rats at 500 mg/kg, urinary excretion of TCOH/TCOG and TCA at 24 hrs was underpredicted (twofold), although within the 95% CI. With bile-cannulated rats at the same dose, the amount of TCOG in bile was well within the 95% CI. Urinary TCOH/TCOG was still underpredicted by about twofold, but again still within the 95% CI.
Jakobson et al. (1986)	31		The only data from the experiment (500 ppm in female rats) were venous blood concentrations during exposure. There were somewhat overpredicted at early times (outside of 95% CI for first 30 min) but was well predicted at the termination of exposure. This suggests some discrepancies in uptake to tissues that reach equilibrium quickly—the model approaches the peak concentration at a faster rate than the data suggest.

Table A-2. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in rats (continued)

Reference	Simulation #	Calibration data	Discussion
Kaneko et al. (1994)	32–35		In these inhalation experiments (50–1,000 ppm), urinary excretion of TCOH/TCOG and TCA are consistently overpredicted, particularly at lower doses. The discrepancy decreases systematically as dose increases, with TCA excretion accurately predicted at 1,000 ppm (TCOH/TCOG excretion slightly below near the lower 95% CI at this dose). This suggests a discrepancy in the dose-dependence of TCOH, TCOG, and TCA formation and excretion. On the other hand, venous blood TCE concentrations postexposure are well predicted. TCE blood concentrations right at the end of the exposure are overpredicted; however, concentrations are rapidly declining at this point, so even a few minutes delay in obtaining the blood sample could explain the discrepancy.
Keys et al. (2003)	36–39		These experiments collected extensive data on TCE in blood and tissues following i.a., oral, and inhalation exposures. For the i.a. exposure, blood and tissue concentrations were very well predicted by the model, even with the use of the rapidly perfused tissue concentration as a surrogate for brain, heart, kidney, liver, lung, and spleen concentrations. Similarly accurate predictions were found with the higher (500 ppm) inhalation exposure. At the lower inhalation exposure (50 ppm), there was some minor overprediction of concentrations (twofold), particularly in fat, but values were still within the 95% CIs. For oral exposure, the GI absorption parameters needed to be revised substantially to obtain a good fit. When the values reported by Keys et al. (2003) were used, the model generally had accurate predictions. Two exceptions were the values in the gut and fat in the first 30 min after exposure. In addition, the liver concentration was overpredicted in the first 30 min, and underpredicted at 2–4 hrs, but still within the 95% CI during the entire period.
Kimmerle and Eben (1973b)	40–44		In these inhalation experiments (49–3,160 ppm), urinary excretion of TCOH/TCOG was systematically overpredicted (>twofold; outside 95% CI), while excretion of TCA was accurately predicted. In addition, elimination by exhaled breath was substantially overpredicted at the lowest exposure. Blood TCOH levels were accurately predicted, but blood TCE levels were overpredicted at the 55 ppm. Part of the discrepancies may be due to limited analytic sensitivities at the lower exposures.
Larson and Bull (1992a)	12–14	√	The digitization in the calibration file did not appear to be accurate, as there was a 10-fold discrepancy with the original paper in the TCOH data. The data were replaced this those used by Clewell et al. (2000) and Bois (2000b). Except for the TCOH data, differences between the digitizations were ≤20%. Adequate posterior predictions were obtained for these data (oral dosing from 200 to 3,000 mg/kg). All predictions were within the 95% CI of posterior predictions. Better fits were obtained using subject-specific posterior parameters, for which gut absorption and TCA urinary excretion parameters were more highly identified.

Table A-2. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in rats (continued)

Reference	Simulation #	Calibration data	Discussion
Lash et al. (2006)	45–46		In these corn-oil gavage experiments, almost all of the measurements appeared to be systematically low, sometimes by many orders of magnitude. For example, at the lowest dose (263 mg/kg), urinary excretion of TCOH/TCOG and TCA, and blood concentrations of TCOH were overpredicted by the model by around $>10^5$-fold. TCE concentrations in blood and tissues at 2, 4, and 8 hrs were underpredicted by 10^3- to 10^4-fold. Many studies, including those using the corn oil gavage (Hissink et al., 2002; Green and Prout, 1985), with similar ranges of oral doses show good agreement with the model, it seems likely that these data are aberrant.
Lee et al. (1996)	47–61		This extensive set of experiments involved multiroute administration of TCE (oral, i.v., i.a., or portal vein), with serial measurements of arterial blood concentrations. For the oral route (8–64 mg/kg), the GI absorption parameters had to be modified. The values from Keys et al. (2003) were used, and the resulting predictions were quite accurate, albeit a more prominent peak was predicted. Predictions >30 min after dosing were highly accurate. For the i.v. route (0.71–64 mg/kg), predictions were also highly accurate in almost all cases. At the lower doses (0.71 and 2 mg/kg), there was slight overprediction in the first 30 min after dosing. At highest dose (64 mg/kg), there was slight underprediction between 1 and 2 hrs after dosing. In all cases, the values were within the 95% CI. For the i.a. route (0.71–16 mg/kg), all predictions were very accurate. For the p.v. route (0.7–64 mg/kg), predictions still remained in the 95% CI, although there was more variation. At the lowest dose, there was overprediction in the first 30 min after dosing. At the highest two doses (16 and 64 mg/kg), there was slight underprediction between 1 and 5 hrs after dosing. This may in part be because a pharmacodynamic change in metabolism (e.g., via direct solvent injury proposed by Lee et al., 2000a).
Lee et al. (2000a)	62–69		In the p.v. and i.v. exposures, blood and liver concentrations were accurately predicted. For oral exposures, the GI absorption parameters needed to be changed. While the values from Keys et al. (2003) led to accurate predictions for lower doses (2–16 mg/kg), at the higher doses (48–432 mg/kg), much slower absorption was evident. Comparisons at these higher dose are not meaningful without calibration of absorption parameters.
Prout et al. (1985)	15	√	Adequate posterior fits were obtained for these data—rat dosing at 1,000 mg/kg in corn oil. All predictions were within the 95% CI of posterior predictions. Better fits were obtained using subject-specific posterior parameters, for which gut absorption and TCA urinary excretion parameters were more highly identified.
Stenner et al. (1997)	70		As with other oral exposures, different GI absorption parameters were necessary. Again, the values from Keys et al. (2003) were used, with some success. Blood TCA levels were accurately predicted, while TCOH blood levels were systematically underpredicted (up to 10-fold). Additional data with TCOH and TCA dosing, including naive and bile-cannulated rats, can be added when those exposure routes are added to the model. These could be useful in better calibrating the enterohepatic recirculation parameters.

Table A-2. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in rats (continued)

Reference	Simulation #	Calibration data	Discussion
Templin et al. (1995b)	16	√	Adequate posterior fits were obtained for blood TCA from these data—oral dosing at 100 mg/kg in Tween. Blood levels of TCOH were underpredicted, while the time-course of TCE in blood exhibited an earlier peak. Better fits were obtained using subject-specific posterior parameters, for which gut absorption and TCA urinary excretion parameters (and to a lesser extent glucuronidation of TCOH and biliary excretion of TCOG) were more highly identified.

NAc-1,2-DCVC = N-acetyl-S-(1,2-dichlrovinyl)-L-cysteine; NAc-2,2-DCVC = N-acetyl-S-(2,2-dichlrovinyl)-L-cysteine; NAcDCVC = NAc-1,2-DCVC and NAc-2,2-DCVC.

A.2.3.2.1.1. Subject-specific predictions and calibration data

(See "Supplementary data for TCE assessment: Hack mouse subject calibration," 2011)

A.2.3.2.1.2. Population-based predictions and calibration and additional evaluation data

(See "Supplementary data for TCE assessment: Hack mouse subject calibration," 2011)

A.2.3.2.2. Conclusions regarding rat model

A.2.3.2.2.1. TCE concentrations in blood and tissues generally well-predicted

The PBPK model for the parent compound appears to be robust. Multiple data sets not used for calibration with TCE measurements in blood and tissues were simulated, and overall the model gave very accurate predictions. A few data sets seemed somewhat anomalous—Dallas et al. (1991), Kimmerle and Eben (1973b), and Lash et al. (2006). However, data from Kaneko et al. (1994), Keys et al. (2003), and Lee et al. (2000a; 1996) were all well simulated, and corroborated the data used for calibration (Templin et al., 1995b; Larson and Bull, 1992a; Fisher et al., 1991; Prout et al., 1985). Particularly important is the fact that tissue concentrations from Keys et al. (2003) were well simulated.

A.2.3.2.2.2. Total metabolism probably well simulated, but ultimate disposition is less certain

Closed-chamber data are generally thought to provide a good indicator of total metabolism. Two closed-chamber studies not used for calibration were available—Barton et al. (1995) and Fisher et al. (1989). Additional experimental information is required to analyze the Barton et al. (1995) data, but the predictions for the Fisher et al. (1989) data were quite accurate.

However, the ultimate disposition of metabolized TCE is much less certain. Clearly, the flux through the GSH pathway is not well constrained, with apparent discrepancies between the N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine (NAc-1,2-DCVC) data of Bernauer et al. (1996) and Birner et al. (1993). Moreover, each of these data has limitations—in particular, the Bernauer et al. (1996) data show that excretion is still substantial at the end of the reporting period, so that the total flux of mercapturates has not been collected. Moreover, there is some question as to the consistency of the Bernauer et al. (1996) data (see Table 2 vs. Figures 6 and 7), since a direct comparison seems to imply a very low creatinine excretion rate. The Birner et al. (1993) data only report concentrations—not total excretion—so a urinary flow rate needs to be assumed.

In addition, no data are directly informative as to the fraction of total metabolism in the lung or the amount of “untracked” hepatic oxidative metabolism (parameterized as “FracDCA”). The lung metabolism could just as well be located in other extrahepatic tissues, with little change in calibration. In addition, there is a degeneracy between untracked hepatic oxidative metabolism and GSH conjugation, particularly at low doses.

The ultimate disposition of TCE as excreted TCOH/TCOG or TCA is also poorly estimated in some cases, as discussed in more detail below.

A.2.3.2.2.3. **TCOH/TCOG submodel requires revision and recalibration**

TCOH blood levels of TCOH were inconsistently predicted in noncalibration data sets (well predicted for Larson and Bull (1992a); Kimmerle and Eben (1973b); but not Stenner et al. (1997)] or Lash et al. (2006), and the amount of TCE ultimately excreted as TCOG/TCOH also appeared to be poorly predicted. The model generally underpredicted TCOG/TCOH urinary excretion (underpredicted Green and Prout (1985), overpredicted Kaneko et al. (1994), Kimmerle and Eben (1973b), and Lash et al. (2006)). This may in part be due to discrepancies in the Bernauer et al. (1996) data as to the conversion of excretion relative to creatinine.

Moreover, there are relatively sparse data on TCOH in combination with a relatively complex model, so the identifiability of various pathways—conversion to TCA, enterohepatic recirculation, and excretion in urine—is questionable.

This could be improved by the ability to incorporate TCOH dosing data from Merdink et al. (1999) and Stenner et al. (1997), the latter of which included bile duct cannulation to better estimate enterohepatic recirculation parameters. However, the TCOH dosing in these studies is by the i.v. route, whereas with TCE dosing, TCOH first appears in the liver. Thus, the model needs to ensure that any first-pass effect is accounted for appropriately. Importantly, the estimated clearance rate for glucuronidation of TCOH is substantially greater than hepatic blood flow. That is, since TCOH is formed in the liver from TCE, and TCOH is also glucuronidated in the liver to TCOG, a substantial portion of the TCOH may be glucuronidated before reaching systemic circulation. Thus, suggests that a liver compartment for TCOH is necessary. Furthermore, because substantial TCOG can be excreted in bile from the liver prior to systemic circulation, a liver compartment for TCOG may also be necessary to address that first-pass effect.

The addition of the liver compartment will necessitate several changes to model parameters. The distribution volume for TCOH will be replaced by two parameters: the liver:blood and body:blood partition coefficients. Similarly for TCOG, liver:blood and body:blood partition coefficients will need to be added. Clearance of TCOH to TCA and TCOG can be redefined as occurring in the liver, and urinary clearance can be redefined as coming from the rest of the body.

Finally, additional clearance of TCOH (not to TCA or urine—e.g., to DCA or some other untracked metabolite) is possible. This may in part explain the discrepancy between the accurate predictions to blood data along with poor predictions to urinary excretion (i.e., there is a missing pathway). This pathway can be considered for inclusion, and limits can be placed on it using the available data.

A.2.3.2.2.4. TCA submodel would benefit from revised submodel and incorporating TCA dosing studies

While blood levels of TCA were well predicted in the one noncalibration data set (Stenner et al., 1997), the urinary excretion of TCA was inconsistently predicted (underpredicted in Green and Prout (1985); overpredicted in Kaneko et al. (1994) and Lash et al. (2006); accurately predicted in Kimmerle and Eben (1973b)]). Because TCA is, in part, derived from TCOH, a more accurate TCOH/TCOG submodel would probably improve the TCA submodel.

In addition, there are a number of TCA dosing studies that could be used to isolate the TCA kinetics from the complexities of TCE and TCOH. These could be readily incorporated into the TCA submodel.

Finally, as with TCOH, additional clearance of TCA (not to urine—e.g., to DCA or some other untracked metabolite) is possible. This may in part explain the discrepancy between the accurate predictions to blood data along with poor predictions to urinary excretion (i.e., there is a missing pathway). As with TCOH, this pathway can be considered for inclusion, and limits can be placed on it using the available data.

A.2.3.3. Human Model

A.2.3.3.1. Subject-specific and population-based predictions

As with the mouse and rat models, initially, the sampled subject-specific parameters were used to generate predictions for comparison to the calibration data. Because these parameters were "optimized" for each subject, these "subject-specific" predictions should be accurate by design. However, unlike for the rat, this was not the case for some experiments (this is partially responsible for the slower convergence), although the inaccuracies were generally less than those in the mouse. For example, alveolar air concentrations were systematically overpredicted for several data sets. There was also variability in the ability to predict the precise time-course of TCA and TCOH blood levels, with a few data sets more difficult for the model to accommodate. These data are discussed further in Table A-3. Next, only samples of the population parameters (means and variances) were used, and "new subjects" were sampled from appropriate distribution using these population means and variances. These "new subjects" then represent the predicted population distribution, incorporating both variability as well as uncertainty in the population means and variances. These "population-based" predictions were then compared to both the data used in calibration, as well as the additional data identified that was not used in calibration. The Hack et al. (2006) PBPK model was modified to accommodate some of the different outputs (e.g., arterial blood, intermittently collected urine, retained dose) and exposure routes (TCA i.v., oral TCA, and TCOH) used in the "noncalibration" data, but otherwise unchanged.

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in humans

Reference	Simulation number	Calibration data	Discussion
Bartonicek (1962)	38–45		The measured minute-volume was multiplied by a factor of 0.7 to obtain an estimate for alveolar ventilation rate, which was fixed for each subject. These data are difficult to interpret because they consist of many single data points. It is easiest to go through the measurements one at a time: *Alveolar retention* (1—exhaled dose/inhaled dose during exposure) and *Retained dose* (inhaled dose—exhaled dose during exposure): Curiously, retention was generally underpredicted, which in many cases retained dose was accurately predicted. However, alveolar retention was an adjustment of the observed total retention: TotRet = (CInh – CExh)/CInh = QAlv × (CInh – CAlv)/(MV × CInh), so that AlvRet = TotRet × (QAlv/MV), with QAlv/MV assumed to be 0.7. Because retained dose is the more relevant quantity, and is less sensitive to assumptions about QAlv/MV, then this is the better quantity to use for calibration. *Urinary TCOG*: This was generally underpredicted, although generally within the 95% CI. Thus, these data will be informative as to intersubject variability. *Urinary TCA*: Total collection (at 528 hrs) was accurately predicted, although the amount collected at 72 hrs was generally underpredicted, sometimes substantially so. *Plasma TCA*: Generally well predicted.
Bernauer et al. (1996)	1–3	√	Subject-specific predictions were good for the time-courses of urinary TCOG and TCA, but poor for total urinary TCOG+TCA and for urinary NAc-1,2-DCVC. One reason for the discrepancy in urinary excretion of TCA and TCOG is that the urinary time-course data (see Figures 4-5 in the manuscript) for TCA, TCOG, and NAc-1,2-DCVC was given in concentration units (mg/mg creat-hr), whereas total excretion at 48 hrs (see Table 2 in the manuscript) was given in molar units (mmol excreted). In the original calibration files, the conversion from concentration to cumulative excretion was not consistent (i.e., the amount excreted at 48 hrs was different). For population-based predictions, the data were revised using a conversion that forced consistency. One concern, however, is that this conversion amounts to 400–500 mg creatinine over 48 hrs, or 200–250 mg/d, which seems rather low. For instance, Araki (1978) reported creatinine excretion of 11.5 ± 1.8 mmol/24 hrs (mean ± SD) in nine subjects, corresponding to 1,300 ± 200 mg/d. In addition, for population-based predictions, the data were revised include both the NAc-1,2-DCVC and the N acetyl-S-(2,2-dichlorovinyl)-L-cysteine isomer (the combination denoted NAcDCVC), since the goal of the GSH pathway is primarily to constrain the total flux. Furthermore, because of the extensive interorgan processing of GSH conjugates, and the fact that excretion was still ongoing at the end of the study (48 hrs), the amount of NAcDCVC recovered can only be a lower bound on the amount ultimately excreted in urine. However, the model does not attempt to represent the excretion time-course of GSH conjugates—it merely models the total flux. This is evinced by the fact that the model predicts complete excretion by the first time point of 12 hrs, whereas in the data, there is still substantial excretion occurring at 48 hrs.

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in humans (continued)

Reference	Simulation number	Calibration data	Discussion
Bernauer et al. (1996) (continued)	1–3 (continued)		Population-based posterior fits to these data were quite good for urinary TCA and TCOH, but not for NAcDCVC in urine. Because of the incomplete capture of NAcDCVC in urine, unless the model can accurately portray the time-course of NAcDCVC in urine, it should probably not be used for calibration of the GSH pathway, except perhaps as a lower bound.
Bloemen et al. (2001)	72–75		Like Bartonicek (1962), these data are more difficult to interpret due to their being single data points for each subject and exposure. However, in general, posterior population-based estimates of retained dose, urinary TCOG, and urinary TCA were fairly accurate, staying within the 95% CI, and mostly inside the interquartile range. The data on GSH mercapturates are limited—first they are all nondetects. In addition, because of the 48–56 hrs collection period, excretion of GSH mercapturates is probably incomplete, as noted above in the discussion of Bernauer et al. (1996).
Chiu et al. (2007)	66–71		The measured minute-volume was multiplied by a factor of 0.7 to obtain an estimate for alveolar ventilation rate, which was fixed for each subject. Alveolar air concentrations of TCE were generally well predicted, especially during the exposure period. Postexposure, the initial drop in TCE concentration was generally further than predicted, but the slope of the terminal phase was similar. Blood concentrations of TCE were consistently overpredicted for all subjects and occasions. Blood concentrations of TCA were consistently overpredicted, though mostly staying in the lower 95% confidence region. Blood TCOH (free) levels were generally overpredicted, in many cases falling below the 95% confidence region, though in some cases the predictions were accurate. On the other hand, total TCOH (free+glucuronidated) was well predicted (or even underpredicted) in most cases—in the cases where free TCOH was accurately predicted, total TCOH was underpredicted. The free and total TCOH data reflect the higher fraction of TCOH as TCOG than previously reported (e.g., Fisher et al. (1998) reported no detectable TCOG in blood). Data on urinary TCA and TCOG were complicated by some measurements being saturated, as well as the intermittent nature of urine collection after d 3. Thus, only the nonsaturated measurements for which the time since the last voiding was known were included for direct comparison to the model predictions. Saturated measurements were kept track of separately for comparison, but were considered only rough lower bounds. TCA excretion was generally overpredicted, whether looking at unsaturated or saturated measurements (the latter, would of course, be expected). Urinary excretion of TCOG generally stayed within the 95% confidence range.
Fernandez et al. (1977)			Alveolar air concentrations are somewhat overestimated. Other measurements are fairly well predicted.

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in humans (continued)

Reference	Simulation number	Calibration data	Discussion
Fisher et al. (1998)	13–33	√	The majority of the data used in the calibration (both in terms of experiments and data points) came from this study. In general, the subject-specific fits to these data were good, with the exception of alveolar air concentrations, which were consistently overpredicted. In addition, for some subjects, the shape of the TCOH time-course deviated from the predictions (#14, 24, 29, and 30)—the predicted peak was too "sharp," with underprediction at early times. Simulation #23 showed the most deviation from predictions, with substantial inaccuracies in blood TCA, TCOH, and urinary TCA. Interestingly, in the population-based predictions, in some cases the predictions were not very accurate—indicating that the full range of population variability is not accounted for in the posterior simulations. This is particularly the case with venous blood TCE concentrations, which are generally underpredicted in population estimates (although in some cases the predictions are accurate). One issue with the way in which these data were utilized in the calibration is that in some cases, the same subject was exposed to two different concentrations, but in the calibration, they were treated as separate "subjects." Thus, parameters were allowed to vary between exposures, mixing intersubject and interoccasion variability. It is recommended that in subsequent calibrations, the different occasions with the same subject be modeled together. This will also allow identification of any dose-related changes in parameters (e.g., saturation).
Kimmerle and Eben (1973a)	46–57		Blood TCE levels are generally overpredicted for both single and multiexposure experiments. However, levels at the end of exposure are rapidly changing, so some of those values may be better predicted if the "exact" time after cessation of exposure were known. Blood TCOH levels are fairly accurately predicted, although in some subjects in single exposure experiments, there is a tendency to overpredict at early times and underpredict at late times. In multiexposure experiments, the decline after the last exposure was somewhat steeper than predicted. Urinary excretion of TCA and TCOH was well predicted. Only grouped data on alveolar air concentrations were available, so they were not used.
Laparé et al. (1995)	34	√	Predictions for these data were not accurate. However, there was an error in some of the exposure concentrations used in the original calibration. In addition, the last exposure "occasion" in these experiments involved exercise/workload, and so should be excluded. Finally, subject data are available for these experiments.
	62–65 (individual data)		Taking into account these changes, population-based predictions were somewhat more accurate. However, alveolar air concentrations and venous blood TCE concentrations were still overpredicted.

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in humans (continued)

Reference	Simulation number	Calibration data	Discussion
Monster et al. (1976)	5–6 (summary data)	√	Subject-specific predictions were quite good, except that for blood TCA concentrations exhibited a higher peak that predicted. However, TCOH values were entered as free TCOH, whereas the TCOH data were actually total (free + glucuronidated) TCOH. Therefore, for population-based predictions, this change was made. In addition, as with the Monster et al. (1979a) data, minute-volume and exhaled air concentrations were measured and incorporated for population-based predictions. Finally, subject-specific data are available, so, in this case, those data should replace the grouped data in any revised calibration. These individual data also included estimates of retained dose based on complete inhaled and exhaled air samples during exposure. For population-based predictions, as with the Monster et al. (1979a) data, grouped urinary and blood TCOH/TCOG was somewhat underpredicted in the population-based predictions, and grouped alveolar and blood TCE concentrations were somewhat overpredicted.
	58–61 (individual data)		The results for the individual data were similar, but exhibited substantially greater variability that predicted. For instance, in subject A, blood TCOH levels were generally greater than the 95% CI at both 70 and 140 ppm, whereas predictions for blood TCOH in subject D were quite good. In another example, for blood TCE levels, predictions for subject B were quite good, but those for subject D were poor (substantially overpredicted). Thus, it is anticipated that adding these individual data will be substantially informative as to intersubject variability, especially since all four individuals were exposed at two different doses.
Monster et al. (1979a)	4	√	Subject-specific predictions for these data were quite good. However, TCA values were entered as plasma, whereas the TCA data were actually in whole blood. Therefore, for population-based predictions, this change was made. In addition, two additional time-courses were available that were not used in calibration: exhaled air concentrations and total TCOH blood concentrations. These were added for population-based predictions. In addition, the original article had data on ventilation rate, which as incorporated into the model. The minute volume needed to be converted to alveolar ventilation rate for the model, but this required adjusted for an extra dead space volume of 0.15 L due to use of a mask, as suggested in the article. The measured mean minute volume was 11 L/min, and with a breathing rate of 14 breaths/min (assumed in the article), this corresponding to a total volume of 0.79 L. Subtracting the 0.15 L of mask dead space and 0.15 L of physiological dead space (suggested in the article) gives 0.49 L of total physiological dead space. Thus, the minute volume of 11 L/min was adjusted by the factor 0.49/0.79 to give an alveolar ventilation rate of 6.8 L/min, which is a reasonably typical value at rest. Due to extra nonphysiological dead space issue, some adjustment to the exhaled air predictions also needed to be made. The alveolar air concentration CAlv was, therefore, estimated based on the formula

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in humans (continued)

Reference	Simulation number	Calibration data	Discussion
Monster et al. (1979a) (continued)	4 (continued)		CAlv = (CExh × VTot – CInh × VDs)/VAlv where CExh is the measured exhaled air concentration, VTot is the total volume (alveolar space VAlv of 0.49 L, physiological dead space of 0.15 L, and mask dead space of 0.15 L), VDs is the total dead space of 0.3 L, and CInh is the inhaled concentration. Population-based predictions for these data lead to slight underestimation urinary TCOG and blood TCOH levels, as well as some overprediction of alveolar air and venous blood concentrations by factors of 3~10-fold.
Muller et al. (1975; 1974, 1972)	7–10	√	Subject-specific predictions for these data were good, except for alveolar air concentrations. However, several problems were found with these data as utilized in the original calibration: • Digitization problems, particular with the time axis in the multiday exposure study (Simulation 9) that led to measurements taken prior to an exposure modeled as occurring during the exposure. The original digitization from Bois (2000b) and Clewell et al. (2000) was used for population-based estimates. • Original article showed TCA as measured in plasma, not blood as was assumed in the calibration. • Blood was taken from the earlobe, which is thought to be indicative of arterial blood concentrations, rather than venous blood concentrations. • TCOH in blood was free, not total, as Ertle et al. (1972) (cited in Methods) had no use of β-glucuronidase in analyzing blood samples. Separate free and total measurements were done in plasma (not whole blood), but these data were not included. • Simulation 9, contiguous data on urinary excretion were only available out to 6 d, so only that data should be included. • Simulation 10, is actually the same as the first day of simulation 9, from Muller et al. (1975; 1972) (the data were reported in both papers), and, thus, should be deleted. These were corrected in the population-based estimates. Alveolar air concentration measurements remained overpredicted, while the change to arterial blood led to overprediction of those measurements during exposure (but postexposure predictions were accurate).
Muller et al. (1974)	81–82 (TCA and TCOH dosing)		The experiment with TCA showed somewhat more rapid decline in plasma levels than predicted, but still well within the 95% confidence range. Urinary excretion was well predicted, but only accounted for 60% of the administered dose—this is not consistent with the rapid decline in TCA plasma levels (10-fold lower than peak at the end of exposure), which would seem to suggest the majority of TCA has been eliminated. With TCOH dosing, blood levels of TCOH were overpredicted in the first 5 hrs, perhaps due to slower oral absorption (the augmented model used instantaneous and complete absorption). TCA plasma and urinary excretion levels were fairly well predicted. However, urinary excretion of TCOG was near the bottom of the 95% CI; while, in the same individuals with TCE dosing (Simulation 7), urinary excretion of TCOG was substantially greater (near slightly above the interquartile region). Furthermore, total TCA and TCOG urinary excretion accounted for <40% of the administered dose.

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for in vivo data in humans (continued)

Reference	Simulation number	Calibration data	Discussion
Paykoc and Powell (1945)	35–37		Population-based fits were good, within the inner quartile region.
Sato et al. (1977)	76		Both alveolar air and blood concentrations are overpredicted in this model. Urinary TCA and TCOG, on the other hand, are well predicted.
Stewart et al. (1970)	11	√	Subject-specific predictions for these data were good, except for some alveolar air concentrations. However, a couple of problems were found with these data as utilized in the original calibration: • The original article noted that individuals took a lunch break during which there was no exposure. This was not accounted for in the calibration runs, which a assumed a continuous 7-hr exposure. The exposures were, therefore, revised with a 3-hr morning exposure (9–12), a 1 hr lunch break (12–1), and 4-hr afternoon exposure (1–5), to mimic a typical workday. The times of the measurements had to be revised as well, since the article gave "relative" rather than "absolute" times (e.g., *x* hr postexposure). • Contiguous data on urinary excretion were only available out to 11 d, so only that data should be included (see Table 2). With these changes, population-based predictions of urinary TCA and TCOG were still accurate, but alveolar air concentrations were overpredicted.
Triebig et al. (1976)	12	√	Only two data points are available for alveolar air, and blood TCA and TCOH. Only one data point is available on blood TCE. Alveolar air was underpredicted at 24 hrs. Blood TCA and TCOH were within the 95% confidence ranges. Blood TCE was overpredicted substantially (outside 95% confidence range).

A.2.3.3.1.1. Subject-specific predictions and calibration data

(See "Supplementary data for TCE assessment: Hack mouse subject calibration," 2011)

A.2.3.3.1.2. Population-based predictions and calibration and additional evaluation data

(See "Supplementary data for TCE assessment: Hack mouse subject calibration," 2011)

A.2.3.3.2. Conclusions regarding human model

A.2.3.3.2.1. TCE concentrations in blood and air are often not well-predicted

Except for the Chiu et al. (2007) during exposure, TCE alveolar air levels were consistently overpredicted. Even in Chiu et al. (2007), TCE levels postexposure were overpredicted, as the drop-off after the end of exposure was further than predicted. Because predictions for retained dose appear to be fairly accurate, this implies that less clearance is occurring via exhalation than predicted by the model. This could be the result of additional metabolism or storage not accounted for by the model.

Except for the Fisher et al. (1998) data, TCE blood levels were consistently overpredicted. Because the majority of the data used for calibration was from Fisher et al. (1998), this implies that the Fisher et al. (1998) data had blood concentrations that were consistently higher than the other studies. This could be due to differences in metabolism and/or distribution among studies.

Interestingly, the mouse inhalation data also exhibited inaccurate prediction of blood TCE levels. Hypotheses such as fractional uptake or presystemic elimination due to local metabolism in the lung have not been tested experimentally, nor is it clear that they can explain the discrepancies.

Due to the difficulty in accurately predicted blood and air concentrations, there may be substantial uncertainty in tissue concentrations of TCE. However, such potential model errors can be characterized estimated and estimated as part of a revised calibration.

A.2.3.3.2.2. TCA blood concentrations well predicted following TCE exposures, but some uncertainty in TCA flux and disposition

TCA blood and plasma concentrations and urinary excretion, following TCE exposure, are generally well predicted. Even though the model's central estimates overpredicted the Chiu et al. (2007) TCA data, the CIs were still wide enough to encompass those data.

However, the total flux of TCA may not be correct, as evidenced by TCA dosing studies, none of which were included in the calibration. In these studies, total recovery of urinary TCA was found to be substantially less than the administered dose. However, the current model assumes that urinary excretion is the only source of clearance of TCA. This leads to overestimation of urinary excretion. This fact, combined with the observation that under TCE

dosing, the model appears to give accurate predictions of TCA urinary excretion for several data sets, strongly suggests a discrepancy in the amount of TCA formed from TCE. That is, since the model appears to overpredict the fraction of TCA that appears in urine, it may be reducing TCA production to compensate. Inclusion of the TCA dosing studies, along with inclusion of a nonrenal clearance pathway, would probably be helpful in reducing these discrepancies. Finally, improvements in the TCOH/TCOG submodel, below, should also help to insure accurate estimates of TCA kinetics.

A.2.3.3.2.3. **TCOH/TCOG submodel requires revision and recalibration**

Blood levels of TCOH and urinary excretion of TCOG were generally well predicted. Additional individual data show substantial intersubject variability than can be incorporated into the calibration. Several errors as to the measurement of free or total TCOH in blood need to be corrected.

A few inconsistencies with noncalibration data sets stand out. The presence of substantial TCOG in blood in the Chiu et al. (2007) data are not predicted by the model. Interestingly, only two studies that included measurements of TCOG in blood (rather than just total TCOH or just free TCOH)—Muller et al. (1975), which found about 17% of total TCOH to be TCOG, and Fisher et al. (1998), who could not detect TCOG. Both of these studies had exposures at 100 ppm. Interestingly, Muller et al. (1975) reported increased TCOG (as fraction of total TCOH) with ethanol consumption, hypothesizing the inhibition of a glucuronyl transferase that slowed glucuronidation. This also would result in a greater half-life for TCOH in blood with ethanol consumptions, which was observed.

An additional concern is the overprediction of urinary TCOG following TCOH administration from the Muller et al. (1974) data. Like the case of TCA, this indicates that some other source of TCOH clearance (not to TCA or urine—e.g., to DCA or some other untracked metabolite) is possible. This pathway can be considered for inclusion, and limits can be placed on it using the available data.

Also, as for TCA, the fact that blood and urine are relatively well predicted from TCE dosing strongly suggests a discrepancy in the amount of TCOH formed from TCE. That is, since the model appears to overpredict the fraction of TCOH that appears in urine, it may be reducing TCOH production to compensate.

Finally, as with the rat and mice, the model needs to ensure that any first-pass effect is accounted for appropriately. Particularly for the Chiu et al. (2007) data, in which substantial TCOG appears in blood, since TCOH is formed in the liver from TCE, and TCOH is also glucuronidated in the liver to TCOG, a substantial portion of the TCOH may be glucuronidated before reaching systemic circulation. Thus, suggests that a liver compartment for TCOH is necessary. Furthermore, because substantial TCOG can be excreted in bile from the liver prior to systemic circulation, a liver compartment for TCOG may also be necessary to address that

first-pass effect. In addition, in light of the Chiu et al. (2007) data, it may be useful to expand the prior range for the K_M of TCOH glucuronidation.

The addition of the liver compartment will necessitate several changes to model parameters. The distribution volume for TCOH will be replaced by two parameters: the liver:blood and body:blood partition coefficients. Similarly for TCOG, liver:blood and body:blood partition coefficients will need to be added. Clearance of TCOH to TCA and TCOG can be redefined as occurring in the liver, and urinary clearance can be redefined as coming from the rest of the body. Fortunately, there are in vitro partition coefficients for TCOH. It may be important to incorporate the fact that Fisher et al. (1998) found no TCOG in blood. This can be included by having the TCOH data be used for both free and total TCOH (particularly since that is how the estimation of TCOG was made—by taking the difference between total and free).

A.2.3.3.2.4. Uncertainty in estimates of total metabolism

Estimates of total recovery after TCE exposure (TCE in exhaled air, TCA and TCOG in urine) have been found to be only 60–70% (Chiu et al., 2007; Monster et al., 1979a, 1976). Even estimates of total recovery after TCA and TCOH dosing have found 25–50% unaccounted for in urinary excretion (Muller et al., 1974; Paykoc and Powell, 1945). Bartonicek (1962) found some TCOH and TCA in feces, but this was about 10-fold less than that found in urine, so this cannot account for the discrepancy. Therefore, it is likely that additional metabolism of TCE, TCOH, and/or TCA are occurring. Additional metabolism of TCE could account for the consistent overestimation of TCE in blood and exhaled breath found in many studies. However, no data are *directly* informative as to the fraction of total metabolism in the lung, the amount of "untracked" hepatic oxidative metabolism (parameterized as "FracDCA"), or any other extrahepatic metabolism. The lung metabolism as currently modeled could just as well be located in other extrahepatic tissues, with little change in calibration. In addition, it is difficult to distinguish between untracked hepatic oxidative metabolism and GSH conjugation, particularly at low doses.

A.3. PRELIMINARY ANALYSIS OF MOUSE GAS UPTAKE DATA: MOTIVATION FOR MODIFICATION OF RESPIRATORY METABOLISM

Potential different model structures can be investigated using the core PBPK model containing averaged input parameters, since this approach saves computational time and is more efficient when testing different structural hypotheses. This approach is particularly helpful for quick comparisons of data with model predictions. During the calibration process, this approach was used for different routes of exposure and across all three species. For both mice and rats, the closed-chamber inhalation data resulted in fits that were considered not optimal when visually examined. Although closed-chamber inhalation usually combines multiple animals per experiment, and may not be as useful in differentiating between individual and experimental

uncertainty (Hack et al., 2006), closed-chamber data do describe in vivo metabolism and have been historically used to quantify averaged in vivo Michaelis-Menten kinetics in rodents.

There are several assumptions used when combining PBPK modeling and closed-chamber data to estimate metabolism via regression. The key experimental principles require a tight, sealed, or air-closed system where all chamber variables are controlled to known set points or monitored, that is all except for metabolism. For example, the inhalation chamber is calibrated without an animal, to determine normal absorption to the empty system. This empty chamber calibration is then followed with a dead animal experiment, identical in every way to the in vivo exposure, and is meant to account for every factor other than metabolism, which is zero in the dead animal. When the live animal(s) are placed in the chamber, oxygen is provided for, and carbon dioxide accumulated during breathing is removed by absorption with a chemical scrubber. A bolus injection of the parent chemical, TCE, is given and this injection time starts the inhalation exposure. The chemical inside the chamber will decrease with time, as it is absorbed by the system and the metabolic process inside the rodent. Since all known processes contributing to the decline are quantified, except for metabolism, the metabolic parameters can be extracted from the total chamber concentration decline using regression techniques.

The basic structure for the PBPK model that is linked to closed-chamber inhalation data has the same basic structure as described before. The one major difference is the inclusion of one additional equation that accounts for mass balance changes inside the inhalation chamber or system, and connects the chamber with the inhaled and exhaled concentrations breathed in and out by the animal:

$$\frac{dA_{Ch}}{dt} = RATS\left(Q_P\right)\left(C_X - \frac{A_{Ch}}{V_{Ch}}\right) - K_{LOSS}A_{Ch} \qquad \text{(Eq. A-4)}$$

where

$RATS$ = number of animals in the chamber
Q_P = alveolar ventilation rate
C_X = exhaled concentration
A_{Ch} = net amount of chemical inside chamber
V_{Ch} = volume of chamber
K_{LOSS} = loss rate constant to glassware.

An updated model was developed that included updated physiological and chemical-specific parameters as well as GSH metabolism in the liver and kidney, as discussed in Chapter 3. The PBPK model code was translated from MCSim to use in Matlab© (version 7.2.0.232, R2006a, Natick, MA) using their m language. This PBPK model made use of fixed or constant, averaged values for physiological, chemical and other input parameters; there were no statistical distributions attached to each average value. As an additional step in quality

control, mass balance was checked for the MCSim code, and comparisons across both sets of code were made to ensure that both sets of predictions were the same.

The resulting simulations were compared to mice gas uptake data (Fisher et al., 1991) after some adjustments of the fat compartment volumes and flows based on visual fits, and limited least-squares optimization of just V_{MAX} (different for males and females) and K_M (same for males and females). The results are shown in the top panels of Figures A-3 and A-4, which showed poor fits particularly at lower chamber concentrations. In particular, metabolism is observed to be faster than predicted by simulation. This is directly related to metabolism of TCE being limited by hepatic blood flow at these exposures. Indeed, Fisher et al. (1991) was able to obtain adequate fits to these data by using cardiac output and ventilation rates that were about twofold higher than is typical for mice. Although their later publication reporting inhalation experiments (Greenberg et al., 1999) used the lower values from Brown et al. (1997) for these parameters, they did not revisit the Fisher et al. (1991) data with the updated model. In addition, the Hack et al. (2006) model estimated the cardiac output and ventilation rate and for these experiments to be about twofold higher than typical. However, it seems unlikely that cardiac output and ventilation rate were really as high as used in these models, since TCE and other solvents typically have CNS-depressing effects. In the mouse, after the liver, the lung has the highest rate of oxidative metabolism, as assessed by in vitro methods (see footnote in Section 3.5.4.2 for a discussion of why kidney oxidative metabolism is likely to be minor quantitatively). In addition, TCE administered via inhalation is available to the lung directly, as well as through blood flow. Therefore, it was hypothesized that a more refined treatment of respiratory metabolism may be necessary to account for the additional metabolism.

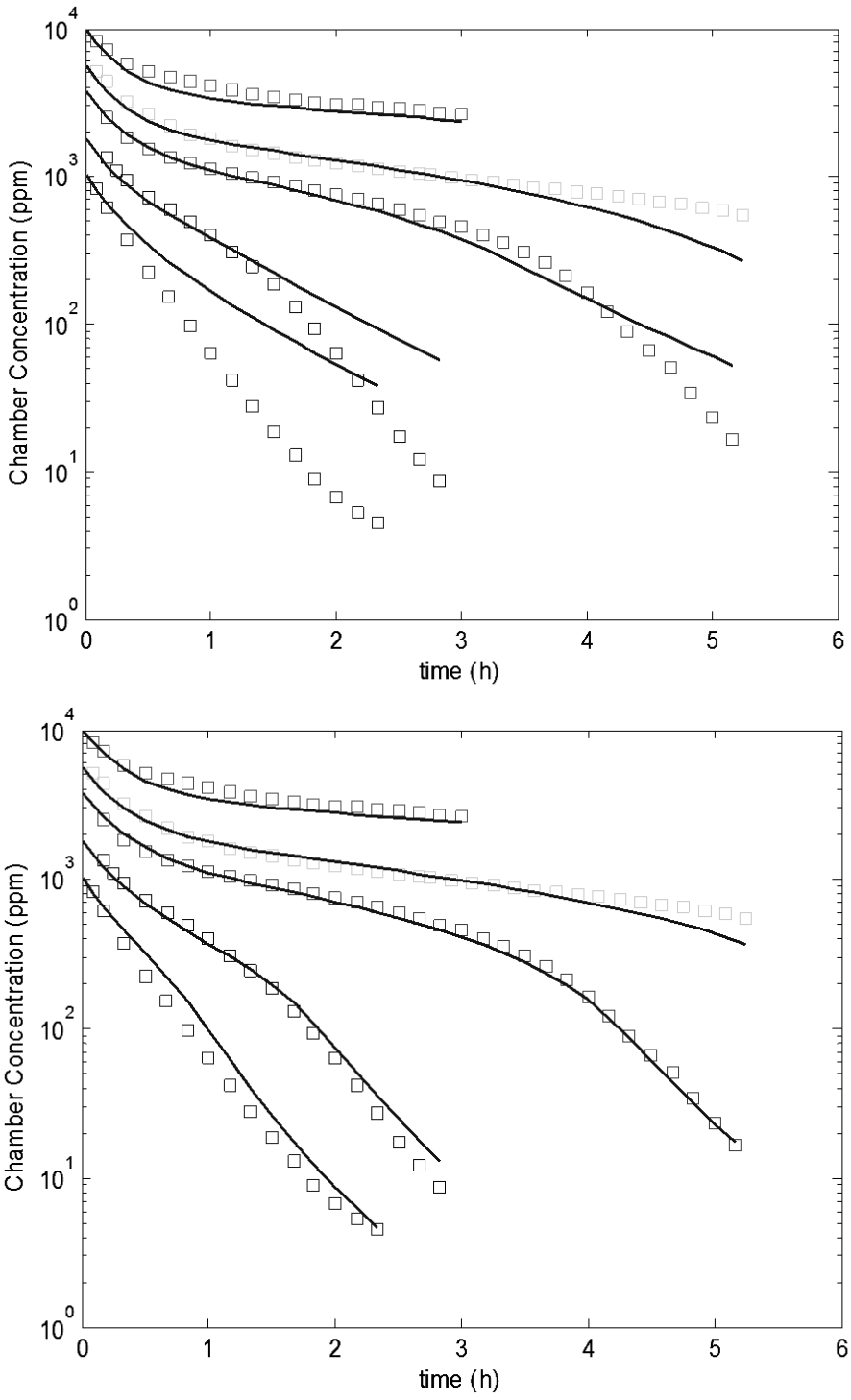


Figure A-3. Limited optimization results for male closed-chamber data from Fisher et al. (1991) without (top) and with (bottom) respiratory metabolism.

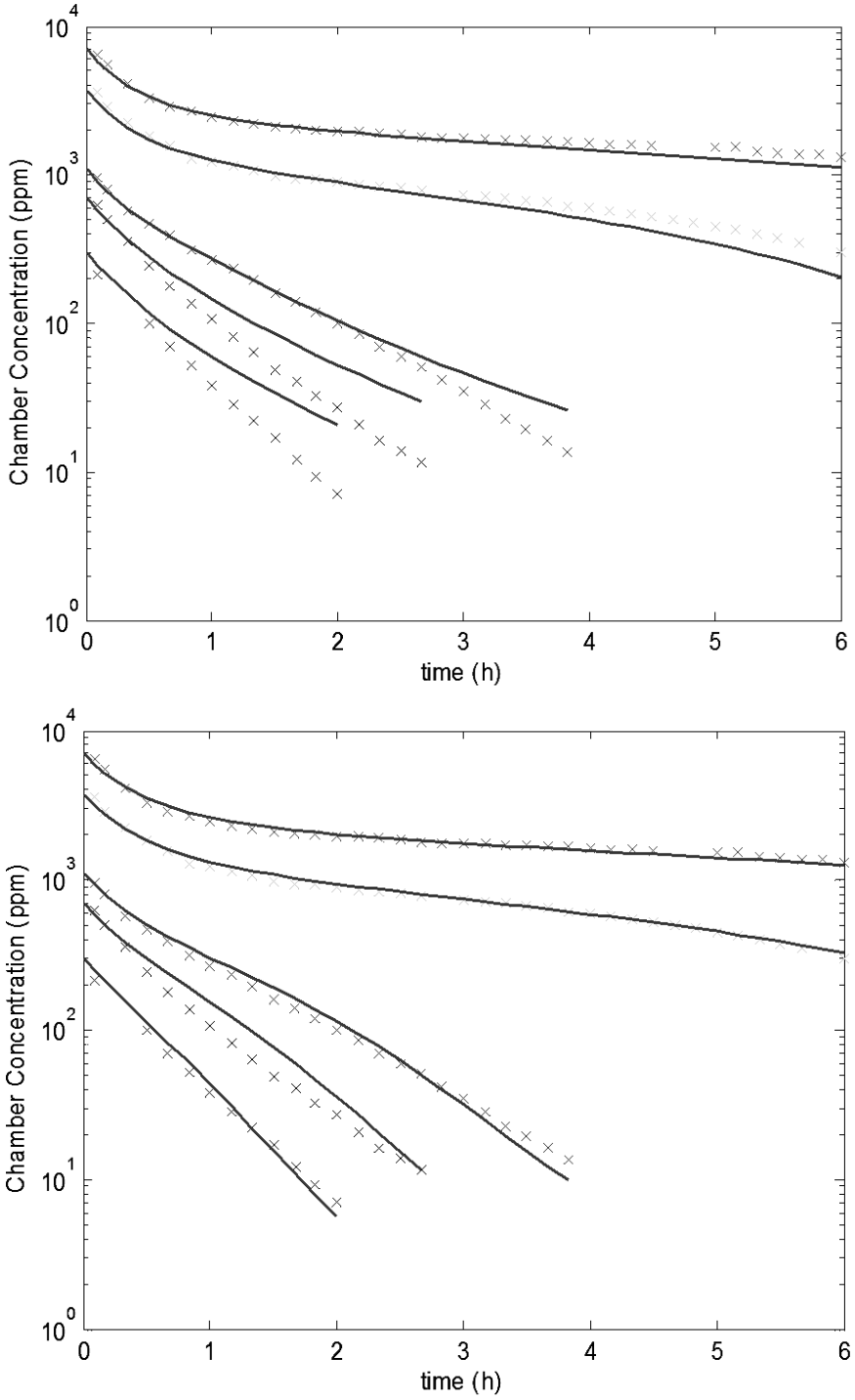


Figure A-4. Limited optimization results for female closed-chamber data from Fisher et al. (1991) without (top) and with (bottom) respiratory metabolism.

The structure of the updated respiratory metabolism model is shown in Figure A-5, with the mathematical formulation shown in the model code in Section A.6, where the "D" is the diffusion rate, "concentrations" and "amounts" are related by the compartment volume, and the other symbols have their standard meanings in the context of PBPK modeling. In brief, this is a more highly "lumped" version of the Sarangapani et al. (2003) respiratory metabolism model for styrene combined with a "continuous breathing" model to account for a possible wash-in/wash-out effect. In brief, upon inhalation (at a rate equal to the full minute volume, not just the alveolar ventilation), TCE can either: (1) diffuse between the respiratory tract lumen and the respiratory tract tissue; (2) remain in the dead space; or (3) enter the gas exchange region. In the respiratory tract tissue, TCE can either be "stored" temporarily until exhalation, during which it diffuses to the "exhalation" respiratory tract lumen, or be metabolized. In the dead space, TCE is transferred directly to the "exhalation" respiratory tract lumen at a rate equal to the minute-volume minus the alveolar ventilation rate, where it mixes with the other sources. In the gas exchange region, it undergoes transfer to and from blood, as is standard for PBPK models of volatile organics. Therefore, if respiratory metabolism is absent (V_{MAX}Clara = 0), then the model reduces to a wash-in/wash-out effect where TCE is temporarily adsorbed to the respiratory tract tissue, the amount of which depends on the diffusion rate, the volume of the tissue, and the partition coefficients.

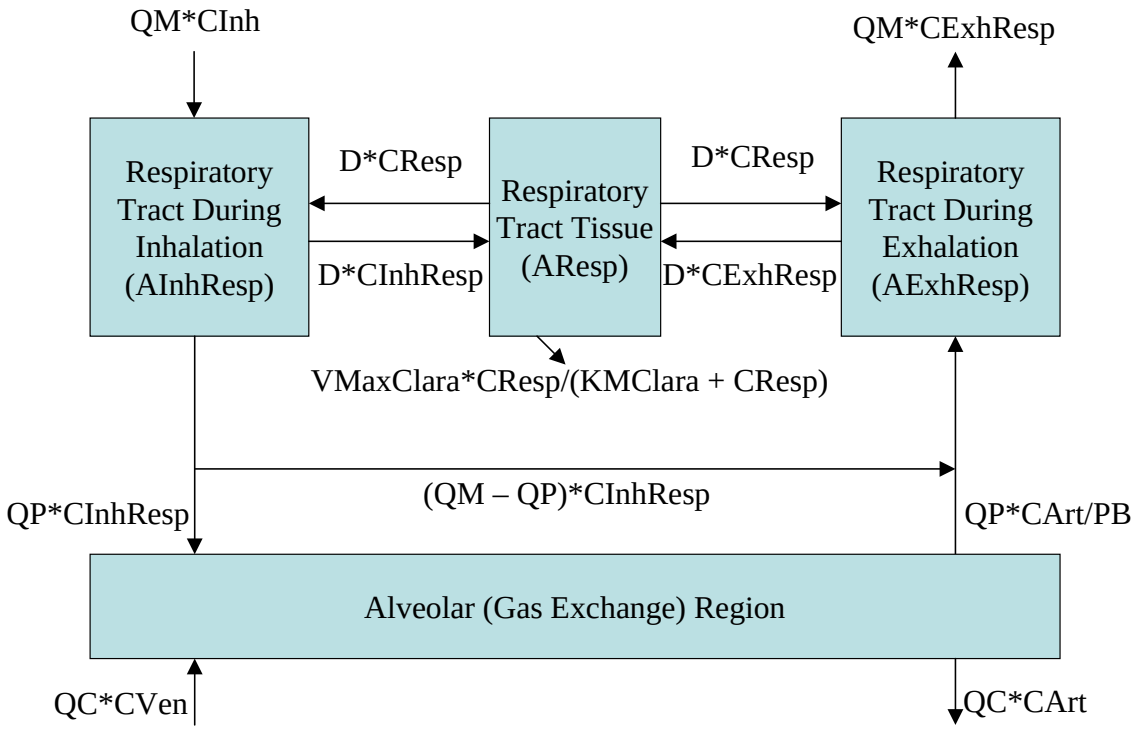


Figure A-5. Respiratory metabolism model for updated PBPK model.

The results of the same limited optimization, now with additional parameters V_{MAX}Clara, K_MClara, and D being estimated simultaneously with the hepatic V_{MAX} and K_M, are shown in the bottom panels of Figures A-2 and A-3. The improvement in the model fits is obvious, and these results served as a motivation to include this respiratory metabolism model for analysis by the more formal Bayesian methods.

A.4. DETAILS OF THE UPDATED PBPK MODEL FOR TCE AND ITS METABOLITES

The structure of the updated PBPK model and the statistical population model are shown graphically in Chapter 3, with the model code shown below in Section A.7. Details as to the model structure, equations, and parameter values and prior distributions are given below.

A.4.1. PBPK Model Structure and Equations

The equations below, along with the parameters defined in Table A-4, specify the PBPK model. The ordinary differential equations are shown in **bold**, with the remaining equations being algebraic definitions. The same equations are in the PBPK model code, with some additional provisions for unit conversions (e.g., ppm to mg/L) or numerical stability (e.g., truncating small values at 10^{-15}, so states are never negative). For reference, the stoichiometric adjustments for molecular weights are given by the following:

```
# Molecular Weights
        TCE:        MWTCE = 131.39
        DCVC:       MWDCVC = 216.1
        TCA:        MWTCA = 163.5
        TCOH:       MWTCOH = 149.5
        TCOG:       MWTCOHGluc = 325.53
        NAcDCVC:    MWNADCVC = 258.8
# Stoichiometry
        StochTCATCE = MWTCA/MWTCE;
        StochTCATCOH = MWTCA/MWTCOH;
        StochTCOHTCE = MWTCOH/MWTCE;
        StochGlucTCOH = MWTCOHGluc/MWTCOH;
        StochTCOHGluc = MWTCOH/MWTCOHGluc;
        StochTCEGluc = MWTCE/MWTCOHGluc;
        StochDCVCTCE = MWDCVC/MWTCE;
        StochN = MWNADCVC/MWDCVC;
```

Table A-4. PBPK model parameters, baseline values, and scaling relationships

Parameter	Description (units)	Formula	Baseline value or parameter	Description	Mouse	Rat	Human F/M	Scaling parameter	Sources(s)
Body weight	Body weight (kg)	-	Body weight$_0$	Standard body weight	0.03	0.3	60/70	–	[a]
Flows									
QC	Cardiac output (L/hr)	QC = QCC$_0$ × exp(lnQCC) × body weight$^{0.75}$	QCC$_0$	Cardiac output allometrically scaled	11.6	13.3	16/16	lnQCC	[b]
QP	Alveolar ventilation (L/hr)	QP = QC × VPR$_0$ × exp(lnVPR)	VPR$_0$	Ventilation-perfusion ratio	2.5	1.9	0.96/0.96	lnVPRC	[c]
DResp	Diffusion clearance rate (L/hr)	DResp = QP × exp(lnDRespC)	–	–	–	–	–	lnDRespC	[d]
Physiological blood flows to tissues									
QFat	Blood flow to fat (L/hr)	QFat = QC × QFatC$_0$ × QFatC	QFatC$_0$	Fraction of blood flow to fat	0.07	0.07	0.085/0.05	QFatC	[e]
QGut	Blood flow to gut (L/hr)	QGut = QC × QGutC$_0$ × QGutC	QGutC$_0$	Fraction of blood flow to gut	0.141	0.153	0.21/0.19	QGutC	[e]
QLiv	Hepatic artery blood flow (L/hr)	QLiv = QC × QLivC$_0$ × QLivC	QLivC$_0$	Fraction of blood flow to hepatic artery	0.02	0.021	0.065/0.065	QLivC	[e]
QSlw	Blood flow to slowly perfused tissues (L/hr)	QSlw = QC × QSlwC$_0$ × QSlwC	QSlwC$_0$	Fraction of blood flow to slowly perfused tissues	0.217	0.336	0.17/0.22	QSlwC	[e]
QKid	Blood flow to kidney (L/hr)	QKid = QC × QKidC$_0$ × QKidC	QKidC$_0$	Fraction of blood flow to kidney	0.091	0.141	0.085/0.05	QKidC	[e]
QRap	Blood flow to rapidly perfused tissues (L/hr)	QRap = QC–(QFat + QGut + QLiv + QSlw + QKid)	–	–	–	–	0.21/0.19	–	[e]
FracPlas	Fraction of blood that is plasma	FracPlas = FracPlas$_0$ × FracPlasC	FracPlas$_0$	Fraction of blood that is plasma	0.52	0.53	0.065/0.065	FracPlasC	[f]

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Parameter	Description (units)	Formula	Baseline value or parameter	Description	Mouse	Rat	Human F/M	Scaling parameter	Sources(s)
Physiological volumes									
VFat	Volume of fat (L)	VFat = body weight × $VFatC_0$ × VFatC	$VFatC_0$	Fraction of body weight that is fat	0.07	0.07	0.317/0.199	VFatC	[g]
VGut	Volume of gut (L)	VGut = body weight × $VGutC_0$ × VGutC	$VGutC_0$	Fraction of body weight that is gut	0.049	0.032	0.022/0.02	VGutC	[g]
VLiv	Volume of liver (L)	VLiv = body weight × $VLivC_0$ × VLivC	$VLivC_0$	Fraction of body weight that is liver	0.055	0.034	0.023/0.025	VLivC	[g]
VRap	Volume of rapidly perfused tissues (L)	VRap = body weight × $VRapC_0$ × VRapC	$VRapC_0$	Fraction of body weight that is rapidly perfused	0.1	0.088	0.093/0.088	VRapC	[g]
VRespLum	Volume of respiratory tract lumen (L)	VRespLum = body weight × $VRespLumC_0$ × VRespLumC	$VRespLumC_0$	Respiratory lumen volume as fraction body weight	0.004667	0.004667	0.002386/0.002386	VRespLumC	[g]
VResp	Volume of respiratory tract tissue (L)	VResp = body weight × $VRespC_0$ × VRespC	$VRespC_0$	Fraction of body weight that is respiratory tract	0.0007	0.0005	0.00018/0.00018	VRespC	[g]
VRespEff	Effective air volume of respiratory tract tissue	VRespEff = VResp × PResp × PB	–	–	–	–	–	–	[g]
VKid	Volume of kidney (L)	VKid = body weight × $VKidC_0$ × VKidC	$VKidC_0$	Fraction of body weight that is kidney	0.017	0.007	0.0046/0.0043	VKidC	[g]
VBld	Volume of blood (L)	VBld = body weight × $VBldC_0$ × VBldC	$VBldC_0$	Fraction of body weight that is blood	0.049	0.074	0.068/0.077	VBldC	[g]
VSlw	Volume of slowly perfused tissue (L)	VSlw = body weight × $VperfC_0$ – (VFat + VGut + VLiv + VRap + VResp + VKid + VBld)	$VperfC_0$	Fraction of body weight that is blood perfused	0.8897	0.8995	0.85778/0.8560	–	[g]
VPlas	Volume of plasma (L)	VPlas = FracPlas × VBld	–	–	–	–	–	–	[h]

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Parameter	Description (units)	Formula	Baseline value or parameter	Description	Mouse	Rat	Human F/M	Scaling parameter	Sources(s)
VBod	Volume body for TCA submodel (L)	VBod = VFat + VGut + VRap + VResp + VKid + VSlw	–	–	–	–	–	–	[i]
VBodTCOH	Volume body for TCOH and TCOG submodels (L)	VBodTCOH = VBod + VBld	–	–	–	–	–	–	[j]
TCE distribution/partitioning									
PB	TCE blood-air partition coefficient	$PB=PB_0 \times PBC$	PB_0	TCE blood-air partition coefficient	15	22	9.5	PBC	[k]
PFat	TCE fat-blood partition coefficient	$PFat=PFatC_0 \times \exp(PFatC)$	$PFatC_0$	TCE fat-blood partition coefficient	36	27	67	PFatC	[l]
PGut	TCE gut-blood partition coefficient	$PGut=(PGutC_0) \times \exp(lnPGutC)$	$PGutC_0$	TCE gut-blood partition coefficient	1.9	1.4	2.6	lnPGutC	[m]
PLiv	TCE liver-blood partition coefficient	$PLiv = (PLivC_0) \times \exp(lnPLivC)$	$PLivC_0$	TCE liver-blood partition coefficient	1.7	1.5	4.1	lnPLivC	[n]
PRap	TCE rapidly perfused-blood partition coefficient	$PRap = (PRapC_0) \times \exp(lnPRapC)$	$PRapC_0$	TCE rapidly perfused-blood partition coefficient	1.9	1.3	2.6	lnPRapC	[o]
PResp	TCE respiratory tract tissue-blood partition coefficient	$Presp = (PRespC_0) \times \exp(lnPRespC)$	$PRespC_0$	TCE respiratory tract tissue-blood partition coefficient	2.6	1.0	1.3	lnPRespC	[p]
PKid	TCE kidney-blood partition coefficient	$PKid = (PKidC_0) \times \exp(lnPKidC)$	$PKidC_0$	TCE kidney-blood partition coefficient	2.1	1.3	1.6	lnPKidC	[q]
PSlw	TCE slowly perfused-blood partition coefficient	$PSlw = (PSlwC_0) \times \exp(lnPSlwC)$	$PSlwC_0$	TCE slowly perfused-blood partition coefficient	2.4	0.58	2.1	lnPSlwC	[r]

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Parameter	Description (units)	Formula	Baseline value or parameter	Description	Mouse	Rat	Human F/M	Scaling parameter	Sources(s)
TCA distribution/partitioning									
TCAPlas	TCA blood-plasma concentration ratio	TCAPlas = FracPlas + (1 – FracPlas) × $PRBCPlasTCA_0$ × exp(lnPRBCPlasTCAC)	$PRBCPlasTCA_0$	TCA red blood cell-plasma partition coefficient	0.5	0.5	0.5/0.5	lnPRBCPlasTCAC	[s]
PBodTCA	Free TCA body-plasma partition coefficient	PBodTCA = TCAPlas × $PBodTCAC_0$ × exp(lnPBodTCAC)	$PBodTCAC_0$	Free TCA body-blood partition coefficient	0.88	0.88	0.52	lnPBodTCAC	[t]
PLivTCA	Free TCA liver-plasma partition coefficient	PLivTCA = TCAPlas × $PLivTCAC_0$ × exp(lnPLivTCAC)	$PLivTCAC_0$	Free TCA liver-blood partition coefficient	1.18	1.18	0.66	lnPLivTCAC	[t]
TCA plasma binding									
kDissoc	Protein TCA dissociation constant (microM)	kDissoc = $kDissoc_0$ × exp(lnkDissocC)	$kDissoc_0$	Protein TCA dissociation constant (microM)	107	275	182	lnkDissocC	[u]
BMax	Protein concentration (microM)	BMax = $BMaxkD_0$ × kDissoc × exp(lnBMaxkDC)	$BMaxkD_0$	BMax/kDissoc ratio	0.88	1.22	4.62	lnBMaxkDC	[u]
TCOH and TCOG distribution/partitioning									
PBodTCOH	TCOH body-blood partition coefficient	PBodTCOH = $PBodTCOH_0$ × exp(lnPBodTCOHC)	$PBodTCOH_0$	TCOH body-blood partition coefficient	1.11	1.11	0.91	lnPBodTCOHC	[v]
PLivTCOH	TCOH liver-blood partition coefficient	PBodTCOH = $PLivTCOH_0$ × exp(lnPLivTCOHC)	$PLivTCOH_0$	TCOH liver-blood partition coefficient	1.3	1.3	0.59	lnPLivTCOHC	[v]
PBodTCOG	TCOG body-blood partition coefficient	PBodTCOG = $PBodTCOG_0$ × exp(lnPBodTCOGC)	$PBodTCOG_0$	TCOG body-blood partition coefficient	1.11	1.11	0.91	lnPBodTCOGC	[w]
PLivTCOG	TCOG liver-blood partition coefficient	PBodTCOG = $PLivTCOG_0$ × exp(lnPLivTCOGC)	$PLivTCOG_0$	TCOG liver-blood partition coefficient	1.3	1.3	0.59	lnPLivTCOGC	[w]
DCVG distribution/partitioning									
VDCVG	DCVG distribution volume (L)	VDCVG = VBld + (VBod+VLiv) × exp(lnPeffDCVG)	–	–	–	–	–	lnPeffDCVG	[x]

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Parameter	Description (units)	Formula	Baseline value or parameter	Description	Mouse	Rat	Human F/M	Scaling parameter	Sources(s)
TCE metabolism									
V_{MAX}	V_{MAX} for TCE hepatic oxidation (mg/hr)	$V_{MAX} = V_{MAX0} \times VLiv \times \exp(lnV_{MAX}C)$	V_{MAX0}	V_{MAX} per kg liver for TCE hepatic oxidation (mg/hr/kg liver)	2,700	600	255	$lnV_{MAX}C$	[y]
KM	KM for TCE hepatic oxidation (mg/L blood)	$KM = KM_0 \times \exp(lnKMC)$ [Mouse and Rat]	KM_0	KM for TCE hepatic oxidation (mg/L)	36	21	–	lnKMC	[y]
		$KM = V_{MAX}/(ClC_0 \times VLiv \times \exp(lnClC))$ [Human]	ClC_0	V_{MAX}/KM per kg liver for TCE hepatic oxidation (L blood/hr/kg liver)	–	–	66	lnClC	[y]
FracOther	Fraction of TCE oxidation not to TCA or TCOH	FracOther = exp(lnFracOtherC)/ (1+exp(lnFracOtherC))	–	–	–	–	–	lnFracOtherC	[z]
FracTCA	Fraction of TCE oxidation to TCA	$FracTCA = (1\text{-}FracOther) \times logitFracTCA_0 \times \exp(lnFracTCAC)/ (1 + logitFracTCA_0 \times \exp(lnFracTCAC))$	$logitFracTCA_0$	Log of ratio of fraction to TCA to fraction not to TCA	0.32	0.32	0.32	lnFracTCAC	[aa]
V_{MAX}DCVG	V_{MAX} for TCE hepatic GSH conjugation (mg/hr)	$V_{MAX}DCVG = V_{MAX}DCVG_0 \times VLiv \times \exp(lnV_{MAX}DCVGC)$ [Mouse and Rat]	$V_{MAX}DCVG_0$	V_{MAX} per kg liver for TCE GSH conjugation (mg/hr/kg liver)	300	66	–	lnV_{MAX}DCVGC	[bb]
		$V_{MAX}DCVG = VLiv \times ClDCVG_0 \times \exp(lnClDCVGC) \times KMDCVG_0 \times \exp(lnKMDCVGC)$ [Human]	$ClDCVG_0$	V_{MAX}/KM per kg liver for TCE GSH conjugation (L blood/hr/kg liver)	–	–	19	lnClDCVGC	[bb]
			$KMDCVG_0$	KM for TCE GSH conjugation (mg/L blood)	–	–	2.9	lnKMDCVGC	[bb]

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Parameter	Description (units)	Formula	Baseline value or parameter	Description	Mouse	Rat	Human F/M	Scaling parameter	Sources(s)
KMDCVG	KM for TCE hepatic GSH conjugation (mg/L blood)	KMDCVG = V_{MAX}DCVG/ ($ClDCVG_0$ × exp(lnClDCVGC) [Mouse and Rat]	$ClDCVG_0$	V_{MAX}/KM per kg liver for TCE hepatic GSH conjugation (L blood/hr/kg liver)	1.53	0.25	–	lnClDCVGC	[bb]
		KMDCVG = $KMDCVG_0$ × exp(lnKMDCVGC) [Human]	$KMDCVG_0$	KM for TCE GSH conjugation (mg/L blood)	–	–	2.9	lnKMDCVGC	[bb]
V_{MAX}KidDCVG	V_{MAX} for TCE kidney GSH conjugation (mg/hr)	V_{MAX}KidDCVG = $V_{MAX}KidDCVG_0$ × VKid × exp(lnV_{MAX}KidDCVGC) [Mouse and Rat]	$V_{MAX}KidDCVG_0$	V_{MAX} per kg kidney for TCE GSH conjugation (mg/hr/kg kidney)	60	6.0	–	lnV_{MAX}KidDCVGC	[bb]
		V_{MAX}KidDCVG = VKid × $ClKidDCVG_0$ × exp(lnClKidDCVGC) × $KMKidDCVG_0$ × exp(lnKMKidDCVGC) [Human]	$ClKidDCVG_0$	V_{MAX}/KM per kg kidney for TCE GSH conjugation (L blood/hr/kg liver)	–	–	230	lnClKidDCVGC	[bb]
			$KMKidDCVG_0$	KM for TCE GSH conjugation (mg/L blood)	–	–	2.7	lnKMKidDCVGC	[bb]
KMKidDCVG	KM for TCE kidney GSH conjugation (mg/L blood)	KMKidDCVG = V_{MAX}KidDCVG/ ($ClKidDCVG_0$ × exp(lnClKidDCVGC) [Mouse and Rat]	$ClKidDCVG_0$	V_{MAX}/KM per kg kidney for TCE kidney GSH conjugation (L blood/hr/kg liver)	0.34	0.026	–	lnClDCVGC	[bb]
		KMKidDCVG = $KMKidDCVG_0$ × exp(lnKMKidDCVGC) [Human]	$KMKidDCVG_0$	KM for TCE GSH conjugation (mg/L blood)	–	–	2.7	lnKMKidDCVGC	[bb]

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Parameter	Description (units)	Formula	Baseline value or parameter	Description	Mouse	Rat	Human F/M	Scaling parameter	Sources(s)
TCE metabolism (respiratory tract)									
KMClara	KM for TCE lung oxidation (mg/L air)	KMClara = exp(lnKMClara)	–	–	–	–	–	–	[cc]
V_{MAX}Clara	V_{MAX} for TCE lung oxidation (mg/hr)	V_{MAX}Clara = V_{MAX} × V_{MAX}LungLiv$_0$ × exp(lnV_{MAX}LungLivC)	V_{MAX}LungLiv$_0$	Ratio of lung to liver total V_{MAX} (mg/hr per mg/hr)	0.07	0.0144	0.0138/ 0.0128	lnV_{MAX}LungLivC	[cc]
FracLungSys	Fraction of respiratory oxidation entering systemic circulation	FracLungSys = exp(lnFracLungSysC)/ (1+exp(lnFracLungSysC))	–	–	–	–	–	lnFracLungSysC	[dd]
TCOH metabolism									
V_{MAX}TCOH	V_{MAX} for TCOH oxidation to TCA (mg/hr)	V_{MAX}TCOH= body weight^{¾} × exp(lnV_{MAX}TCOHC) [Mouse and Rat]	–	–	–	–	–	lnV_{MAX}TCOHC	
		V_{MAX}TCOH = body weight^{¾} × exp(lnClTCOHC + lnKMTCOHC) [Human]	–	–	–	–	–	lnClTCOHC lnKMTCOHC	
KMTCOH	KM for TCOH oxidation to TCA (mg/L air)	KMTCOH = exp(lnKMTCOHC)	–	–	–	–	–	lnKMTCOHC	
V_{MAX}Gluc	V_{MAX} for TCOH glucuroni-dation (mg/hr)	V_{MAX}Gluc = body weight^{¾} × exp(lnV_{MAX}GlucC) [Mouse and Rat]	–	–	–	–	–	lnV_{MAX}GlucC	
		V_{MAX}Gluc = body weight^{¾} × exp(lnClGlucC + lnKMGlucC) [Human]	–	–	–	–	–	lnClGlucC lnKMGlucC	
KMGluc	KM for TCOH glucuroni-dation (mg/L air)	KMGluc = exp(lnKMGlucC)	–	–	–	–	–	lnKMGlucC	
kMetTCOH	Rate constant for TCOH other clearance	kMetTCOH = body weight$^{-¼}$ × exp(lnkMetTCOHC)	–	–	–	–	–	lnkMetTCOHC	

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Parameter	Description (units)	Formula	Baseline value or parameter	Description	Mouse	Rat	Human F/M	Scaling parameter	Sources(s)
	(/hr)								
TCA metabolism/clearance									
kUrnTCA	Rate constant for TCA excretion to urine (/hr)	kUrnTCA = GFR_body weight exp(lnkUrnTCAC) × body weight /VPlas	GFR_body weight	Glomerular filtration rate per kg body weight (L/h/kg)	0.6	0.522	0.108	lnkUrnTCAC	[ee]
kMetTCA	Rate constant for other TCA clearance (/hr)	kMetTCA = body weight$^{-¼}$ × exp(lnkMetTCAC)	–	–	–	–	–	lnkMetTCAC	
TCOG metabolism/clearance									
kBile	Rate constant for other TCOG excretion to bile (/hr)	kBile = body weight$^{-¼}$ × exp(lnkBileC)	–	–	–	–	–	lnkBileC	
kEHR	Rate constant for other bile TCOG reaborption as TCOH (/hr)	kEHR = body weight$^{-¼}$ × exp(lnkEHRC)	–	–	–	–	–	lnkEHRC	
kUrnTCOG	Rate constant for TCOH excretion to urine (/hr)	kUrnTCOG = GFR_body weight exp(lnkUrnTCOGC) × body weight/(VBodTCOH × PBodTCOG)	GFR_body weight	Glomerular filtration rate per kg body weight (L/hr/kg)	0.6	0.522	0.108	lnkUrnTCOGC	[ee]
DCVG metabolism									
kDCVG	Rate constant for DCVC formation from DCVG (/hr)	kDCVG = body weight$^{-¼}$ × exp(lnkDCVGC)						lnkDCVGC	[ff]
kNAT	Rate constant for urinary excretion of NAcDCVC (/hr)	kNAT = body weight$^{-¼}$ × exp(lnkNATC)	–	–	–	–	–	lnkNATC	[gg]
kBioact	Rate constant for other bio-activation of DCVC (/hr)	kKidBioact = body weight$^{-¼}$ × exp(lnkKidBioactC)	–	–	–	–	–	lnkKidBioactC	[gg]

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Parameter	Description (units)	Formula	Baseline value or parameter	Description	Mouse	Rat	Human F/M	Scaling parameter	Sources(s)
Oral uptake/transfer coefficients									
kTSD	TCE gavage stomach-duodenum transfer coefficient (/hr)	kTSD = exp(lnkTSD)	1.4	–	–	–	–	lnkTSD	[hh]
kAS	TCE gavage stomach-absorption coefficient (/hr)	kAS = exp(lnkAS)	1.4	–	–	–	–	lnkAS	[hh]
kAD	TCE gavage duodenum-absorption coefficient (/hr)	kAD = exp(lnkAD)	0.75	–	–	–	–	lnkAD	[hh]
kASTCA	TCA stomach absorption coefficient (/hr)	kASTCA = exp(lnkASTCA)	0.75	–	–	–	–	lnkASTCA	[hh]
kASTCOH	TCOH stomach absorption coefficient (/hr)	kASTCOH = exp(lnkASTCOH)	0.75	–	–	–	–	lnkASTCOH	[hh]

Explanatory note. Unless otherwise noted, the model parameter is obtained by multiplying: (1) the "baseline value" (equals one if not specified); (2) the scaling parameter (or for those beginning with "ln," which are natural-log transformed, exp[lnXX]); and (3) any additional scaling as noted in the second to last column. Unless otherwise noted, all log-transformed scaling parameters have baseline value of 0 (i.e., exp[lnXX] has baseline value of 1) and all other scaling parameters have baseline parameters of 1.

[a]Use measured value if available.
[b]If QP is measured, then scale by QP using VPR. Baseline values are from Brown et al. (1997) (mouse and rat) and International Commission on Radiological Protection (ICRP) Publication 89 (2003) (human).
[c]Use measured QP, if available; otherwise scale by QC using alveolar VPR. Baseline values are from Brown et al. (1997) (mouse and rat) and ICRP Publication 89 (2003) (human).
[d]Scaling parameter is relative to alveolar ventilation rate.
[e]Fat represents adipose tissue only. Gut is the GI tract, pancreas, and spleen (all drain to the portal vein). Slowly perfused tissue is the muscle and skin. Rapidly perfused tissue is the rest of the organs, plus the bone marrow and lymph nodes, the blood flow for which is calculated as the difference between the cardiac output (QC) and the sum of the other blood flows. Baseline values are from Brown et al. (1997) (mouse and rat) and ICRP Publication 89 (2003) (human).
[f]This is equal to 1 minus the hematocrit (measured value used if available). Baseline values from control animals in (Hejtmancik et al., 2002) (mouse and rat) and ICRP Publication 89 (2003) (human).

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

[g]Fat represents adipose tissue only, and the measured value is used, if available. Gut is the GI tract, pancreas, and spleen (all drain to the portal vein). Rapidly perfused tissue is the rest of the organs, plus the bone marrow and lymph nodes, minus the tracheobronchial region. The respiratory tissue volume is tracheobronchial region, with an effective air volume given by multiplying by its tissue:air partition coefficient (= tissue:blood times blood:air). The slowly perfused tissue is the muscle and skin. This leaves a small (10–15% of body weight) unperfused volume that consists mostly of bone (minus marrow) and the GI tract contents. Baseline values are from Brown et al. (1997) (mouse and rat) and ICRP Publication 89 (2003) (human), except for volumes of the respiratory lumen, which are from Sarangapani et al. (2003).
[h]Derived from blood volume using FracPlas.
[i]Sum of all compartments except the blood and liver.
[j]Sum of all compartments except the liver.
[k]Mouse value is from pooling Abbas and Fisher (1997) and Fisher et al. (1991). Rat value is from pooling Sato et al. (1977), Gargas et al. (1989), Barton et al. (1995), Simmons et al. (2002), Koizumi (1989), and Fisher et al. (1989). Human value is from pooling Sato and Nakajima (1979), Sato et al. (1977), Gargas et al. (1989), Fiserova-Bergerova et al. (1984), Fisher et al. (1998), and Koizumi (1989).
[l]Mouse value is from Abbas and Fisher (1997). Rat value is from pooling Barton et al. (1995), Sato et al. (1977), and Fisher et al. (1989). Human value is from pooling Fiserova-Bergerova et al. (1984), Fisher et al. (1998), and Sato et al. (1977).
[m]Value is the geometric mean of liver and kidney (relatively high uncertainty) values.
[n]Mouse value is from Fisher et al. (1991). Rat value is from pooling Barton et al. (1995), Sato et al. (1977), and Fisher et al. (1989). Human value is from pooling Fiserova-Bergerova et al. (1984) and Fisher et al. (1998).
[o]Mouse value is geometric mean of liver and kidney values. Rat value is the brain value from Sato et al. (1977). Human value is the brain value from Fiserova-Bergerova et al. (1984).
[p]Mouse value is the lung value from Abbas and Fisher (1997). Rat value is the lung value from Sato et al. (1977). Human value is from pooling lung values from Fiserova-Bergerova et al. (1984) and Fisher et al. (1998).
[q]Mouse value is from Abbas and Fisher (1997). Rat value is from pooling Barton et al. (1995) and Sato et al. (1977). Human value is from pooling Fiserova-Bergerova et al. (1984) and Fisher et al. (1998).
[r]Mouse value is the muscle value from Abbas and Fisher (1997). Rat value is the muscle value from pooling Barton et al. (1995), Sato et al. (1977), and Fisher et al. (1989). Human value is the muscle value from pooling Fiserova-Bergerova et al. (1984) and Fisher et al. (1998).
[s]Scaling parameter is the effective partition coefficient between red blood cells and plasma. Thus, the TCA blood-plasma concentration ratio depends on the plasma fraction. Baseline value is based on the blood-plasma concentration ratio of 0.76 in rats (Schultz et al., 1999).
[t]In vitro partition coefficients were determined at high concentration, when plasma binding is saturated, so should reflect the free blood:tissue partition coefficient. To get the plasma partition coefficient, the partition coefficient is multiplied by the blood:plasma concentration ratio (TCAPlas). In vitro values were from Abbas and Fisher (1997) in the mouse (used for both mouse and rat) and from Fisher et al. (1998). Body values based on measurements in muscle.
[u]Values are based on the geometric mean of estimates based on data from Lumpkin et al. (2003), Schultz et al. (1999), Templin et al. (1995b; 1993), and Yu et al. (2000). Scaling parameter for B_{MAX} is actually the ratio of B_{MAX}/kD, which determines the binding at low concentrations.
[v]Data are from Abbas and Fisher (1997) in the mouse (used for the mouse and rat) and Fisher et al. (1998) (human).
[w]Used in vitro measurements in TCOH as a proxy, but higher uncertainty is noted.
[x]The scaling parameter (only used in the human model) is the effective partition coefficient for the "body" (nonblood) compartment, so that the distribution volume VDCVG is given by VBld + exp(lnPeffDCVG) × (VBod + VLiv).

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

[y]Baseline values have the following units: for V_{MAX}, mg/hr/kg liver; for K_M, mg/L blood; and for clearance (Cl), L/hr/kg liver (in humans, K_M is calculated from $K_M = V_{MAX}/(\exp(\ln ClC) \times Vliv)$. Values are based on in vitro (microsomal and hepatocellular preparations) from Elfarra et al. (1998), Lipscomb et al. (1998b; 1998c, 1997). Scaling from in vitro data based on 32 mg microsomal protein/g liver and 99 × 106 hepatocytes/g liver (Barter et al., 2007). Scaling of K_M from microsomes were based on two methods: (1) assuming microsomal concentrations equal to liver tissue concentrations and (2) using the measured microsome:air partition coefficient and a central estimate of the blood:air partition coefficient. For K_M from human hepatocyte preparations, the measured hepatocyte:air partition coefficient and a central estimate of the blood:air partition coefficient was used.

[z]Scaling parameter is ratio of "DCA" to "non-DCA" oxidative pathway (where DCA is a proxy for oxidative metabolism not producing TCA or TCOH). Fraction of "other" oxidation is exp(lnFracOtherC)/(1 + exp[lnFracOtherC]).

[aa]Scaling parameter is ratio of TCA to TCOH pathways. Baseline value based on geometric mean of Lipscomb et al. (1998b) using fresh hepatocytes and Bronley-DeLancey et al. (2006) using cryogenically-preserved hepatocytes. Fraction of oxidation to TCA is (1 – FracOther) × exp(lnFracTCAC)/(1 + exp[lnFracTCAC]).

[bb]Baseline values are based on in vitro data. In the mouse and rat, the only in vitro data are at 1 or 2 mM (Lash et al., 1998b; Lash et al., 1995). In most cases, rates at 2 mM were increased over the same sex/species at 1 mM, indicating V_{MAX} has not yet been reached. These data therefore put lower bounds on both V_{MAX} (in units of mg/hr/kg tissue) and clearance (in units of L/hr/kg tissue), so those are the scaling parameters used, with those bounds used as baseline values. For humans, data from Lash et al. (1999a) in the liver (hepatocytes) and the kidney (cytosol) and Green et al. (1997b) (liver cytosol) was used to estimate the clearance in units of L/hr/kg tissue and K_M in units of mg/L in blood.

[cc]Scaling parameter is the ratio of the lung to liver V_{MAX} (each in units of mg/hr), with baseline values based on microsomal preparations (mg/hr/mg protein) assayed at ~1 mM (Green et al., 1997b), further adjusted by the ratio of lung to liver tissue masses (Publication 89, ICRP, 2003; Brown et al., 1997).

[dd]Scaling parameter is the ratio of respiratory oxidation entering systemic circulation (translocated to the liver) to that locally cleared in the lung. Fraction of respiratory oxidation entering systemic circulation is exp(lnFracLungSysC)/(1 + exp[lnFracLungSysC]).

[ee]Baseline parameters for urinary clearance (L/hr) were based on glomular filtration rate per unit body weight (L/hr/kg body weight) from Lin (1995), multiplied by the body weights cited in the study. For TCA, these were scaled by plasma volume to obtain the rate constant (/hr), since the model clears TCA from plasma. For TCOG, these were scaled by the effective distribution volume of the body (VBodTCOH × PBodTCOG) to obtain the rate constant (/hr), since the model clears TCOG from the body compartment.

[ff]Human model only.

[gg]Rat and human models only.

[hh]Baseline value for oral absorption scaling parameter are as follows: kTSD and kAS, 1.4/hr, based on human stomach half time of 0.5 hr; kAD, kASTCA, and kASTCOH, 0.75/hr, based on human small intestine transit time of 4 hrs (Publication 89, ICRP, 2003). These are noted to have very high uncertainty.

A.4.1.1. TCE Submodel

The TCE submodel is a whole-body, flow-limited PBPK model, with gas respiratory exchange, oral absorption, and metabolizing and nonmetabolizing tissues (see Figures A-6 and A-7).

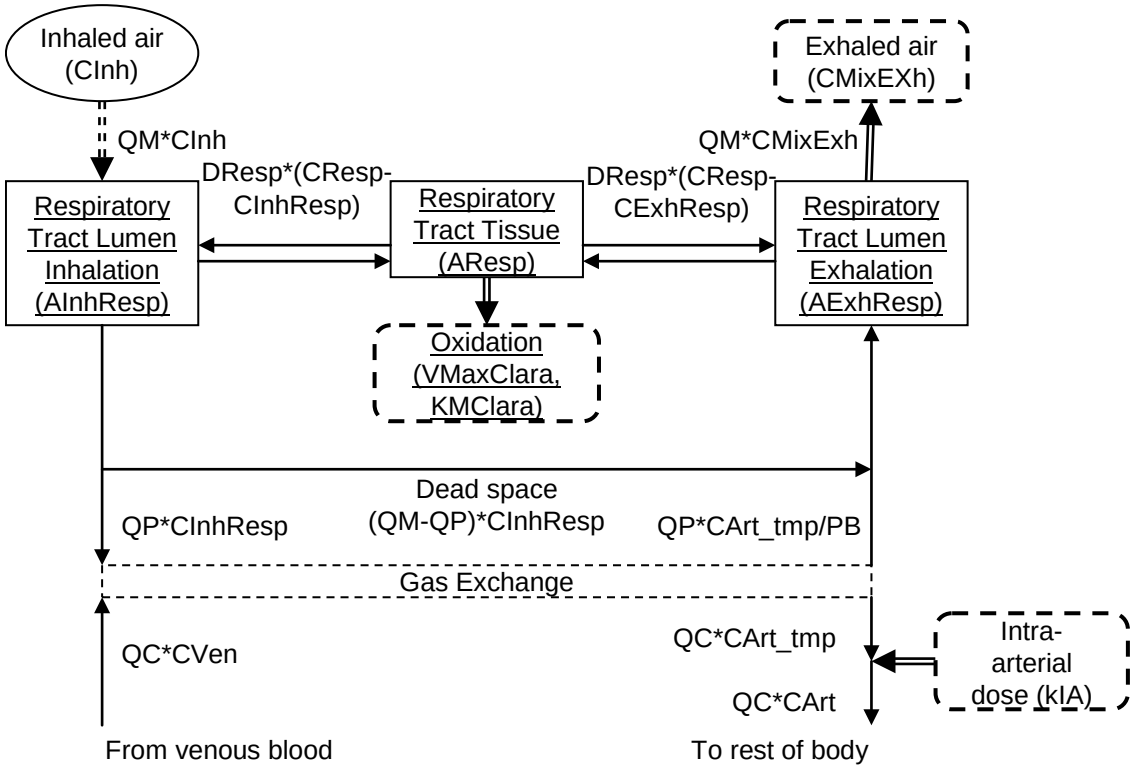


Figure A-6. Submodel for TCE gas exchange, respiratory metabolism, and arterial blood concentration.

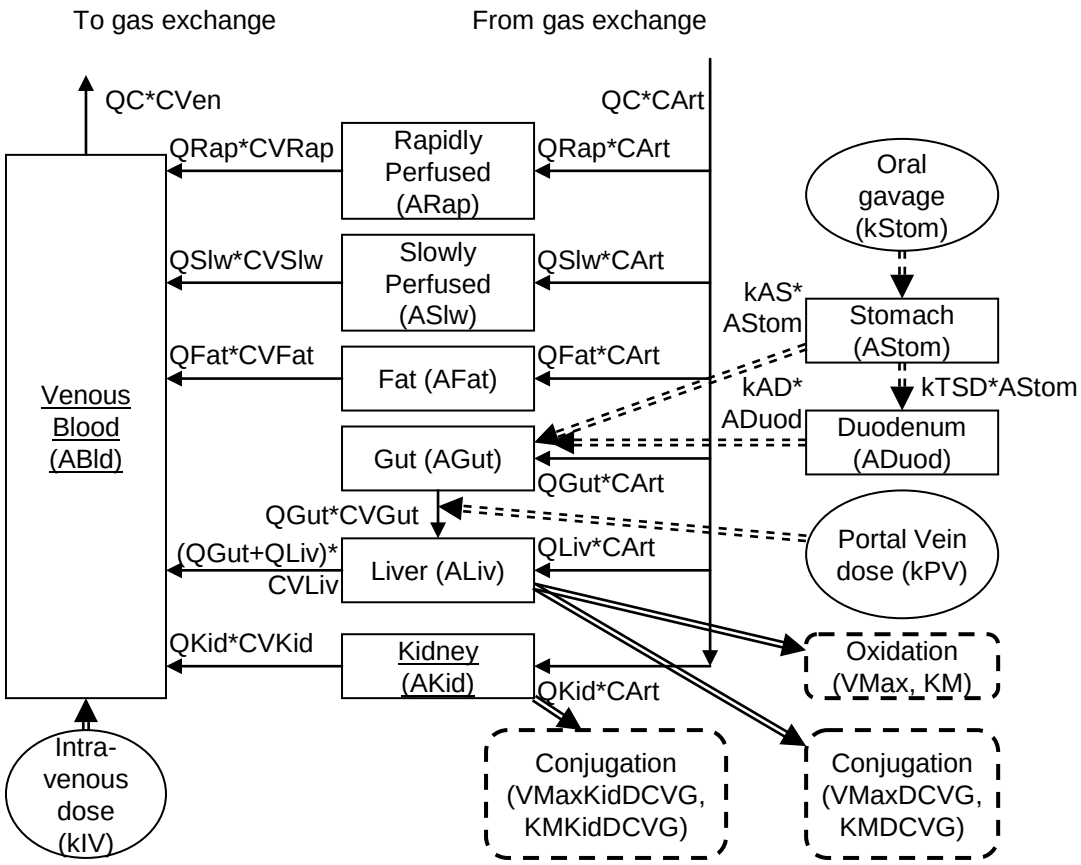


Figure A-7 Submodel for TCE oral absorption, tissue distribution, and metabolism.

A.4.1.1.1. Gas exchange, respiratory metabolism, arterial blood concentration, and closed-chamber concentrations

For an open-chamber concentration and a closed-chamber concentration of ACh/VCh, the rates of change for the amount in the respiratory lumen during inhalation (AInhResp, in mg), the amount in the respiratory tract tissue (AResp, in mg), and the respiratory lumen during exhalation (AExhResp, in mg) are given by the following:

$$d(AInhResp)/dt = (QM \times CInh + DResp \times (CResp - CInhResp) - QM \times CInhResp) \quad \text{(Eq. A-5)}$$

$$d(AResp)/dt = (DResp \times (CInhResp + CExhResp - 2 \times CResp) - RAMetLng) \quad \text{(Eq. A-6)}$$

$$d(AExhResp)/dt = (QM \times (CInhResp - CExhResp) + QP \times (CArt\_tmp/PB-CInhResp) + DResp \times (CResp-CExhResp)) \quad \text{(Eq. A-7)}$$

where

CInh = inhaled concentration (mg/L) = ACh/VCh + Conc
QM = minute volume (L/hour) = QP/0.7
CInhResp = concentration in respiratory lumen during inhalation (mg/L)
= AInhResp/VRespLum
CResp = concentration in respiratory tract tissue (mg/L)
= AResp/VRespEff
CExhResp = concentration in respiratory lumen during exhalation (mg/L)
= AExhResp/VRespLum
RAMetLng = rate of metabolism in respiratory tract tissue
= (V_{MAX}Clara × CResp)/(KMClara + CResp)
CArt_tmp = arterial blood concentration after gas exchange
= (QC × CVen + QP × CInhResp)/(QC + (QP/PB))

Because alveolar breath concentrations can include desorption from the respiratory tract tissue, the concentration at the alveolae (CArt_tmp/PB) may not equal the measured concentration in end-exhaled breath. It is therefore assumed that the ratio of the measured end-exhaled breath concentration to the concentration in the absence of desorption is the same as the ratio of the rate of TCE leaving the lumen to the rate of TCE entering the lumen:

$$CAlv/(CArt\_tmp/PB) = (QM \times CMixExh)/\{(QP \times CArt\_tmp/PB + (QM-QP) \times CInhResp)\} \quad \text{(Eq. A-8)}$$

That is, it is assumed that desorption occurs proportionally throughout the "breath." The concentration of arterial blood entering circulation needs to add the contribution from the i.a. dose (IADose in mg/kg, infused over a time period TChng):

$$CArt = CArt\_tmp + kIA/QC \quad \text{(Eq. A-9)}$$

where

kIA = (IADose × body weight)/TChng

For closed-chamber experiments, the additional differential equation for the amount in the chamber (ACh, in mg) is:

$$\mathbf{d(ACh)/dt = Rodents \times (QM \times CMixExh - QM \times ACh/VCh) - kLoss \times Ach} \quad \textbf{(Eq. A-10)}$$

where rodents is the number of animals in the chamber, and kLoss is the chamber loss rate (per hour).

A.4.1.1.2. Oral absorption to gut compartment

For oil-based gavage, the dose PDose is defined in terms of units of mg/kg, entering the stomach during a time TChng, with rates of change in the stomach (AStom, in mg) and duodenum (ADuod, in mg):

$$\mathbf{d(AStom)/dt = kStom - AStom \times (kAS + kTSD)} \qquad \textbf{(Eq. A-11)}$$

$$\mathbf{d(ADuod)/dt = (kTSD \times AStom) - kAD \times ADuod} \qquad \textbf{(Eq. A-12)}$$

where

kStom = rate of TCE entering stomach (mg/hour) = (PDose × body weight)/TChng

Note that there is absorption to the gut from both the stomach and duodenal compartments. Analogous equations are defined for aqueous gavage, with the expectation that absorption and transfer coefficients would differ with the different vehicle. In particular, the aqueous gavage dose PDoseAq is defined in terms of units of mg/kg, entering the stomach during a time TChng, with rates of change in the stomach (AStomAq, in mg) and duodenum (ADuodAq, in mg):

$$\mathbf{d(AStomAq)/dt = kStomAq - AStomAq \times (kASAq + kTSDAq)} \qquad \textbf{(Eq. A-13)}$$

$$\mathbf{d(ADuodAq)/dt = (kTSDAq \times AStomAq) - kADAq \times ADuodAq} \qquad \textbf{(Eq. A-14)}$$

where

kStomAq = rate of TCE entering stomach (mg/hour) = (PDoseAq × body weight)/TChng

For drinking water, the rate Drink is defined in terms of mg/kg-day, and it is assumed that absorption is direct to the gut:

$$kDrink = (Drink \times body\ weight)/24.0 \qquad \text{(Eq. A-15)}$$

Therefore, the total rate of absorption to the gut via oral exposure (RAO, in mg/hour) is:

$$RAO = kDrink + (kAS \times AStom) + (kAD \times ADuod) + (kASAq \times AStomAq) + (kADAq \times ADuodAq) \qquad \text{(Eq. A-16)}$$

The differential equation for the gut compartment (AGut, in mg) is, therefore, given by:

$$\mathbf{d(AGut)/dt = QGut \times (CArt - CVGut) + RAO} \qquad \textbf{(Eq. A-17)}$$

where

CVGut = concentration in the gut (mg/L) = AGut/VGut/PGut

A.4.1.1.3. Nonmetabolizing tissues

The differential equations for nonmetabolizing tissues (rapidly perfused, ARap, in mg; slowly perfused, ASlw, in mg; and fat, AFat, in mg) follow the standard flow-limited form:

$$\mathbf{d(ARap)/dt = QRap \times (CArt - CVRap)} \qquad \textbf{(Eq. A-18)}$$

$$\mathbf{d(ASlw)/dt = QSlw \times (CArt - CVSlw)} \qquad \textbf{(Eq. A-19)}$$

$$\mathbf{d(AFat)/dt = QFat \times (CArt - CVFat)} \qquad \textbf{(Eq. A-20)}$$

where

CVRap = venous blood concentration leaving rapidly perfused issues
= ARap/VRap/PRap
CVSlw = venous blood concentration leaving slowly perfused issues
= ASlw/VSlw/PSlw
CVFat = venous blood concentration leaving fat
= AFat/VFat/PFat

A.4.1.1.4. Liver compartment

The liver has two metabolizing pathways:

RAMetLiv1 = Rate of TCE oxidation by P450 in liver (mg/hour) (Eq. A-21)
$= (V_{MAX} \times CVLiv)/(KM + CVLiv)$

RAMetLiv2 = Rate of TCE metabolized to S-dichlorovinyl glutathione (DCVG_ in liver (mg/hour)
$= (V_{MAX}DCVG \times CVLiv)\ (KMDCVG + CVLiv)$ (Eq. A-22)

Some experiments also had portal vein dosing (PVDose in mg/kg, infused over a time period TChng), with a rate entering the liver of:

$$kPV = (PVDose \times body\ weight)/TChng \qquad \text{(Eq. A-23)}$$

The differential equation for TCE in liver (ALiv, in mg) is thus:

$$\mathbf{d(ALiv)/dt = (QLiv \times (CArt - CVLiv)) + (QGut \times (CVGut - CVLiv)) - RAMetLiv1 - RAMetLiv2 + kPV} \qquad \textbf{(Eq. A-24)}$$

where

CVLiv = venous blood concentration leaving liver
= ALiv/VLiv/PLiv

A.4.1.1.5. Kidney compartment

The kidney has one metabolizing pathway, GSH conjugation:

RAMetKid = Rate of TCE metabolized to DCVG in kidney (mg/hour) (Eq. A-25)
$= (V_{MAX}KidDCVG \times CVKid)/(KMKidDCVG + CVKid)$

The differential equation for TCE in kidney (AKid, in mg) is thus:

$$\mathbf{d(AKid)/dt = (QKid \times (CArt - CVKid)) - RAMetKid} \qquad \textbf{(Eq. A-26)}$$

where

CVKid = venous blood concentration leaving kidney = AKid/VKid/PKid

A.4.1.1.6. Venous blood compartment

The venous blood compartment (ABld, in mg) has inputs both from the venous blood exiting tissues as well as from an IV dose (IVDose in mg/kg infused during a time TChng), and output to the gas exchange region:

$$d(ABld)/dt = (QFat \times CVFat + QGutLiv \times CVLiv + QSlw \times CVSlw + QRap \times CVRap + QKid \times CVKid) + kIV - CVen \times QC \quad \textbf{(Eq. A-27)}$$

where

kIV = IV infusion rate
= (IVDose × body weight)/TChng

CVen = concentration in mixed venous blood
= ABld/VBld

A.4.1.2. TCOH Submodel

The TCOH submodel is a simplified whole-body, flow-limited PBPK model, with only a body (ABodTCOH, in mg) and liver (ALivTCOH, in mg) compartment (see Figure A-8).

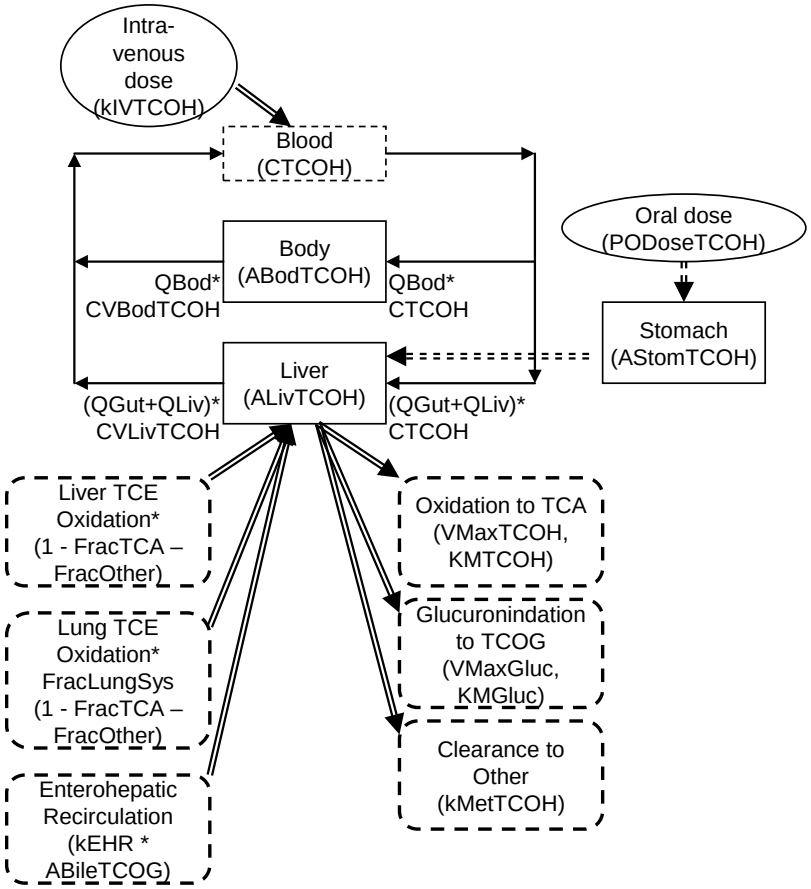


Figure A-8. Submodel for TCOH.

A.4.1.2.1. **Blood concentration**

The venous blood concentration, including an IV dose (IVDoseTCOH in mg/kg infused during a time TChng), is given by

$$CTCOH = (QBod \times CVBodTCOH + QGutLiv \times CVLivTCOH + kIVTCOH)/QC \quad \text{(Eq. A-28)}$$

where

CVBodTCOH = ABodTCOH/VBodTCOH/PBodTCOH
CVLivTCOH = ALivTCOH/VLiv/PLivTCOH
kIVTCOH = IV infusion rate
= (IVDoseTCOH × body weight)/TChng

and the partition coefficients for the body:blood and liver:blood are PBodTCOH and PLivTCOH, respectively, QGutLiv is the sum of the portal vein and hepatic artery blood flows, QBod is the remaining blood flow, VLiv is the liver volume, and VBodTCOH is the remaining perfused volume.

A.4.1.2.2. **Body compartment**

The rate of change of the amount of TCOH in the body compartment is

$$\mathbf{d(ABodTCOH)/dt = QBod \times (CTCOH - CVBodTCOH)} \quad \textbf{(Eq. A-29)}$$

A.4.1.2.3. **Liver compartment**

The liver has three metabolizing pathways:

RAMetTCOHTCA = Rate of oxidation of TCOH to TCA (mg/hour) (Eq. A-30)
$= (V_{MAX}TCOH \times CVLivTCOH)/(KMTCOH + CVLivTCOH)$

RAMetTCOHGluc = Amount of glucuronidation to TCOG (mg/hour) (Eq. A-31)
$= (V_{MAX}Gluc \times CVLivTCOH)/(KMGluc + CVLivTCOH)$

RAMetTCOH = Amount of TCOH metabolized to other (e.g., DCA) (Eq. A-32)
= kMetTCOH × ALivTCOH

Some experiments also had oral dosing (PODoseTCOH in mg/kg, entering the stomach over a time TChng):

$$\mathbf{d(AStomTCOH)/dt = kStomTCOH - AStomTCOH \times kASTCOH} \quad \textbf{(Eq. A-33)}$$

kStomTCOH = (PODoseTCOH × body weight)/TChng; (Eq. A-34)

TCOH PO dose rate into stomach

kPOTCOH = AStomTCOH × kASTCOH; # TCOH oral absorption rate (mg/hour) (Eq. A-35)

In addition, there are three additional sources of TCOH:

Production in the liver from TCE (a fraction of hepatic oxidation) = (1.0 – FracOther – FracTCA) × StochTCOHTCE × RAMetLiv1 (Eq. A-36)

Production in the lung from TCE (a fraction of lung oxidation) = (1.0 – FracOther – FracTCA) × StochTCOHTCE × FracLungSys × RAMetLng (Eq. A-37)

Enterohepatic recirculation (rate kEHR) from TCOG in the bile (amount ABileTCOG) = StochTCOHGluc × RARecircTCOG = StochTCOHGluc × kEHR × ABileTCOG (Eq. A-38)

Note that StochTCOHTCE is the ratio of molecular weights of TCOH and TCE, StochTCOHGluc is the ratio of molecular weights of TCOH and TCOG, FracOther is the fraction of TCE oxidation not producing TCA or TCOH, FracTCA is the fraction of TCE oxidation producing TCA, and FracLungSys is the fraction of lung TCE oxidation that is translocated to the liver and not locally cleared.

The differential equation for TCOH in liver (ALivTCOH, in mg) is thus:

d(ALivTCOH)/dt = kPOTCOH + QGutLiv × (CTCOH – CVLivTCOH)
- RAMetTCOH – RAMetTCOHTCA – RAMetTCOHGluc
+ ((1.0 – FracOther – FracTCA) × StochTCOHTCE
× (RAMetLiv1 + FracLungSys × RAMetLng))
+ (StochTCOHGluc × RARecircTCOG) **(Eq. A-39)**

A.4.1.3. TCOG Submodel

The TCOG submodel is a simplified whole-body, flow-limited PBPK model, with body (ABodTCOG, in mg), liver (ALivTCOG, in mg), and bile (ABileTCOG) compartments (see Figure A-9).

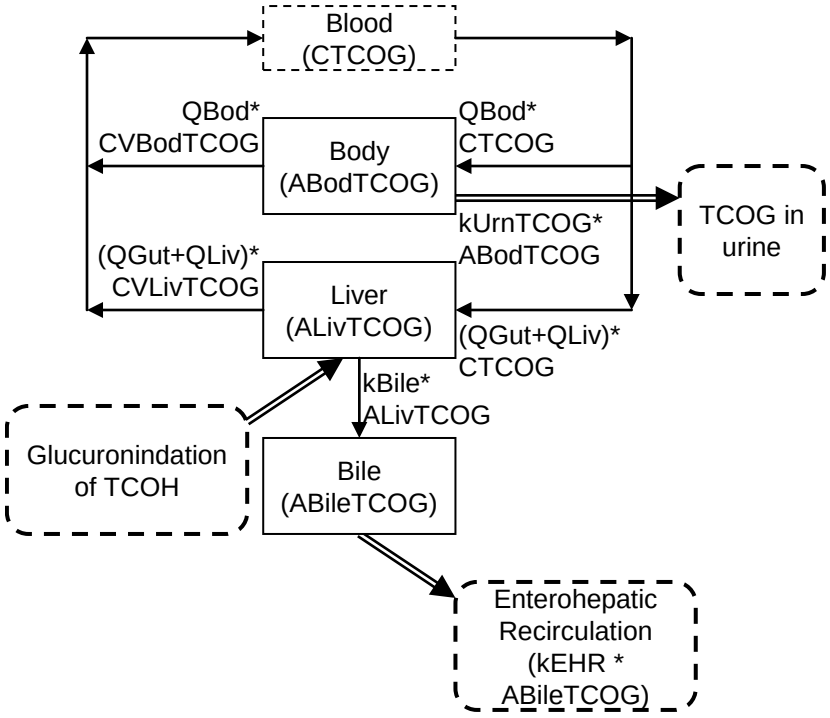


Figure A-9. Submodel for TCOG.

A.4.1.3.1. Blood concentration

The venous blood concentration is given by:

$$CTCOG = (QBod \times CVBodTCOG + QGutLiv \times CVLivTCOG)/QC \quad \text{(Eq. A-40)}$$

where

$CVBodTCOG = ABodTCOG/VBodTCOH/PBodTCOG$
$CVLivTCOG = ALivTCOG/VLiv/PLivTCOG$

and the partition coefficients for the body:blood and liver:blood are PBodTCOG and PLivTCOG, respectively, QGutLiv is the sum of the portal vein and hepatic artery blood flows, QBod is the remaining blood flow, VLiv is the liver volume, and VBodTCOH is the remaining perfused volume.

A.4.1.3.2. Body compartment

The body compartment is flow limited, with urinary excretion rate (mg/hour):

$$RUrnTCOG = kUrnTCOG \times ABodTCOG \quad \text{(Eq. A-41)}$$

So the rate of change of the amount of TCOG in the body compartment is:

$$\mathbf{d(ABodTCOG)/dt = QBod \times (CTCOG - CVBodTCOG) - RUrnTCOG} \quad \textbf{(Eq. A-42)}$$

Thus, the amount excreted in urine (AUrnTCOG, mg) is given by:

$$\mathbf{d(AUrnTCOG)/dt = RUrnTCOG} \qquad \textbf{(Eq. A-43)}$$

A.4.1.3.3. Liver compartment

The liver is flow limited, with one input, glucuronidation of TCOH (defined above in the TCOH submodel):

$$\text{StochGlucTCOH} \times \text{RAMetTCOHGluc} \qquad \text{(Eq. A-44)}$$

and one additional output, excretion in bile:

$$\text{RBileTCOG} = \text{rate of excretion in bile (mg/hour)} = \text{kBile} \times \text{ALivTCOG} \qquad \text{(Eq. A-45)}$$

The rate of change of the amount of TCOG in the liver is, therefore:

$$\mathbf{d(ALivTCOG)/dt = QGutLiv \times (CTCOG - CVLivTCOG) + (StochGlucTCOH \times RAMetTCOHGluc) - RBileTCOG} \qquad \textbf{(Eq. A-46)}$$

A.4.1.3.4. Bile compartment

The bile compartment has one input, excretion of TCOG in bile from the liver (defined above) and one output, enterohepatic recirculation to TCOH in the liver (defined above in the TCOH submodel), with rate of change:

$$\mathbf{d(ABileTCOG)/dt = RBileTCOG - RARecircTCOG} \qquad \textbf{(Eq. A-47)}$$

A.4.1.4. TCA Submodel

The TCA submodel is the same as that in Hack et al. (2006), with an error in the plasma flow to the liver corrected (see Figure A-10). In brief, TCA in plasma is assumed to undergo saturable plasma protein binding. TCA in tissues is assumed to be flow limited, but with the tissue partition coefficient reflecting equilibrium with the free concentration of TCA in plasma.

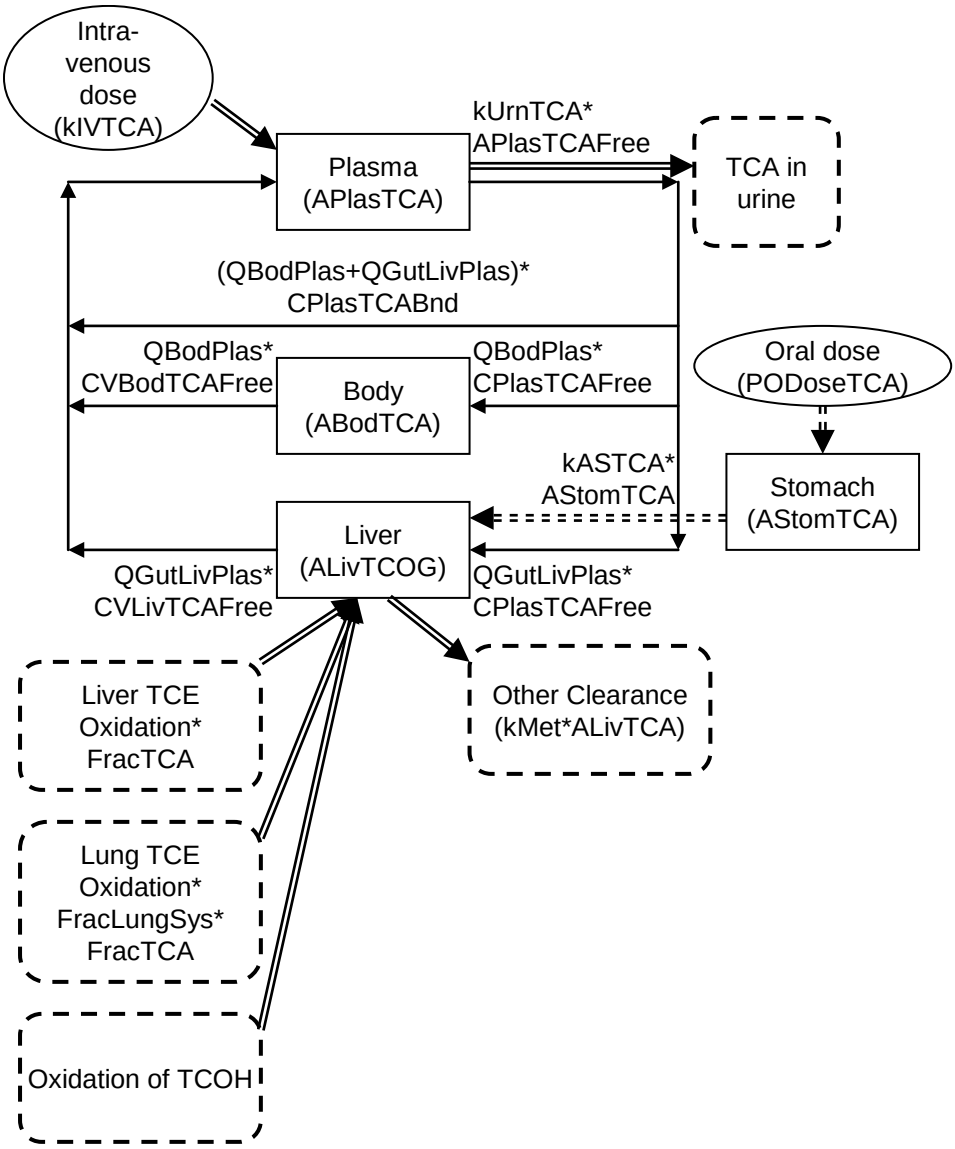


Figure A-10. **Submodel for TCA.**

A.4.1.4.1. Plasma binding and concentrations

For an i.v. dose of TCA given by IVDoseTCA (mg/kg during an infusion period of TChng), the rate of the change of the amount of total TCA in plasma (APlasTCA, in mg) is:

$$\mathbf{d(APlasTCA)/dt = kIVTCA + (QBodPlas \times CVBodTCA) + (QGutLivPlas \times CVLivTCA) - (QCPlas \times CPlasTCA) - RUrnTCAplas} \quad \textbf{(Eq. A-48)}$$

where

kIVTCA	= rate of IV infusion of TCA = (IVDoseTCA × body weight)/TChng
QBodPlas	= plasma flow from body = QBod × FracPlas
QGutLivPlas	= plasma flow from liver = (QGut + QLiv) × FracPlas
CVBodTCA	= venous concentration leaving body = CPlasTCABnd + CVBodTCAFree

CVBodTCAFree = free venous concentration leaving body
= (ABodTCA/VBod/PBodTCA)
CVLivTCA = venous concentration leaving liver
= CPlasTCABnd + CVLivTCAFree
CVLivTCAFree = free venous concentration leaving liver
= (ALivTCA/VLiv/PLivTCA)
QCPlas = total plasma flow
= QC × FracPlas
RUrnTCAplas = rate of urinary excretion of TCA from plasma
= kUrnTCA × APlasTCAFree

The free (CPlasTCAFree) and bound (CPlasTCABnd) concentrations are calculated from the total concentration (CPlasTCA = APlasTCA/VPlas) by solving the equations:

CPlasTCABndMole = BMax × CPlasTCAFreeMole/(kDissoc + CPlasTCAFreeMole) (Eq. A-49)

CPlasTCABndMole = CPlasTCAMole – CPlasTCAFreeMole (Eq. A-50)

Here the suffix "Mole" means that all concentrations are in micromole/L, because BMax and kDissoc in Table A-4 are given in those units. These lead to explicit solutions of:

CPlasTCAFreeMole = (sqrt(a × a + b) – a)/2 (Eq. A-51)

where
a = kDissoc + BMax – CPlasTCAMole
b = 4.0 × kDissoc × CPlasTCAMole
CPlasTCABlasTCAMoleCPlasTCAFreeMole

These concentrations are converted to mg/L (CPlasTCABnd, CPlasTCAFree) by multiplying by the molecular weight in mg/µmoles. The amount of free TCA in plasma is, thus:

APlasTCAFree = CPlasTCAFree × VPlas. (Eq. A-52)

Here, VPlas is derived from the blood volume and hematocrit (see Table A-4).

A.4.1.4.2. **Urinary excretion**

Urinary excretion is modeled as coming from the plasma compartment, so the rate of change of TCA in urine (AUrnTCA, in mg) is:

d(AUrnTCA)/dt = RUrnTCA **(Eq. A-53)**

where
RUrnTCA = RUrnTCAplas

For some human data (Chiu et al., 2007), urinary excretion was only collected during certain time periods, with data missing in other time periods. Thus, a switch UrnMissing was defined, which equals 0 during times of urine collection and 1 when urinary data are missing. The total amount of urinary TCA "collected" (AUrnTCA_collect, in mg) is, thus, given by:

$$\mathbf{d(AUrnTCA\_collect)/dt = (1\text{-}UrnMissing) \times RUrnTCA} \qquad \textbf{(Eq. A-54)}$$

A.4.1.4.3. Body compartment

The body compartment is flow limited, with the rate of change for the amount of TCA in the body (ABodTCA, in mg) given by:

$$\mathbf{d(ABodTCA)/dt = QBodPlas \times (CPlasTCAFree - CVBodTCAFree)} \qquad \textbf{(Eq. A-55)}$$

A.4.1.4.4. Liver compartment

The rate of change for the amount of TCA in the liver (ALivTCA, in mg) is given by:

$$\begin{aligned}\mathbf{d(ALivTCA)/dt} &= \mathbf{QGutLivPlas \times (CPlasTCAFree - CVLivTCAFree)} \qquad \textbf{(Eq. A-56)}\\ &+ \mathbf{(FracTCA \times StochTCATCE \times (RAMetLiv1 + FracLungSys \times RAMetLng))}\\ &+ \mathbf{(StochTCATCOH \times RAMetTCOHTCA) - RAMetTCA + kPOTCA}\end{aligned}$$

The first term reflects the free TCA in plasma flowing into and out of the liver compartment, the second term reflects production of TCA from liver (adjusted for molecular weights and fractional yield of TCA) and lung (adjusted for molecular weights, fraction of lung metabolism translocated to the liver, and fractional yield of TCA) metabolism of TCE, the third term reflects production of TCA from TCOH, the fourth term reflects other clearance of TCA from the liver, and the fifth term reflects absorption from the stomach of TCA. The contribution from liver metabolism of TCE is adjusted for molecular weights and production of oxidative metabolites other than TCA. The rate of clearance of TCA is given by:

$$\text{RAMetTCA} = \text{kMetTCA} \times \text{ALivTCA} \qquad \text{(Eq. A-57)}$$

The oral intake rate of TCA (mg/hour) includes a one-compartment stomach. So for an oral dose of PODoseTCA (in mg/kg), occurring over a time TChng, the rate of change of TCA in the stomach (AStomTCA, in mg) is given by:

$$\mathbf{d(AStomTCA)/dt = kStomTCA - AStomTCA \times kASTCA} \qquad \textbf{(Eq. A-58)}$$

where

kStomTCA = rate of input into stomach
= (PODoseTCA × body weight)/TChng

The rate of absorption into the liver is, thus,

$$kPOTCA = AStomTCA \times kASTCA \quad \text{(Eq. A-59)}$$

A.4.1.5. GSH Conjugation Submodel

The GSH conjugation submodel only tracks DCVG, DCVC, and urinary excretion of NAc-DCVC (see Figure A-11).

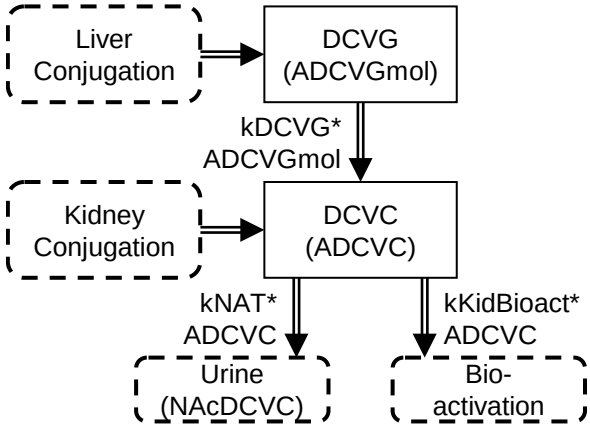


Figure A-11. Submodel for TCE GSH conjugation metabolites.

The rate of change for DCVG (ADCVGmol, in mmoles) depends on production from TCE in the liver and metabolism to DCVC:

$$\mathbf{d(ADCVGmol)/dt = RAMetLiv2/MWTCE - RAMetDCVGmol} \quad \textbf{(Eq. A-60)}$$

where

RAMetDCVGmol = rate of metabolism of DCVG to DCVC
= kDCVG × ADCVGmol

The rate of change of DCVC (ADCVC, in mg) depends on the production from TCE in the kidney (adjusted for molecular weights), production from DCVG, urinary excretion as NAc-DCVC (rate constant kNAT), and other bioactivation (rate constant kKidBioact):

$$\mathbf{d(ADCVC)/dt = RAMetDCVGmol \times MWDCVC + RAMetKid \times StochDCVCTCE - ((kNAT + kKidBioact) \times ADCVC)} \quad \textbf{(Eq. A-61)}$$

where

RAUrnDCVC = Rate of NAcDCVC excretion into urine
= kNAT × ADCVC

The rate of change of the amount of NAc-DCVC excreted (AUrnNDCVC, in mg) is given (adjusted for molecular weights) by:

$$\mathbf{d(AUrnNDCVC)/dt = StochN \times RAUrnDCVC} \qquad \textbf{(Eq. A-62)}$$

For the rat model, the DCVG compartment is "turned off" by setting kDCVG to an arbitrarily high value.

A.4.2. Model Parameters and Baseline Values

The multipage Table A-4 describes all the parameters of the updated PBPK model, their baseline values (which are used as central estimates in the prior distributions for the Bayesian analysis), and any scaling relationship used in their calculation. More detailed notes are included in the comments of the model code (see Section A.7).

A.4.3. Statistical Distributions for Parameter Uncertainty and Variability

A.4.3.1. Initial Prior Uncertainty in Population Mean Parameters

The following multipage Table A-5 describes the initial prior distributions for the population mean of the PBPK model parameters. For selected parameters, rat prior distributions were subsequently updated using the mouse posterior distributions, and human prior distributions were then updated using mouse and rat posterior distributions (see Section A.4.3.2).

A.4.3.2. Interspecies Scaling to Update Selected Prior Distributions in the Rat and Human

As shown in Table A-5, for several parameters, there is little or no in vitro or other prior information available to develop informative prior distributions, so many parameters had lognormal or log-uniform priors that spanned a wide range. Initially, the PBPK model for each species was run with the initial prior distributions in Table A-5, but, in the time available for analysis (up to about 100,000 iterations), only for the mouse did all of these parameters achieve adequate convergence. Additional preliminary runs indicated replacing the log-uniform priors with lognormal priors and/or requiring more consistency between species could lead to adequate convergence. However, an objective method of "centering" the lognormal distributions that did not rely on the in vivo data (e.g., via visual fitting or limited optimization) being calibrated against was necessary in order to minimize potential bias.

Therefore, the approach taken was to consider three species sequentially, from mouse to rat to human, and to use a model for interspecies scaling to update the prior distributions across species (the original prior distributions define the prior bounds). This sequence was chosen because the models are essentially "nested" in this order—the rat model adds to the mouse model the "downstream" GSH conjugation pathways, and the human model adds to the rat model the intermediary DCVG compartment. Therefore, for those parameters with little or no independent

data *only*, the mouse posteriors were used to update the rat priors, and both the mouse and rat posteriors were used to update the human priors. A list of the parameters for which this scaling was used to update prior distributions is contained in Table A-6, with the updated prior distributions. The correspondence between the "scaling parameters" and the physical parameters generally follows standard practice, and were explicitly described in Table A-4. For instance, V_{MAX} and clearance rates are scaled by body weight to the ¾ power, whereas K_M values are assumed to have no scaling, and rate constants (inverse time units) are scaled by body weight to the -¼ power.

Table A-5. Uncertainty distributions for the population mean of the PBPK model parameters

Scaling (sampled) parameter	Mouse			Rat			Human			Notes/ Source
	Distribution[a]	*SD or Min*	*Truncation (± nxSD) or Max*	Distribution	*SD or Min*	*Truncation (± nxSD) or Max*	Distribution	*SD or Min*	*Truncation (± nxSD) or Max*	
Flows										
lnQCC	TruncNormal	0.2	4	TruncNormal	0.14	4	TruncNormal	0.2	4	[a]
lnVPRC	TruncNormal	0.2	4	TruncNormal	0.3	4	TruncNormal	0.2	4	[a]
lnDRespC	Uniform	-11.513	2.303	Uniform	-11.513	2.303	Uniform	-11.513	2.303	[b]
Physiological blood flows to tissues										
QFatC	TruncNormal	0.46	2	TruncNormal	0.46	2	TruncNormal	0.46	2	[a]
QGutC	TruncNormal	0.17	2	TruncNormal	0.17	2	TruncNormal	0.18	2	[a]
QLivC	TruncNormal	0.17	2	TruncNormal	0.17	2	TruncNormal	0.45	2	[a]
QSlwC	TruncNormal	0.29	2	TruncNormal	0.3	2	TruncNormal	0.32	2	[a]
QKidC	TruncNormal	0.32	2	TruncNormal	0.13	2	TruncNormal	0.12	2	[a]
FracPlasC	TruncNormal	0.2	3	TruncNormal	0.2	3	TruncNormal	0.05	3	[c]
Physiological volumes										
VFatC	TruncNormal	0.45	2	TruncNormal	0.45	2	TruncNormal	0.45	2	[a]
VGutC	TruncNormal	0.13	2	TruncNormal	0.13	2	TruncNormal	0.08	2	[a]
VLivC	TruncNormal	0.24	2	TruncNormal	0.18	2	TruncNormal	0.23	2	[a]
VRapC	TruncNormal	0.1	2	TruncNormal	0.12	2	TruncNormal	0.08	2	[a]
VRespLumC	TruncNormal	0.11	2	TruncNormal	0.18	2	TruncNormal	0.2	2	[a]
VRespEffC	TruncNormal	0.11	2	TruncNormal	0.18	2	TruncNormal	0.2	2	[a]
VKidC	TruncNormal	0.1	2	TruncNormal	0.15	2	TruncNormal	0.17	2	[a]
VBldC	TruncNormal	0.12	2	TruncNormal	0.12	2	TruncNormal	0.12	2	[a]

Table A-5. Uncertainty distributions for the population mean of the PBPK model parameters (continued)

Scaling (sampled) parameter	Mouse			Rat			Human			Notes/ Source
	Distribution[a]	***SD or Min***	***Truncation (± nxSD) or Max***	**Distribution**	***SD or Min***	***Truncation (± nxSD) or Max***	**Distribution**	***SD or Min***	***Truncation (± nxSD) or Max***	
TCE distribution/partitioning										
lnPBC	TruncNormal	0.25	3	TruncNormal	0.25	3	TruncNormal	0.2	3	[d]
lnPFatC	TruncNormal	0.3	3	TruncNormal	0.3	3	TruncNormal	0.2	3	
lnPGutC	TruncNormal	0.4	3	TruncNormal	0.4	3	TruncNormal	0.4	3	
lnPLivC	TruncNormal	0.4	3	TruncNormal	0.15	3	TruncNormal	0.4	3	
lnPRapC	TruncNormal	0.4	3	TruncNormal	0.4	3	TruncNormal	0.4	3	
lnPRespC	TruncNormal	0.4	3	TruncNormal	0.4	3	TruncNormal	0.4	3	
lnPKidC	TruncNormal	0.4	3	TruncNormal	0.3	3	TruncNormal	0.2	3	
lnPSlwC	TruncNormal	0.4	3	TruncNormal	0.3	3	TruncNormal	0.3	3	
TCA distribution/partitioning										
lnPRBCPlasTCAC	Uniform	-4.605	4.605	TruncNormal	0.336	3	Uniform	-4.605	4.605	[e]
lnPBodTCAC	TruncNormal	0.336	3	TruncNormal	0.693	3	TruncNormal	0.336	3	[f]
lnPLivTCAC	TruncNormal	0.336	3	TruncNormal	0.693	3	TruncNormal	0.336	3	
TCA plasma binding										
lnkDissocC	TruncNormal	1.191	3	TruncNormal	0.61	3	TruncNormal	0.06	3	[g]
lnBMaxkDC	TruncNormal	0.495	3	TruncNormal	0.47	3	TruncNormal	0.182	3	
TCOH and TCOG distribution/partitioning										
lnPBodTCOHC	TruncNormal	0.336	3	TruncNormal	0.693	3	TruncNormal	0.336	3	
lnPLivTCOHC	TruncNormal	0.336	3	TruncNormal	0.693	3	TruncNormal	0.336	3	
lnPBodTCOGC	Uniform	-4.605	4.605	Uniform	-4.605	4.605	Uniform	-4.605	4.605	
lnPLivTCOGC	Uniform	-4.605	4.605	Uniform	-4.605	4.605	Uniform	-4.605	4.605	
DCVG distribution/partitioning										
lnPeffDCVG	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	[h]
TCE Metabolism										
lnV_{MAX}C	TruncNormal	0.693	3	TruncNormal	0.693	3	TruncNormal	0.693	3	[i]
lnK_MC	TruncNormal	1.386	3	TruncNormal	1.386	3				[i]
lnClC							TruncNormal	1.386	3	[i]
lnFracOtherC	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	[h]

Table A-5. Uncertainty distributions for the population mean of the PBPK model parameters (continued)

Scaling (sampled) parameter	Mouse			Rat			Human			Notes/ Source
	Distribution[a]	*SD or Min*	*Truncation (± nxSD) or Max*	Distribution	*SD or Min*	*Truncation (± nxSD) or Max*	Distribution	*SD or Min*	*Truncation (± nxSD) or Max*	
lnFracTCAC	TruncNormal	1.163	3	TruncNormal	1.163	3	TruncNormal	1.163	3	[j]
lnV_{MAX}DCVGC	Uniform	-4.605	9.21	Uniform	-4.605	9.21				[k]
lnClDCVGC	Uniform	-4.605	9.21	Uniform	-4.605	9.21	TruncNormal	4.605	3	[k]
lnK_MDCVGC							TruncNormal	1.386	3	[k]
lnV_{MAX}KidDCVGC	Uniform	-4.605	9.21	Uniform	-4.605	9.21				[k]
lnClKidDCVGC	Uniform	-4.605	9.21	Uniform	-4.605	9.21	TruncNormal	4.605	3	[k]
lnK_MKidDCVGC							TruncNormal	1.386	3	[k]
lnV_{MAX}LungLivC	TruncNormal	1.099	3	TruncNormal	1.099	3	TruncNormal	1.099	3	[l]
lnK_MClara	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	[h]
lnFracLungSysC	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	[h]
TCOH metabolism										
lnV_{MAX}TCOHC	Uniform	-9.21	9.21	Uniform	-9.21	9.21				[h]
lnClTCOHC							Uniform	-11.513	6.908	
lnK_MTCOH	Uniform	-9.21	9.21	Uniform	-9.21	9.21	Uniform	-9.21	9.21	
lnV_{MAX}GlucC	Uniform	-9.21	9.21	Uniform	-9.21	9.21				
lnClGlucC							Uniform	-9.21	4.605	
lnK_MGluc	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	[h]
lnkMetTCOHC	Uniform	-11.513	6.908	Uniform	-11.513	6.908	Uniform	-11.513	6.908	
TCA metabolism/clearance										
lnkUrnTCAC	Uniform	-4.605	4.605	Uniform	-4.605	4.605	Uniform	-4.605	4.605	[h]
lnkMetTCAC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	
TCOG metabolism/clearance										
lnkBileC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	[h]
lnkEHRC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	
lnkUrnTCOGC	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	
DCVG metabolism										
lnFracKidDCVCC	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	[h]
lnkDCVGC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	

Table A-5. Uncertainty distributions for the population mean of the PBPK model parameters (continued)

Scaling (sampled) parameter	Mouse			Rat			Human			Notes/ Source
	Distribution[a]	***SD or Min***	***Truncation (± nxSD) or Max***	**Distribution**	***SD or Min***	***Truncation (± nxSD) or Max***	**Distribution**	***SD or Min***	***Truncation (± nxSD) or Max***	
DCVC metabolism/clearance										
lnkNATC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	[h]
lnkKidBioactC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	
Oral uptake/transfer coefficients										
lnkTSD	Uniform	-4.269	4.942	Uniform	-4.269	4.942	Uniform	-4.269	4.942	[h]
lnkAS	Uniform	-6.571	7.244	Uniform	-6.571	7.244	Uniform	-6.571	7.244	
lnkTD	Uniform	-4.605	0	Uniform	-4.605	0	Uniform	-4.605	0	
lnkAD	Uniform	-7.195	6.62	Uniform	-7.195	6.62	Uniform	-7.195	6.62	
lnkASTCA	Uniform	-7.195	6.62	Uniform	-7.195	6.62	Uniform	-7.195	6.62	[h]
lnkASTCOH	Uniform	-7.195	6.62	Uniform	-7.195	6.62	Uniform	-7.195	6.62	

Explanatory note. All population mean parameters have either truncated normal (TruncNormal) or uniform distributions. For those with TruncNormal distributions, the mean for the population mean is 0 for natural-log transformed parameters (parameter name starting with "ln") and one for untransformed parameters, with the truncation at the specified number (n) of SDs. All uniformly distributed parameters are natural-log transformed, so their untransformed minimum and maximum are exp(Min) and exp(Max), respectively.

[a]Uncertainty based on coefficient of variation (CV) or range of values in Brown et al. (1997) (mouse and rat) and a comparison of values from ICRP Publication 89 (2003), Brown et al. (1997), and Price et al. (2003) (human).

[b]Noninformative prior distribution intended to span a wide range of possibilities because no independent data are available on these parameters. These priors for the rat and human were subsequently updated (see Section A.4.3.2).

[c]Because of potential strain differences, uncertainty in mice and rat assumed to be 20%. In humans, Price et al. (2003) reported variability of about 5%, and this is also used for the uncertainty in the mean.

[d]For partition coefficients, it is not clear whether interstudy variability is due to intersubject or assay variability, so uncertainty in the mean is based on interstudy variability among in vitro measurements. For single measurements, uncertainty SD of 0.3 was used for fat (mouse) and 0.4 for other tissues was used. In addition, where measurements were from a surrogate tissue (e.g., gut was based on liver and kidney), an uncertainty SD 0.4 was used.

[e]Single in vitro data point available in rats, so a GSD of 1.4 was used. In mice and humans, where no in vitro data was available, a noninformative prior was used.

[f]Single in vitro data points available in mice and humans, so a GSD of 1.4 was used. In rats, where the mouse data was used as a surrogate, a GSD of 2.0 was used, based on the difference between mice and rats in vitro.

[g]GSD for uncertainty based on different estimates from different in vitro studies.

[h]Noninformative prior distribution.

Table A-5. Uncertainty distributions for the population mean of the PBPK model parameters (continued)

[i]Assume twofold uncertainty GSD in V_{MAX}, based on observed variability and uncertainties of in vitro-to-in vivo scaling. For K_M and ClC, the uncertainty is assumed to be fourfold, due to the different methods for scaling of concentrations from TCE in the in vitro medium to TCE in blood.
[j]Uncertainty GSD of 3.2-fold reflects difference between in vitro measurements from Lipscomb et al. (1998b) and Bronley-DeLancey et al. (2006).
[k]In mice and rats, the baseline values are notional lower-limits on V_{MAX} and clearance, however, the lower bound of the prior distribution is set to 100-fold less because of uncertainty in in vitro-in vivo extrapolation, and because Green et al. (1997b) reported values 100-fold smaller than Lash et al. (1998b; 1995). In humans, the uncertainty GSD in clearance is assumed to be 100-fold, due to the difference between Lash et al. (1998b) and Green et al. (1997b). For K_M, the uncertainty GSD of fourfold is based on differences between concentrations in cells and cytosol.
[l]Uncertainty GSD of threefold was assumed due to possible differences in microsomal protein content, the fact that measurements were at a single concentration, and the fact that the human baseline values was based on the limit of detection.

Table A-6. Updated prior distributions for selected parameters in the rat and human

Scaling parameter	Initial prior bounds		Updated rat prior		Updated human prior	
	exp(min)	exp(max)	exp(μ)	exp(σ)	exp(μ)	exp(σ)
lnDRespC	1.0×10^{-5}	1.0×10^{1}	1.22	5.21	1.84	4.18
lnPBodTCOGC	1.0×10^{-2}	1.0×10^{2}	0.42	5.47	0.81	5.10
lnPLivTCOGC	1.0×10^{-2}	1.0×10^{2}	1.01	5.31	2.92	4.31
lnFracOtherC	1.0×10^{-3}	1.0×10^{3}	0.02	6.82	0.14	4.76
lnV_{MAX}DCVGC	1.0×10^{-2}	1.0×10^{4}	2.61	42.52		
lnClDCVGC	1.0×10^{-2}	1.0×10^{4}	0.36	15.03		
lnV_{MAX}KidDCVGC	1.0×10^{-2}	1.0×10^{4}	2.56	22.65		
lnClKidDCVGC	1.0×10^{-2}	1.0×10^{4}	1.22	15.03		
lnV_{MAX}LungLivC	3.7×10^{-2}	2.7×10^{1}	2.77	6.17	2.80	4.71
lnK_MClara	1.0×10^{-3}	1.0×10^{3}	0.01	6.69	0.02	4.85
lnFracLungSysC	1.0×10^{-3}	1.0×10^{3}	4.39	11.13	3.10	8.08
lnV_{MAX}TCOHC	1.0×10^{-4}	1.0×10^{4}	1.65	5.42		
lnClTCOHC	1.0×10^{-5}	1.0×10^{3}			0.37	4.44
lnK_MTCOH	1.0×10^{-4}	1.0×10^{4}	0.93	5.64	4.81	4.53
lnV_{MAX}GlucC	1.0×10^{-4}	1.0×10^{4}	69.41	5.58		
lnClGlucC	1.0×10^{-4}	1.0×10^{2}			3.39	4.35
lnK_MGluc	1.0×10^{-3}	1.0×10^{3}	30.57	6.11	11.13	4.57
lnkMetTCOHC	1.0×10^{-5}	1.0×10^{3}	3.35	5.87	2.39	4.62
lnkUrnTCAC	1.0×10^{-2}	1.0×10^{2}	0.11	5.42	0.09	4.22
lnkMetTCAC	1.0×10^{-4}	1.0×10^{2}	0.61	5.37	0.45	4.26
lnkBileC	1.0×10^{-4}	1.0×10^{2}	1.01	5.70	3.39	4.44
lnkEHRC	1.0×10^{-4}	1.0×10^{2}	0.01	6.62	0.22	4.71
lnkUrnTCOGC	1.0×10^{-3}	1.0×10^{3}	8.58	6.05	16.12	4.81
lnkNATC	1.0×10^{-4}	1.0×10^{2}			0.00	6.11
lnkKidBioactC	1.0×10^{-4}	1.0×10^{2}			0.01	6.49

Notes: updated rat prior is based on the mouse posterior; and the updated human priors are based on combining the mouse and rat posteriors, except in the case of lnkNATC and lnkKidBioactC, which are unidentified in the mouse model. Columns labeled exp(min) and exp(max) are the exponentiated prior bounds; columns labeled exp(μ) and exp(σ) are the exponentiated mean and SD of the updated prior distributions, which are normal distributions truncated at the prior bounds.

The scaling model is given explicitly as follows. If θ_i are the "scaling" parameters (usually also natural-log-transformed) that are actually estimated, and A is the "universal" (species-independent) parameter, then $\theta_i = A + \varepsilon_I$, where ε_i is the species-specific "departure" from the scaling relationship, assumed to be normally distributed with variance σ_ε^2. This "scatter" in the interspecies scaling relationship is assumed to have a SD of 1.15 = ln(3.16), so that the unlogarithmically transformed 95% CI spans about 100-fold (i.e., $\exp(2\sigma) = 10$). This implies that 95% of the time, the species-specific scaling parameter is assumed be within 10-fold higher or lower than the "species-independent" value. However, the prior bounds, which

generally span a wider range, are maintained so that if the data strongly imply an extreme species-specific value, they can be accommodated. In addition, the model transfers the marginal distributions for each parameter across species, so correlations between parameters are not retained. This is a restriction on the software used for conducting MCMC analyses, however, assuming independence will lead to a "broader" joint distribution, given the same marginal distributions. Thus, this assumption tends to reduce the weight of the interspecies scaling as compared to the species-specific calibration data.

Therefore, the mouse model gives an initial estimate of "A," which is used to update the prior distribution for $\theta_r = A + \varepsilon_r$ in the rat (alternatively, since there is only one species at this stage, one could think of this as estimating the rat parameter using the mouse parameter, but with a cross-species variance is twice the allometric scatter variance). The rat and mouse together then give a "better" estimate of A, which is used to update the prior distribution for $\theta_h = A + \varepsilon_h$ in the human, with the assumed distribution for ε_h. This approach is implemented by approximating the posterior distributions by normal distributions, deriving heuristic "data" for the specific-specific parameters, and then using these pseudo-data to derive updated prior distributions for the other species parameters. Specifically, the procedure is as follows:

1. Run the mouse model.
2. Use the mouse posterior to derive the mouse "pseudo-data" D_m (equal to the posterior mean) and its uncertainty σ_m^2 (equal to the posterior variance).
3. Use the D_m as the prior mean for the rat. The prior variance for the rat is $2\sigma_\varepsilon^2 + \sigma_m^2$, which accounts for two components of species-specific departure from "species-independence" (one each for mouse and rat), and the mouse posterior uncertainty.
4. Match the rat posterior mean and variance to the values derived from the normal approximation (posterior mean = $\{D_m/(2\sigma_\varepsilon^2 + \sigma_m^2) + D_r/\sigma_r^2\}/\{1/(2\sigma_\varepsilon^2 + \sigma_m^2) + 1/\sigma_r^2\}$; posterior variance = $\{1/(2\sigma_\varepsilon^2 + \sigma_m^2) + 1/\sigma_r^2\}^{-1}$), and solve for the rat "data" D_r and its uncertainty σ_r^2.
5. Use, σ_m^2, and σ_r^2 to derive the updated prior mean and variance for the human model. For the mean ($=\{D_m/(\sigma_\varepsilon^2 + \sigma_m^2) + D_r/(\sigma_\varepsilon^2 + \sigma_r^2)\}/\{1/(\sigma_\varepsilon^2 + \sigma_m^2) + 1/(\sigma_\varepsilon^2 + \sigma_r^2)\}$), it is the weighted average of the mouse and rat, with each weight including both posterior uncertainty and departure from "species-independence." For the variance ($=\{1/(\sigma_\varepsilon^2 + \sigma_m^2) + 1/(\sigma_\varepsilon^2 + \sigma_r^2)\}^{-1} + \sigma_\varepsilon^2$), it is the variance in the weighted average of the mouse and rat plus an additional component of species-specific departure from "species-independence."

Formally, then, the probability of θ_i given A can be written as:

$$P(\theta_i \mid A) = \varphi(\theta_i - A, \sigma_\varepsilon^2) \qquad \text{(Eq. A-63)}$$

where $\varphi(x, \sigma^2)$ is the normal density centered on 0 with variance σ^2. Let D_i be a heuristic "datum" for species *i*, so the likelihood given θ_i is adequately approximated by:

$$P(D_i \mid \theta_i) = \varphi(D_i - \theta_i, \sigma_i^2) \qquad \text{(Eq. A-64)}$$

Therefore, considering A to have a uniform prior distribution, then running the mouse model gives a posterior of the form:

$$P(A, \theta_m \mid D_m) \propto P(A)\, P(\theta_m \mid A)\, P(D_m \mid \theta_m) \propto \varphi(\theta_m - A, \sigma_\varepsilon^2)\, \varphi(D_m - \theta_m, \sigma_m^2) \qquad \text{(Eq. A-65)}$$

From the MCMC posterior, the values of D_m and σ_m^2 are simply the mean and variance of the scaled parameter θ_m.

Now, adding the rat data gives:

$$P(A, \theta_m, \theta_r \mid D_m, D_r) \propto P(A)\, P(\theta_m \mid A)\, P(D_m \mid \theta_m)\, P(\theta_r \mid A)\, P(D_r \mid \theta_r) \qquad \text{(Eq. A-66)}$$
$$\propto \varphi(\theta_m - A, \sigma_\varepsilon^2)\, \varphi(D_m - \theta_m, \sigma_m^2)\, \varphi(\theta_r - A, \sigma_\varepsilon^2)\, \varphi(D_r - \theta_r, \sigma_r^2)$$

D_r and σ_r^2 can be derived by marginalizing first over θ_m and then over *A*:

$$\int P(A, \theta_m, \theta_r \mid D_m, D_r)\, d\theta_m\, dA \qquad \text{(Eq. A-67)}$$
$$\propto [\int P(A) \{\int P(\theta_m \mid A)\, P(D_m \mid \theta_m)\, d\theta_m\}\, P(\theta_r \mid A)\, dA\,] P(D_r \mid \theta_r)$$
$$= [\int P(A)\, P(D_m \mid A)\, P(\theta_r \mid A)\, dA]\, P(D_r \mid \theta_r)$$
$$\propto [\int P(A \mid D_m)\, P(\theta_r \mid A)\, dA]\, P(D_r \mid \theta_r)$$
$$= P(\theta_r \mid D_m)\, P(D_r \mid \theta_r)$$

So $P(\theta_r \mid D_m)$ can be identified as the prior for θ_r based on the mouse data, and $P(D_r \mid \theta_r)$ as the rat-specific likelihood. The updated prior for the rats is then:

$$P(\theta_r \mid D_m) \propto \int \varphi(\theta_m - A, \sigma_\varepsilon^2)\, \varphi(D_m - \theta_m, \sigma_m^2)\, \varphi(\theta_r - A, \sigma_\varepsilon^2)\, d\theta_m\, dA \qquad \text{(Eq. A-68)}$$
$$= \int \varphi(D_m - A, \sigma_\varepsilon^2 + \sigma_m^2)\, \varphi(\theta_r - A, \sigma_\varepsilon^2)\, dA$$
$$= \varphi(D_m - \theta_r, 2\sigma_\varepsilon^2 + \sigma_m^2)$$

Therefore, for the "mouse-based" prior, use the mean D_m from the mouse, and then the variance from the mouse σ_m^2 plus twice the "allometric scatter" variance σ_ε^2.

The rat "data" and variance, assuming conditional independence of the rat and mouse "pseudo-data," is thus:

$$P(\theta_r \mid D_m, D_r) \propto P(\theta_r \mid D_m)\, P(D_r \mid \theta_r) \qquad \text{(Eq. A-69)}$$
$$\propto \varphi(D_m - \theta_r, 2\sigma_\varepsilon^2 + \sigma_m^2)\, \varphi(D_r - \theta_r, \sigma_r^2)$$

This distribution is also normal with:

$$E(\theta_r) = \{D_m/(2\sigma_\varepsilon^2 + \sigma_m^2) + D_r/\sigma_r^2\}/\{1/(2\sigma_\varepsilon^2 + \sigma_m^2) + 1/\sigma_r^2\} \quad \text{(Eq. A-70)}$$

= weighted mean of D_r

$$\mathrm{VAR}(\theta_r) = \{1/(2\sigma_\varepsilon^2 + \sigma_m^2) + 1/\sigma_r^2\}^{-1} \quad \text{(Eq. A-71)}$$

= harmonic mean of variances

Thus, using the mean and variance of the posterior distribution from the MCMC analysis, D_r and σ_r^2 can be derived.

Now, D_m, σ_m^2, D_r, and σ_r^2 are known, so the analogous "mouse + rat" based prior used in the human model can be derived. As with the rat prior, the human prior is based on a normal approximation of the posterior for *A*, and then incorporates a random term for cross-species variation (allometric scatter):

$$P(A, \theta_m, \theta_r, \theta_h \mid D_m, D_r, D_h) \quad \text{(Eq. A-72)}$$
$$\propto P(A)\, P(\theta_m \mid A)\, P(D_m \mid \theta_m)\, P(\theta_r \mid A)\, P(D_r \mid \theta_r)\, P(\theta_h \mid A)\, P(D_h \mid \theta_h)$$
$$\propto \varphi(\theta_m - A, \sigma_\varepsilon^2)\, \varphi(D_m - \theta_m, \sigma_m^2)\, \varphi(\theta_r - A, \sigma_\varepsilon^2)\, \varphi(D_r - \theta_r, \sigma_r^2)$$
$$\varphi(\theta_h - A, \sigma_\varepsilon^2)\, \varphi(D_h - \theta_h, \sigma_h^2)$$

Consider marginalizing first over θ_m, then over θ_r, and then over A:

$$\int P(A, \theta_m, \theta_r, \theta_h \mid D_m, D_r, D_h)\, d\theta_m\, d\theta_r\, dA \quad \text{(Eq. A-73)}$$
$$\propto [\int P(A)\, \{\int P(\theta_m \mid A)\, P(D_m \mid \theta_m)\, d\theta_m\}\, \{\int P(\theta_r \mid A)\, P(D_r \mid \theta_r)\, d\theta_r\}\, P(\theta_h \mid A)\, dA$$
$$P(D_h \mid \theta_h)$$
$$= [\int P(A)\, P(D_m \mid A)\, P(D_r \mid A)\, P(\theta_h \mid A)\, dA\,]\, P(D_h \mid \theta_h)$$
$$\propto [\int P(A \mid D_m D_r)\, P(\theta_h \mid A)\, dA]\, P(D_h \mid \theta_h)$$
$$= P(\theta_h \mid D_m D_r)\, P(D_h \mid \theta_h)$$

So $P(\theta_h \mid D_m D_r)$ is the prior for θ_h based on the mouse and rat data, and $P(D_h \mid \theta_h)$ as the human-specific likelihood. The prior is used in the MCMC analysis for the humans, and it is derived to be:

$$P(\theta_h \mid D_m D_r) \propto \int \varphi(\theta_m - A, \sigma_\varepsilon^2)\, \varphi(D_m - \theta_m, \sigma_m^2)\, \varphi(\theta_r - A, \sigma_\varepsilon^2)\, \varphi(D_r - \theta_r, \sigma_r^2) \quad \text{(Eq. A-74)}$$
$$\varphi(\theta_h - A, \sigma_\varepsilon^2)\, d\theta_m\, d\theta_r\, dA$$
$$= \int [\varphi(D_m - A, \sigma_\varepsilon^2 + \sigma_m^2)\, \varphi(D_r - A, \sigma_\varepsilon^2 + \sigma_r^2)]\, \varphi(\theta_h - A, \sigma_\varepsilon^2)\, dA$$
$$\propto \int \varphi(D_{m+r} - A, \sigma_{m+r}^2)\, \varphi(\theta_h - A, \sigma_\varepsilon^2)\, dA$$
$$= \varphi(D_{m+r} - \theta_h, \sigma_{m+r}^2 + \sigma_\varepsilon^2)$$

where D_{m+r} and σ_{m+r}^2 are the weighted mean and variances of *A* under the density:

$$[\varphi(D_m - A, \sigma_\varepsilon^2 + \sigma_m^2)\, \varphi(D_r - A, \sigma_\varepsilon^2 + \sigma_r^2)] \quad \text{(Eq. A-75)}$$

which is given by:

$$D_{m+r} = E(A|\, D_m\, D_r) = \{D_m/(\sigma_\varepsilon^2 + \sigma_m^2) + D_r/(\sigma_\varepsilon^2 + \sigma_r^2)\}/\{1/(\sigma_\varepsilon^2 + \sigma_m^2) + 1/(\sigma_\varepsilon^2 + \sigma_r^2)\}$$
$$= \text{weighted mean of } D_m \text{ and } D_r$$
$$\sigma_{m+r}^2 = \text{VAR}(A|\, D_m\, D_r) = \{1/(\sigma_\varepsilon^2 + \sigma_m^2) + 1/(\sigma_\varepsilon^2 + \sigma_r^2)\}^{-1}$$
$$= \text{harmonic mean of variances}$$

At this point, these values are used in the normal approximation of the combined rodent posterior, which will be incorporated into the cross-species extrapolation as described in Step 5 above.

The results of these calculations for the updated prior distributions, are shown in Table A-6. With this methodology for updating the prior distributions, adequate convergence was achieved for the rat and human after 110,000~140,000 iterations.

A.4.3.3. Population Variance: Prior Central Estimates and Uncertainty

The following multipage Table A-7 describes the uncertainty distributions used for the population variability in the PBPK model parameters.

Table A-7. Uncertainty distributions for the population variance of the PBPK model parameters

Scaling (sampled) parameter	Mouse		Rat		Human		Notes/source
	CV	CU	CV	CU	CV	CU	
Flows							
lnQCC	0.2	2	0.14	2	0.2	2	[a]
lnVPRC	0.2	2	0.3	2	0.2	2	
lnDRespC	0.2	0.5	0.2	0.5	0.2	0.5	
Physiological blood flows to tissues							
QFatC	0.46	0.5	0.46	0.5	0.46	0.5	[a]
QGutC	0.17	0.5	0.17	0.5	0.18	0.5	
QLivC	0.17	0.5	0.17	0.5	0.45	0.5	
QSlwC	0.29	0.5	0.3	0.5	0.32	0.5	
QKidC	0.32	0.5	0.13	0.5	0.12	0.5	
FracPlasC	0.2	0.5	0.2	0.5	0.05	0.5	
Physiological volumes							
VFatC	0.45	0.5	0.45	0.5	0.45	0.5	[a]
VGutC	0.13	0.5	0.13	0.5	0.08	0.5	
VLivC	0.24	0.5	0.18	0.5	0.23	0.5	
VRapC	0.1	0.5	0.12	0.5	0.08	0.5	
VRespLumC	0.11	0.5	0.18	0.5	0.2	0.5	
VRespEffC	0.11	0.5	0.18	0.5	0.2	0.5	
VKidC	0.1	0.5	0.15	0.5	0.17	0.5	
VBldC	0.12	0.5	0.12	0.5	0.12	0.5	

Table A-7. Uncertainty distributions for the population variance of the PBPK model parameters (continued)

Scaling (sampled) parameter	Mouse		Rat		Human		Notes/source
	CV	CU	CV	CU	CV	CU	
TCE distribution/partitioning							
lnPBC	0.25	2	0.25	0.333	0.185	0.333	[b]
lnPFatC	0.3	2	0.3	0.333	0.2	1	
lnPGutC	0.4	2	0.4	2	0.4	2	
lnPLivC	0.4	2	0.15	0.333	0.4	1.414	
lnPRapC	0.4	2	0.4	2	0.4	2	
lnPRespC	0.4	2	0.4	2	0.4	2	
lnPKidC	0.4	2	0.3	0.577	0.2	1.414	
lnPSlwC	0.4	2	0.3	0.333	0.3	1.414	
TCA distribution/partitioning							
lnPRBCPlasTCAC	0.336	2	0.336	2	0.336	2	[c]
lnPBodTCAC	0.336	2	0.693	2	0.336	2	[b]
lnPLivTCAC	0.336	2	0.693	2	0.336	2	
TCA plasma binding							
lnkDissocC	1.191	2	0.61	2	0.06	2	[b]
lnBMaxkDC	0.495	2	0.47	2	0.182	2	
TCOH and TCOG distribution/partitioning							
lnPBodTCOHC	0.336	2	0.693	2	0.336	2	[b]
lnPLivTCOHC	0.336	2	0.693	2	0.336	2	[b]
lnPBodTCOGC	0.4	2	0.4	2	0.4	2	[d]
lnPLivTCOGC	0.4	2	0.4	2	0.4	2	[d]
DCVG distribution/partitioning							
lnPeffDCVG	0.4	2	0.4	2	0.4	2	[b]
TCE metabolism							
lnV_{MAX}C	0.824	1	0.806	1	0.708	0.26	[e]
lnK_MC	0.270	1	1.200	1			
lnClC					0.944	1.41	
lnFracOtherC	0.5	2	0.5	2	0.5	2	[f]
lnFracTCAC	0.5	2	0.5	2	1.8	2	[g]
lnV_{MAX}DCVGC	0.5	2	0.5	2			[f]
lnClDCVGC	0.5	2	0.5	2	0.5	2	
lnK_MDCVGC					0.5	2	
lnV_{MAX}KidDCVGC	0.5	2	0.5	2			
lnClKidDCVGC	0.5	2	0.5	2	0.5	2	
lnK_MKidDCVGC					0.5	2	
lnV_{MAX}LungLivC	0.5	2	0.5	2	0.5	2	
lnK_MClara	0.5	2	0.5	2	0.5	2	
lnFracLungSysC	0.5	2	0.5	2	0.5	2	

Table A-7. Uncertainty distributions for the population variance of the PBPK model parameters (continued)

Scaling (sampled) parameter	Mouse		Rat		Human		Notes/source
	CV	CU	CV	CU	CV	CU	
TCOH metabolism							
lnV_{MAX}TCOHC	0.5	2	0.5	2			[f]
lnClTCOHC					0.5	2	
lnK_MTCOH	0.5	2	0.5	2	0.5	2	
lnV_{MAX}GlucC	0.5	2	0.5	2			
lnClGlucC					0.5	2	
lnK_MGluc	0.5	2	0.5	2	0.5	2	
lnkMetTCOHC	0.5	2	0.5	2	0.5	2	
TCA metabolism/clearance							
lnkUrnTCAC	0.5	2	0.5	2	0.5	2	[f]
lnkMetTCAC	0.5	2	0.5	2	0.5	2	
TCOG metabolism/clearance							
lnkBileC	0.5	2	0.5	2	0.5	2	[f]
lnkEHRC	0.5	2	0.5	2	0.5	2	
lnkUrnTCOGC	0.5	2	0.5	2	0.5	2	[f]
DCVG metabolism/clearance							
lnFracKidDCVCC	0.5	2	0.5	2	0.5	2	[f]
lnkDCVGC	0.5	2	0.5	2	0.5	2	
DCVC metabolism/clearance							
lnkNATC	0.5	2	0.5	2	0.5	2	[f]
lnkKidBioactC	0.5	2	0.5	2	0.5	2	
Oral uptake/transfer coefficients							
lnkTSD	2	2	2	2	2	2	[h]
lnkAS	2	2	2	2	2	2	
lnkTD	2	2	2	2	2	2	
lnkAD	2	2	2	2	2	2	
lnkASTCA	2	2	2	2	2	2	
lnkASTCOH	2	2	2	2	2	2	

Explanatory note. All population variance parameters (V_pname, for parameter "pname") have Inverse-Gamma distributions, with the expected value given by CV and coefficient of uncertainty given by CU (i.e., SD of V_pname divided by expected value of V_pname) (notation the same as Hack et al. (2006)). Under these conditions, the Inverse-Gamma distribution has a shape parameter is given by $\alpha = 2 + 1/CU^2$ and scale parameter $\beta = (\alpha I - 1)\ CV^2$. In addition, it should be noted that, under a normal distribution and a uniform prior distribution on the population variance, the posterior distribution for the variance given n data points with a sample variance s^2 is given by and Inverse-Gamma distribution with $\alpha = (n - 1)/2$ and $\beta = \alpha\ s^2$. Therefore, the "effective" number of data points is given by $n = 5 + 2/CU^2$ and the "effective" sample variance is $s^2 = CV^2\ \alpha\omega\eta\alpha\tau/(\alpha - 1)$.

[a]For physiological parameters, CV values generally taken to be equal to the uncertainty SD in the population mean, most of which were based on variability between studies (i.e., not clear whether variability represents uncertainty or variability). Given this uncertainty, CU of 2 assigned to cardiac output and ventilation-perfusion, while CU of 0.5 assigned to the remaining physiological parameters.

Table A-7. Uncertainty distributions for the population variance of the PBPK model parameters (continued)

[b]As discussed above, it is not clear whether interstudy variability is due to intersubject or assay variability, so the same central were assigned to the uncertainty in the population mean as to the central estimate of the population variance. In the cases where direct measurements were available, the CU for the uncertainty in the population variance is based on the actual sample n, with the derivation discussed in the notes preceding this table. Otherwise, a CU of 2 was assigned, reflecting high uncertainty.
[c]Used value from uncertainty in population in mean in rats for all species with high uncertainty.
[d]No data, so assumed CV of 0.4 with high uncertainty.
[e]For mice and rats, based on variability in results from Lipscomb et al. (1998c) and Elfarra et al. (1998) in microsomes. Since only pooled or mean values are available, CU of one was assigned (moderate uncertainty). For humans, based on variability in *individual* samples from Lipscomb et al. (1997) (microsomes), Elfarra et al. (1998) (microsomes), and Lipscomb et al. (1998c) (freshly isolated hepatocytes). High uncertainty in clearance (lnClC) reflects two different methods for scaling concentrations in microsomal preparations to blood concentrations: (1) assuming microsomal concentration equals liver concentration and then using the measured liver:blood partition coefficient to convert to blood and (2) using the measured microsome:air partition coefficient and then using the measured blood:air partition coefficient to convert to blood.
[f]No data on variability, so a CV of 0.5 was assigned, with a CU of 2.
[g]For mice and rats, no data on variability, so a CV of 0.5 was assigned, with a CU of 2. For humans, sixfold variability based on in vitro data from Bronley-DeLancy et al. (2006), but with high uncertainty.
[h]No data on variability, so a CV of 2 was assigned (larger than assumed for metabolism due to possible vehicle effects), with a CU of 2.

A.4.3.4. Likelihood Function and Prior distributions for Residual Error Estimates

From Equation A-3 for the total likelihood function, different measurement types may have different partial likelihoods. In all cases except one, the likelihood was assumed to be lognormal, with probability density for a particular measurement y_{ijkl} at time t_{ijkl} given by:

$$P(y_{ijkl} \mid \theta_i, \sigma_{ijk}^2, t_{ijkl}) = (2\pi\sigma^2)^{-½} \exp[\{-\ln y_{ijkl} - \ln f_{ijkl}(\theta_i, t_{ijkl})\}^2/(2\sigma_{ijk}^2)] \quad \text{(Eq. A-76)}$$

As before, the subject is labeled *i*, the study is labeled *j*, the type of measurement is labeled *k*, and the different time points are labeled *l*. The parameters θ_i are the "scaling parameters" at the subject-level, shown in Table A-4, whereas the parameters σ_{ijk}^2 represent the "residual error" variance σ^2. This error term may include variability due to measurement error, intrasubject and intrastudy heterogeneity, as well as model misspecification. The available in vivo measurements to which the model was calibrated are listed in Table A-8. The variances for each of the corresponding residual errors were given log-uniform distributions. For all measurements, the bounds on the log-uniform distribution were 0.01 and 3.3, corresponding to GSDs bounded by 1.11 and 6.15. The lower bound was set to prevent "over-fitting," as was done in Bois (2000a) and Hack et al. (2006).

Table A-8. Measurements used for calibration

Measurement abbreviation	Mouse	Rat	Human	Measurement description
RetDose			√	Retained TCE dose (mg)
CAlvPPM			√	TCE concentration in alveolar air (ppm)
CInhPPM	√	√		TCE concentration in closed-chamber (ppm)
Cart		√		TCE concentration in arterial blood (mg/L)
CVen	√	√	√	TCE concentration in venous blood (mg/L)
CBldMix	√	√		TCE concentration in mixed arterial and venous blood (mg/L)
CFat	√	√		TCE concentration in fat (mg/L)
CGut		√		TCE concentration in gut (mg/L)
CKid	√	√		TCE concentration in kidney (mg/L)
CLiv	√	√		TCE concentration in liver (mg/L)
CMus		√		TCE concentration in muscle (mg/L)
AExhpost	√	√		Amount of TCE exhaled postexposure (mg)
CTCOH	√	√	√	Free TCOH concentration in blood (mg/L)
CLivTCOH	√			Free TCOH concentration in liver (mg/L)
CPlasTCA	√	√	√	TCA concentration in plasma (mg/L)
CBldTCA	√	√	√	TCA concentration in blood (mg/L)
CLivTCA	√	√		TCA concentration in liver (mg/L)
AUrnTCA	√	√	√	Cumulative amount of TCA excreted in urine (mg)
AUrnTCA_collect			√	Cumulative amount of TCA collected in urine (noncontinuous sampling) (mg)
ABileTCOG		√		Cumulative amount of bound TCOH excreted in bile (mg)
CTCOG		√		Bound TCOH concentration in blood (mg/L)
CTCOGTCOH	√			Bound TCOH concentration in blood in free TCOH equivalents (mg/L)
CLivTCOGTCOH	√			Bound TCOH concentration in liver in free TCOH equivalents (mg/L)
AUrnTCOGTCOH	√	√	√	Cumulative amount of total TCOH excreted in urine (mg)
AUrnTCOGTCOH_collect			√	Cumulative amount of total TCOH collected in urine (noncontinuous sampling) (mg)
CDCVGmol			√	DCVG concentration in blood (mmol/L)
CDCVG_ND			√	DCVG nondetects from Lash et al. (1999b)
AUrnNDCVC		√	√	Cumulative amount of NAcDCVC excreted in urine (mg)
AUrnTCTotMole		√		Cumulative amount of TCA+total TCOH excreted in urine (mmol)
TotCTCOH	√	√	√	Total TCOH concentration in blood (mg/L)

where:

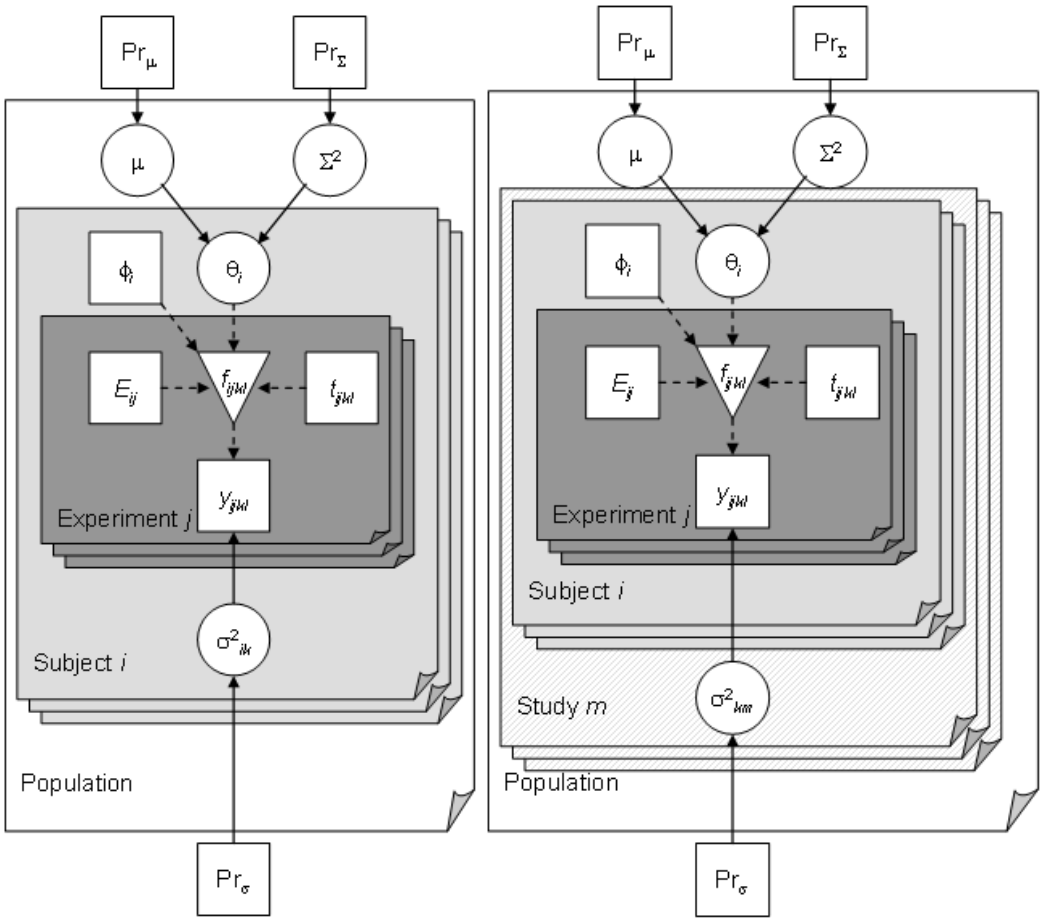
$\Phi(y)$ is the cumulative standard normal distribution.

Nondetects (ND) of DCVG from Lash et al. (1999b) were also included in the data, at it was found that these data were needed to place constraints on the clearance rate of DCVG from blood. The detection limit reported in the study was LD = 0.05 pmol/mL= 5×10^{-5} mmol/L. It

was assumed, as is standard in analytical chemistry, that the detection limit represents a response from a blank sample at 3 SDs. Because detector responses near the detection limit are generally normally distributed, the likelihood for observing a nondetect given a model-predicted value of $f_{ijkl}(\theta_i, t_{ijkl})$ is equal to:

$$P(= \text{ND}| \theta_i, t_{ijkl}) = \Phi(3 \times \{1 - f_{ijkl}(\theta_i, t_{ijkl})/\text{L}\square), \qquad \text{(Eq. A-77)}$$

The rat and human models differed from the mouse model in terms of the hierarchical structure of the residual errors. In the mouse model, all of the studies were assumed to have the same residual error, as shown in Figure A-1, so that the residual error is only indexed by *k*, the type of measurement: σ_k^2. This appeared reasonable because there were fewer studies, and there appeared to be less variation between studies. In the rat and human models, each of which used a much larger database of in vivo studies, residual errors were assumed to be the same within a study, but may differ between studies, and so are labeled by study *j* and the type of measurement *k*: σ_{jk}^2. The updated hierarchical structures are shown in Figure A-12. Initial attempts to use a single set of residual errors led to large residual errors for some measurements, even though fits to many studies appeared reasonable. Residual errors were generally reduced when study-specific errors were used, except for some data sets that appeared to be outliers (discussed below).



Symbols have the same meaning as Figure A-1, with modifications for the rat and human. In particular, in the rat, each "subject" consists of animals (usually comprising multiple dose groups) of the same sex, species, and strain within a study (possibly reported in more than one publication, but reasonably presumed to be of animals in the same "lot"). Animals within each subject are presumed to be "identical," with the same PBPK model parameters, and each such subject is assigned its own set of "residual" error variances σ^2_{ik}. In humans, each "subject" is a single person, possibly exposed in multiple experiments, and each subject is assigned a set of PBPK model parameters drawn from the population. However, in humans, "residual" error variances are assigned at an intermediate level of the hierarchy—the "study" level, σ^2_{km}—rather than the subject or the population level.

Figure A-12. Updated hierarchical structure for rat and human models.

A.4.4. Summary of Bayesian Posterior Distribution Function

As described in Section A.1, the posterior distribution for the unknown parameters is obtained in the usual Bayesian manner by multiplying:

(1) The prior distributions for the population mean of the scaling parameter(μ) (see Sections A.4.3.1–A.4.3.2), the population variance of the scaling parameters(Σ^2) (see Section A.4.3.3), and the "residual" error (σ^2) (see Section A.4.3.4);

(2) The population distribution, assumed to be a truncated normal distribution, for the subject parameters $(\theta \mid \mu, \Sigma^2)$; and

(3) The likelihood functions $(y \mid \theta, \sigma^2)$, (see Section A.4.3.4)

as follows:

$$(\theta, \mu, \Sigma^2, \sigma^2 \mid y) \propto (\mu)(\Sigma^2)\,(\sigma^2)\,(\theta \mid \mu, \Sigma^2)\,(y \mid \theta, \sigma^2) \qquad \text{(Eq. A-78)}$$

Each subject's parameters θ_i have the same sampling distribution (i.e., they are independently and identically distributed), so their joint prior distribution is:

$$(\theta \mid \mu, \Sigma^2) = \prod_{i=1\ldots n} (\theta_i \mid \mu, \Sigma^2) \qquad \text{(Eq. A-79)}$$

Different experiments $j = 1\ldots n_j$ may have different exposure and different data collected and different time points. In addition, different types of measurements $k = 1\ldots n_k$ (e.g., TCE blood, TCE breath, TCA blood, etc.) may have different errors, but errors are otherwise assumed to be independently and identically distributed. Because the subjects are treated as independent given $\theta_{1\ldots n}$, the likelihood function is simply:

$$y \mid \theta, \sigma^2) = \prod_{i=1\ldots n} \prod_{j=1\ldots nij} \prod_{k=1\ldots m} \prod_{l=1\ldots Nijk} (y_{ijkl} \mid \theta_i, \sigma_{ijk}{}^2, t_{ijkl}) \qquad \text{(Eq. A-80)}$$

where n is the number of subjects, n_{ij} is the number of experiments in that subject, m is the number of different types of measurements, N_{ijk} is the number (possibly 0) of measurements (e.g., time points) for subject i of type k in experiment j, and t_{ijkl} are the times at which measurements for subject i of type k were made in experiment j.

The MCSim software (version 5.0.0) was used to sample from this distribution.

A.5. RESULTS OF UPDATED PBPK MODEL

The evaluation of the updated PBPK model was discussed in Chapter 3. Detailed results in the form of tables and figures are provided in this section.

A.5.1. Convergence and Posterior Distributions of Sampled Parameters

For each sampled parameter (population mean and variance and the variance for residual errors), summary statistics (median, [2.5, 97.5%] CI) for the posterior distribution are tabulated in Tables A-9, A-10, A-12, A-13, A-15, and A-16 below. In addition, the potential scale reduction factor R, calculated from comparing four independent chains, is given. For each species, graphs of the prior and posterior distributions for the population mean and variance parameters are shown in Figures A-13 to A-18 for mice, A-19 to A-24 for rats, and A-25 to A-30

for humans. Finally, posterior correlations between population mean parameters are given in Tables A-11, A-14, and A-17, which show parameter pairs with correlation coefficients ≥0.25.

In addition, posterior distributions for the subject-specific parameters are summarized in supplementary figures accessible here:

- **Mouse:** (Supplementary data for TCE assessment: Mouse posterior by subject, 2011)
- **Rat:** (Supplementary data for TCE assessment: Rat posterior by subject, 2011)
- **Human**: (Supplementary data for TCE assessment: Human posterior by subject, 2011)

Table A-9. Posterior distributions for mouse PBPK model population parameters

Sampled parameter[a]	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population GSD	
	Median (2.5, 97.5%)	*R*	Median (2.5, 97.5%)	*R*
lnQCC	1.237 (0.8972, 1.602)	1	1.402 (1.183, 2.283)	1
lnVPRC	0.8076 (0.6434, 1.022)	1	1.224 (1.108, 1.63)	1.001
QFatC	1.034 (0.5235, 1.55)	1	0.436 (0.3057, 0.6935)	1
QGutC	1.183 (1.002, 1.322)	1	0.1548 (0.1101, 0.2421)	1
QLivC	1.035 (0.8002, 1.256)	1	0.1593 (0.1107, 0.2581)	1
QSlwC	0.9828 (0.6043, 1.378)	1	0.275 (0.1915, 0.4425)	1
lnDRespC	1.214 (0.7167, 2.149)	1.002	1.215 (1.143, 1.375)	1
QKidC	0.995 (0.5642, 1.425)	1	0.3001 (0.21, 0.48)	1
FracPlasC	0.8707 (0.5979, 1.152)	1.001	0.1903 (0.1327, 0.3039)	1
VFatC	1.329 (0.8537, 1.784)	1.002	0.4123 (0.2928, 0.6414)	1
VGutC	0.9871 (0.817, 1.162)	1	0.1219 (0.085, 0.1965)	1
VLivC	0.8035 (0.5609, 1.093)	1.013	0.2216 (0.1552, 0.3488)	1
VRapC	0.997 (0.8627, 1.131)	1	0.09384 (0.06519, 0.1512)	1
VRespLumC	0.9995 (0.8536, 1.145)	1	0.1027 (0.07172, 0.1639)	1
VRespEffC	1 (0.8537, 1.148)	1.001	0.1032 (0.07176, 0.1652)	1
VKidC	1.001 (0.8676, 1.134)	1	0.09365 (0.06523, 0.1494)	1
VBldC	0.9916 (0.8341, 1.153)	1.001	0.1126 (0.07835, 0.1817)	1
lnPBC	0.9259 (0.647, 1.369)	1	1.644 (1.278, 3.682)	1
lnPFatC	0.9828 (0.7039, 1.431)	1.001	1.321 (1.16, 2.002)	1.001
lnPGutC	0.805 (0.4735, 1.418)	1	1.375 (1.198, 2.062)	1
lnPLivC	1.297 (0.7687, 2.039)	1	1.415 (1.21, 2.342)	1
lnPRapC	0.9529 (0.5336, 1.721)	1	1.378 (1.203, 2.141)	1
lnPRespC	0.9918 (0.5566, 1.773)	1.001	1.378 (1.2, 2.066)	1
lnPKidC	1.277 (0.7274, 2.089)	1	1.554 (1.265, 2.872)	1
lnPSlwC	0.92 (0.5585, 1.586)	1.001	1.411 (1.209, 2.3)	1.001
lnPRBCPlasTCAC	2.495 (1.144, 5.138)	1.001	1.398 (1.178, 2.623)	1.001
lnPBodTCAC	0.8816 (0.6219, 1.29)	1.003	1.27 (1.158, 1.609)	1
lnPLivTCAC	0.8003 (0.5696, 1.15)	1.003	1.278 (1.157, 1.641)	1.001
lnkDissocC	1.214 (0.2527, 4.896)	1.003	2.71 (1.765, 8.973)	1

Table A-9. Posterior distributions for mouse PBPK model population parameters (continued)

Sampled parameter[a]	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population GSD	
	Median (2.5, 97.5%)	*R*	Median (2.5, 97.5%)	*R*
lnBMaxkDC	1.25 (0.6793, 2.162)	1.002	1.474 (1.253, 2.383)	1
lnPBodTCOHC	0.8025 (0.5607, 1.174)	1	1.314 (1.17, 1.85)	1.001
lnPLivTCOHC	1.526 (0.9099, 2.245)	1	1.399 (1.194, 2.352)	1
lnPBodTCOGC	0.4241 (0.1555, 1.053)	1.004	1.398 (1.207, 2.156)	1
lnPLivTCOGC	1.013 (0.492, 2.025)	1.002	1.554 (1.279, 2.526)	1
lnPeffDCVG	0.9807 (0.008098, 149.6)	1.041	1.406 (1.206, 2.379)	1
lnkTSD	5.187 (0.3909, 69.34)	1.001	5.858 (2.614, 80)	1
lnkAS	1.711 (0.3729, 11.23)	1.001	4.203 (2.379, 18.15)	1
lnkTD	0.1002 (0.01304, 0.7688)	1	5.16 (2.478, 60.24)	1
lnkAD	0.2665 (0.05143, 1.483)	1.003	4.282 (2.378, 20.21)	1
lnkASTCA	3.986 (0.1048, 141.9)	1	5.187 (2.516, 58.72)	1
lnkASTCOH	0.7308 (0.006338, 89.75)	1.001	5.047 (2.496, 54.8)	1
$lnV_{MAX}C$	0.6693 (0.4093, 1.106)	1.005	1.793 (1.49, 2.675)	1
lnK_MC	0.07148 (0.0323, 0.1882)	1	2.203 (1.535, 4.536)	1.001
lnFracOtherC	0.02384 (0.003244, 0.1611)	1.006	1.532 (1.265, 2.971)	1
lnFracTCAC	0.4875 (0.2764, 0.8444)	1.002	1.474 (1.258, 2.111)	1
lnV_{MAX}DCVGC	1.517 (0.02376, 1,421)	1.001	1.53 (1.263, 2.795)	1
lnClDCVGC	0.1794 (0.02333, 79.69)	1.013	1.528 (1.261, 2.922)	1
lnV_{MAX}KidDCVGC	1.424 (0.04313, 704.9)	1.014	1.533 (1.262, 2.854)	1
lnClKidDCVGC	0.827 (0.04059, 167.2)	1.019	1.527 (1.263, 2.874)	1
lnV_{MAX}LungLivC	2.903 (0.487, 12.1)	1.001	4.157 (1.778, 29.01)	1.018
lnK_MClara	0.01123 (0.001983, 0.09537)	1.012	1.629 (1.278, 5.955)	1.003
lnFracLungSysC	3.304 (0.2619, 182.1)	1.011	1.543 (1.266, 3.102)	1.001
lnV_{MAX}TCOHC	1.645 (0.6986, 3.915)	1.005	1.603 (1.28, 2.918)	1
lnK_MTCOH	0.9594 (0.2867, 2.778)	1.007	1.521 (1.264, 2.626)	1
lnV_{MAX}GlucC	65.59 (27.58, 232.5)	1.018	1.487 (1.254, 2.335)	1
lnK_MGluc	31.16 (6.122, 137.3)	1.015	1.781 (1.299, 5.667)	1.002
lnkMetTCOHC	3.629 (0.7248, 9.535)	1.009	1.527 (1.265, 2.626)	1
lnkUrnTCAC	0.1126 (0.04083, 0.2423)	1.012	1.757 (1.318, 3.281)	1.003
lnkMetTCAC	0.6175 (0.2702, 1.305)	1.027	1.508 (1.262, 2.352)	1.002
lnkBileC	0.9954 (0.316, 3.952)	1.003	1.502 (1.26, 2.453)	1
lnkEHRC	0.01553 (0.001001, 0.0432)	1.008	1.534 (1.264, 2.767)	1
lnkUrnTCOGC	7.874 (2.408, 50.28)	1	3.156 (1.783, 12.18)	1.001
lnFracKidDCVCC	1.931 (0.01084, 113.7)	1.018	1.53 (1.264, 2.77)	1
lnkDCVGC	0.2266 (0.001104, 16.46)	1.011	1.525 (1.263, 2.855)	1
lnkNATC	0.1175 (0.0008506, 14.34)	1.024	1.528 (1.264, 2.851)	1
lnkKidBioactC	0.07506 (0.0009418, 12.35)	1.035	1.527 (1.263, 2.84)	1.001

[a]These "sampled parameters" are scaled one or more times (see Table A-4) to obtain a biologically-meaningful parameter, posterior distributions of which are summarized in Tables 3-36 through 3-40). For natural log transformed parameters (name starting with "ln"), values are for the population geometric means and SDs.

Table A-10. Posterior distributions for mouse residual errors

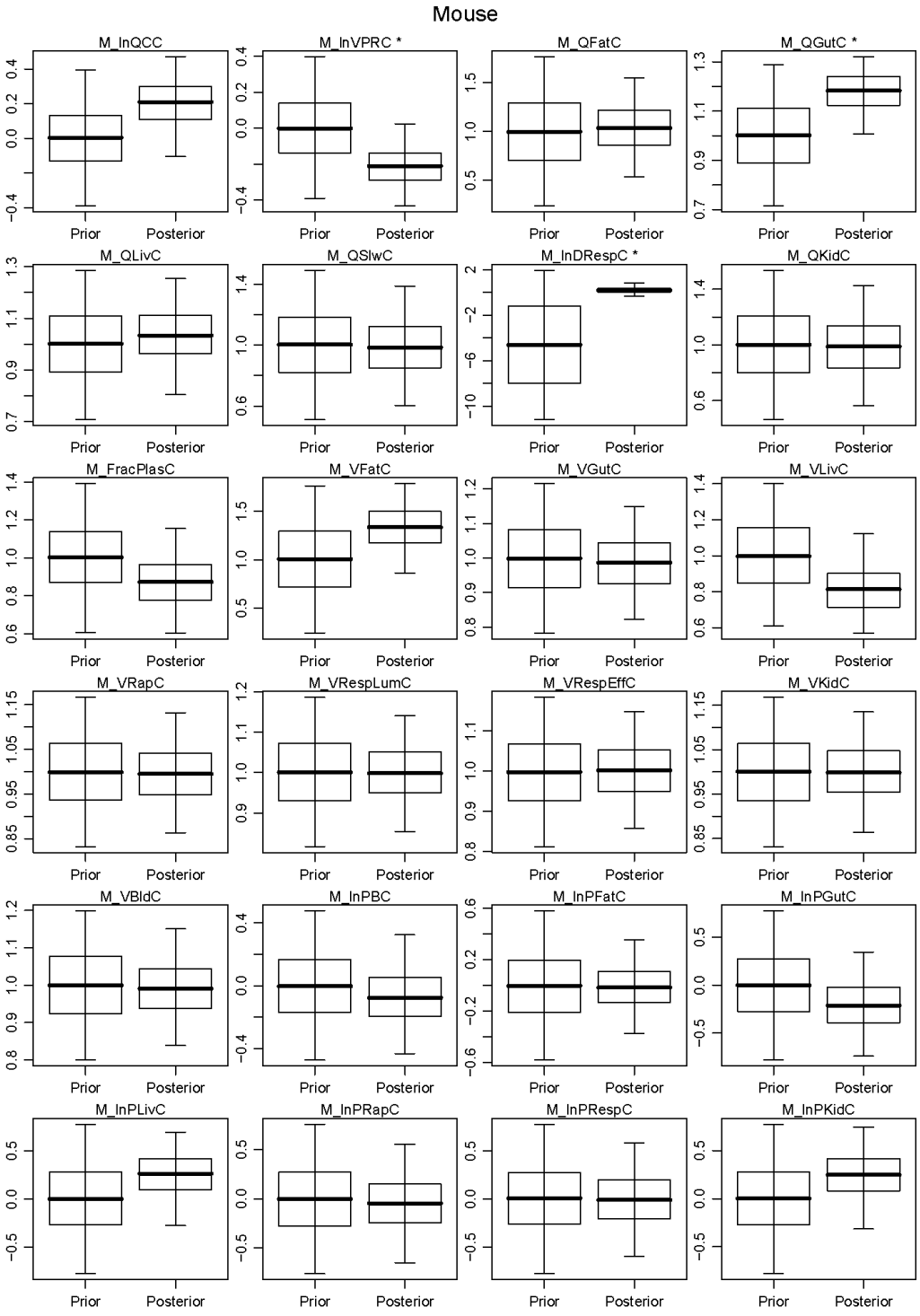
Measurement	Residual error GSD	
	Median (2.5, 97.5%)	***R***
CInhPPM	1.177 (1.16, 1.198)	1.001
CVen	2.678 (2.354, 3.146)	1.001
CBldMix	1.606 (1.415, 1.96)	1.001
CFat	2.486 (2.08, 3.195)	1
CKid	2.23 (1.908, 2.796)	1
CLiv	1.712 (1.543, 1.993)	1
AExhpost	1.234 (1.159, 1.359)	1
CTCOH	1.543 (1.424, 1.725)	1
CLivTCOH	1.591 (1.454, 1.818)	1
CPlasTCA	1.396 (1.338, 1.467)	1.001
CBldTCA	1.488 (1.423, 1.572)	1.001
CLivTCA	1.337 (1.271, 1.43)	1
AUrnTCA	1.338 (1.259, 1.467)	1
CTCOGTCOH	1.493 (1.38, 1.674)	1.001
CLivTCOGTCOH	1.63 (1.457, 1.924)	1
AUrnTCOGTCOH	1.263 (1.203, 1.355)	1
TotCTCOH	1.846 (1.506, 2.509)	1.002

Note: the hierarchical statistical model for residual errors did not separate by subject.

Table A-11. Posterior correlations for mouse population mean parameters

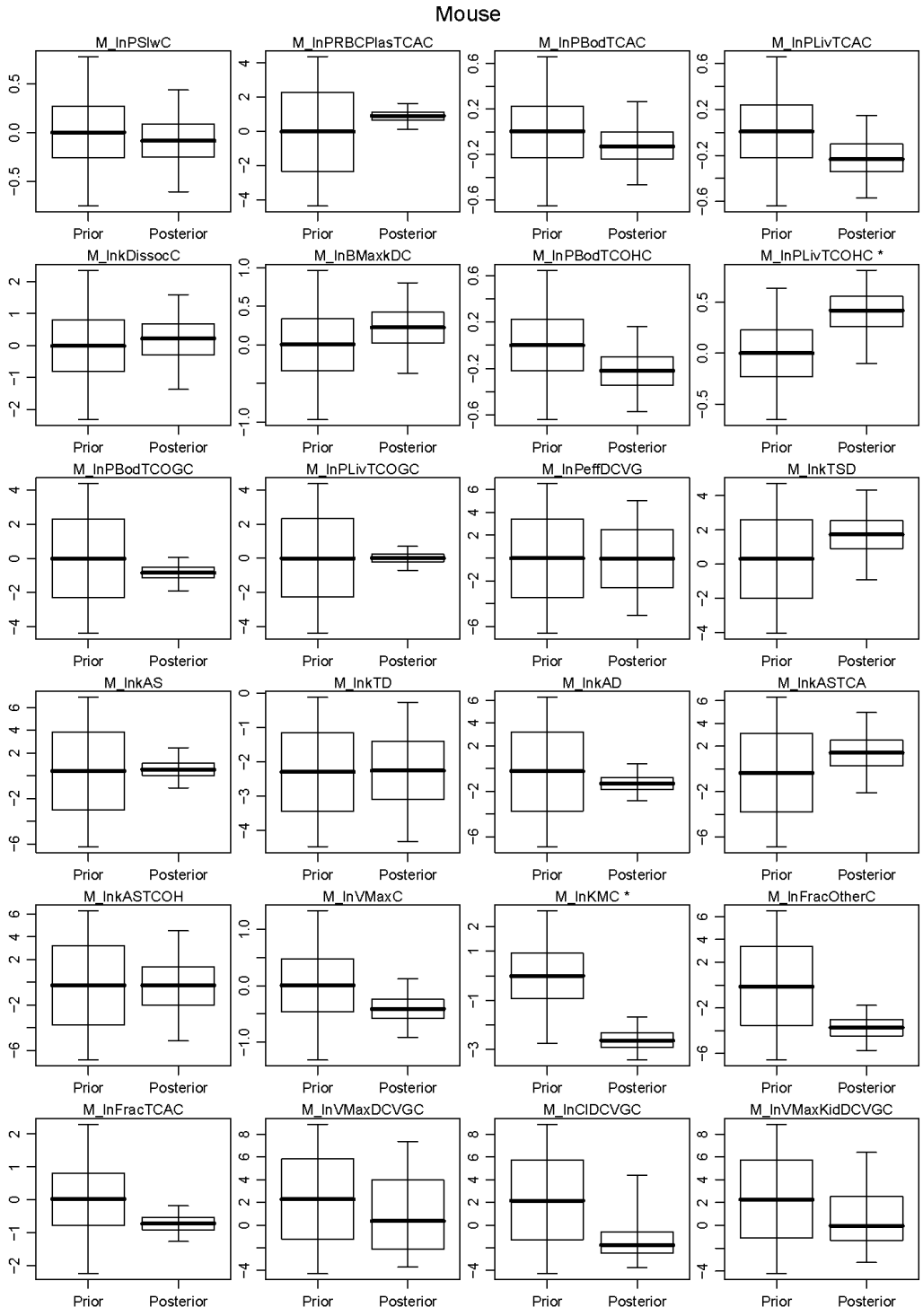
Mouse		
Parameter 1	**Parameter 2**	**Correlation coefficient.**
lnKMGluc	lnV_{MAX}GlucC	0.765
lnClDCVGC	lnV_{MAX}DCVGC	-0.553
lnkMetTCAC	lnkUrnTCAC	-0.488
lnKMTCOH	lnV_{MAX}TCOHC	0.464
lnClKidDCVGC	lnV_{MAX}KidDCVGC	-0.394
lnkUrnTCAC	lnPRBCPlasTCAC	0.358
lnkDissocC	lnPBodTCAC	0.328
lnkEHRC	lnkMetTCOHC	0.314
lnV_{MAX}C	VLivC	-0.305
lnKMClara	lnV_{MAX}LungLivC	0.302
lnBMaxkDC	lnPLivTCAC	0.299
lnKMGluc	lnKMTCOH	0.293
lnkBileC	lnkEHRC	-0.280
lnkEHRC	lnKMTCOH	-0.273
lnPBodTCOGC	lnV_{MAX}GlucC	0.269
lnFracTCAC	lnV_{MAX}TCOHC	-0.267
lnkMetTCAC	lnPBodTCAC	0.264
lnkDissocC	lnPLivTCAC	0.253
lnPSlwC	QFatC	-0.252

Note: only parameter pairs with correlation coefficient ≥0.25 are listed.



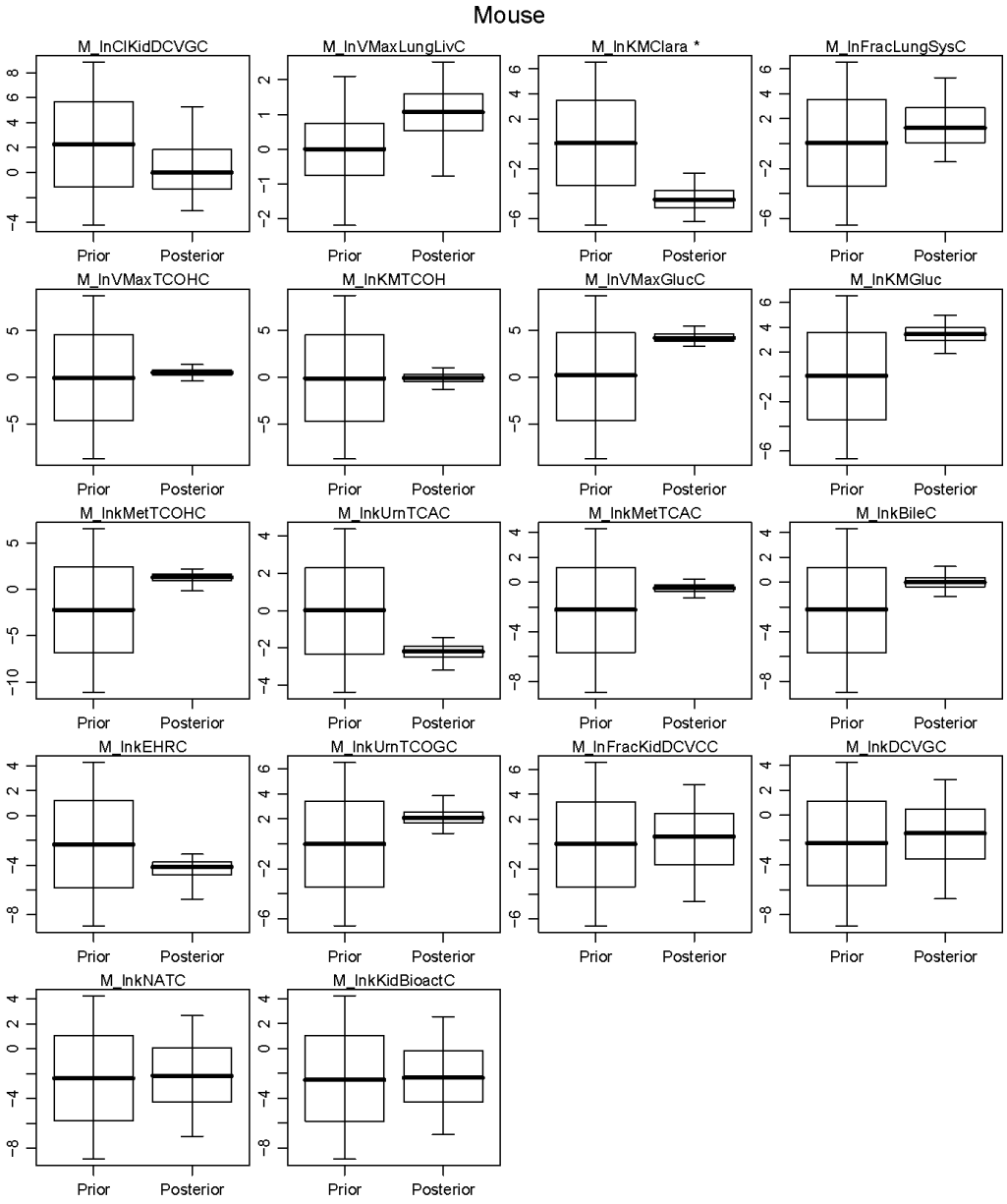
Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-13. Prior and posterior mouse population mean parameters (Part 1).



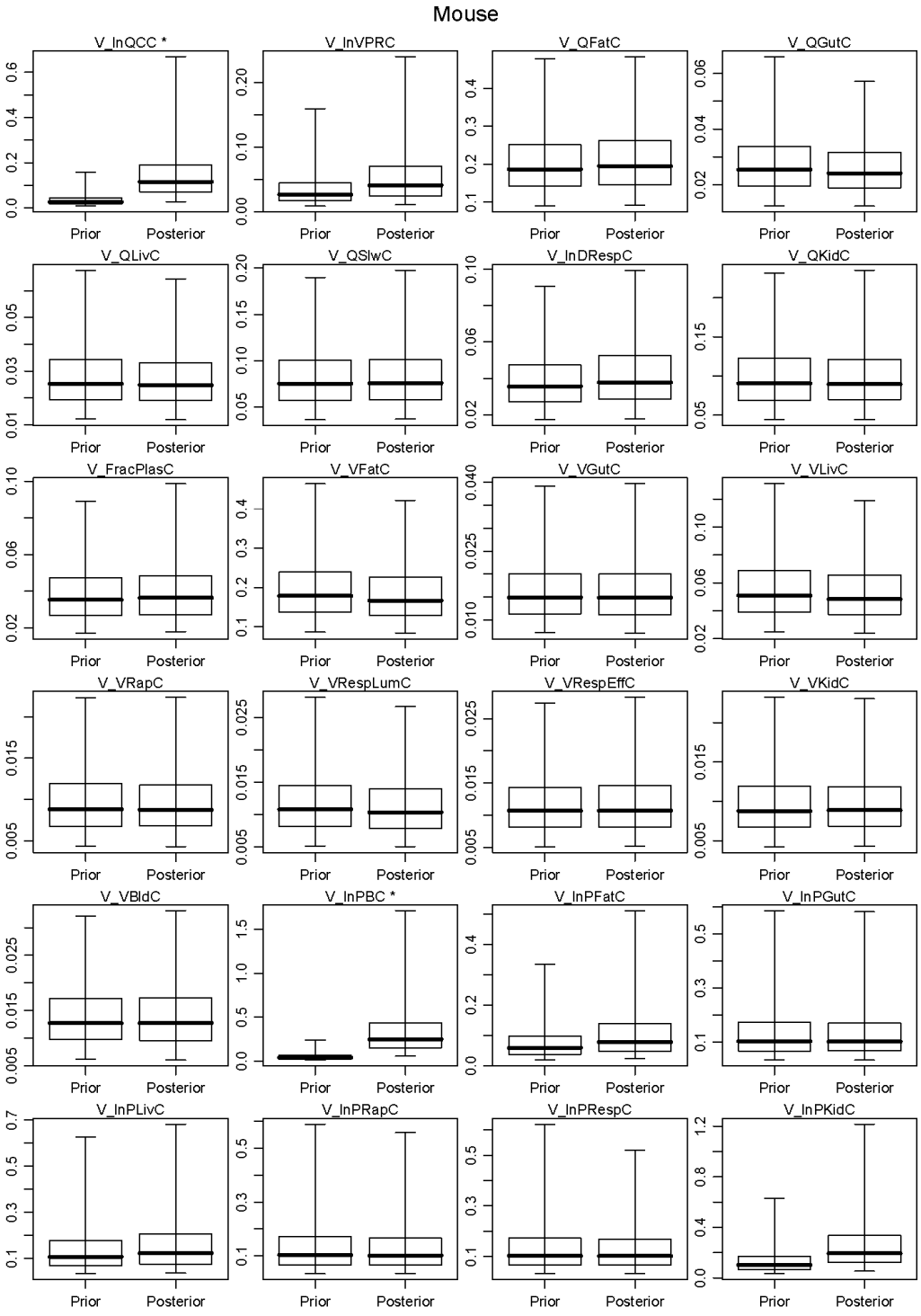
Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-14. Prior and posterior mouse population mean parameters (Part 2).



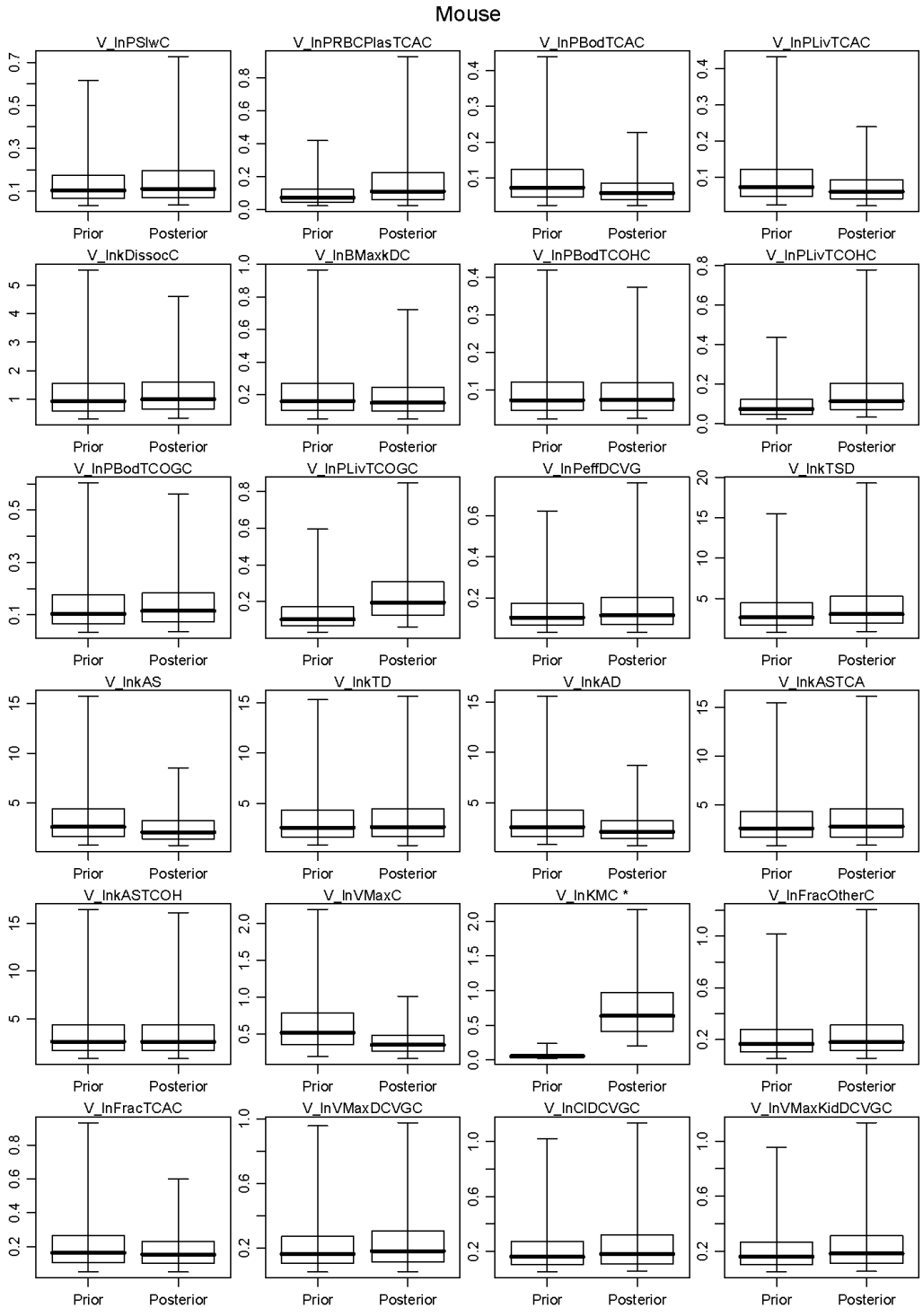
Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-15. Prior and posterior mouse population mean parameters (Part 3).



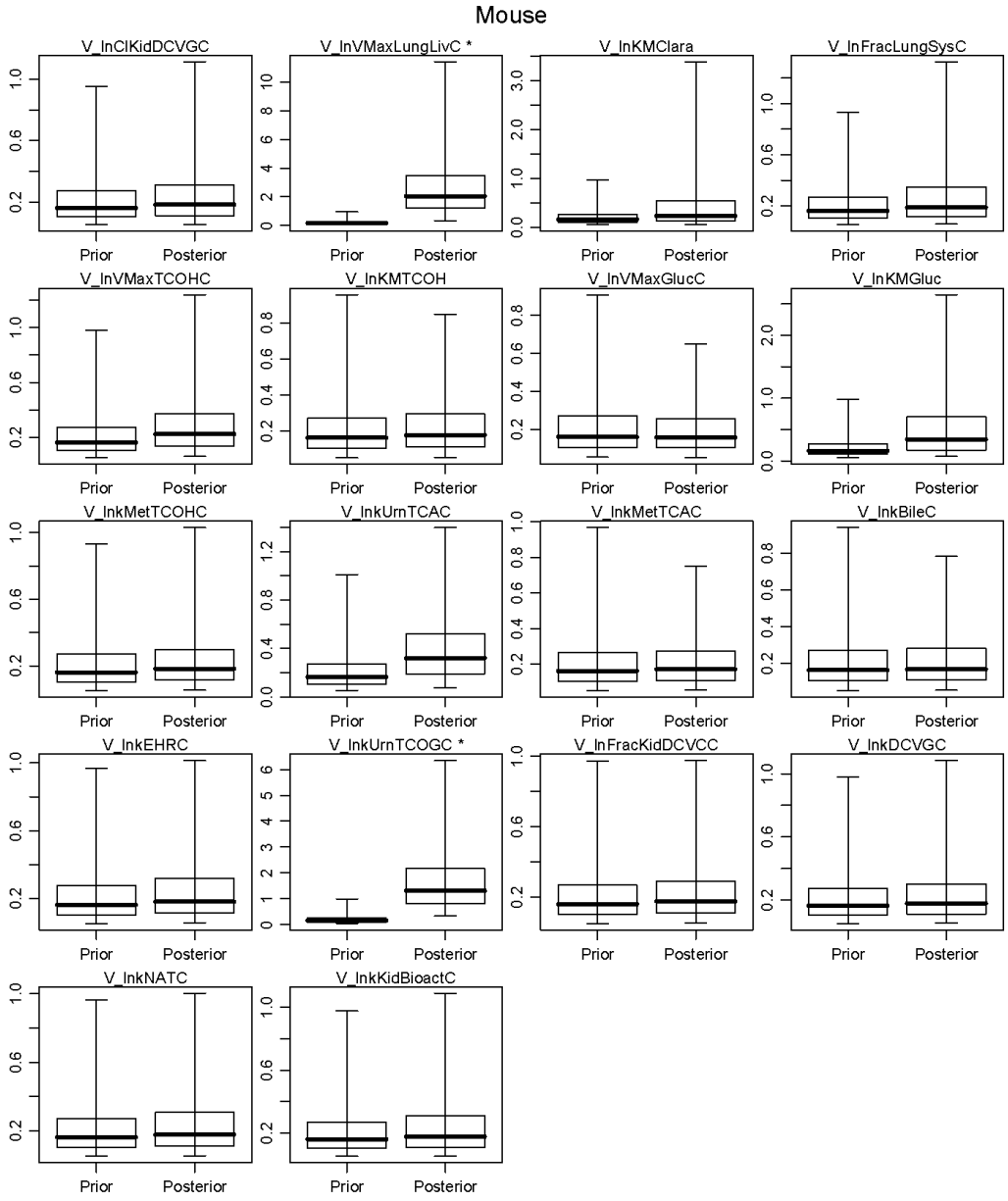
Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-16. Prior and posterior mouse population variance parameters (Part 1).



Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-17. Prior and posterior mouse population variance parameters (Part 2).



Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-18. Prior and posterior mouse population variance parameters (Part 3).

Table A-12. Posterior distributions for rat PBPK model population parameters

	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population GSD	
Sampled parameter	Median (2.5, 97.5%)	*R*	Median (2.5, 97.5%)	*R*
lnQCC	1.195 (0.9285, 1.448)	1.034	1.298 (1.123, 2.041)	1.031
lnVPRC	0.6304 (0.4788, 0.8607)	1.012	1.446 (1.247, 2.011)	1.005
QFatC	1.167 (0.8321, 1.561)	1	0.4119 (0.2934, 0.6438)	1
QGutC	1.154 (0.988, 1.306)	1	0.1613 (0.1132, 0.2542)	1
QLivC	1.029 (0.8322, 1.223)	1.002	0.1551 (0.1092, 0.2483)	1
QSlwC	0.9086 (0.5738, 1.251)	1.001	0.2817 (0.1968, 0.4493)	1
lnDRespC	2.765 (1.391, 5.262)	1.018	1.21 (1.142, 1.358)	1.001
QKidC	1.002 (0.8519, 1.152)	1.001	0.1185 (0.08284, 0.1871)	1
FracPlasC	1.037 (0.8071, 1.259)	1.002	0.1785 (0.1272, 0.2723)	1
VFatC	0.9728 (0.593, 1.378)	1	0.4139 (0.2924, 0.6552)	1.002
VGutC	0.9826 (0.8321, 1.137)	1	0.1187 (0.08296, 0.1873)	1
VLivC	0.9608 (0.7493, 1.19)	1.015	0.1682 (0.1168, 0.2718)	1.001
VRapC	0.9929 (0.8563, 1.133)	1.001	0.1093 (0.07693, 0.175)	1
VRespLumC	1.001 (0.7924, 1.21)	1	0.1636 (0.116, 0.2601)	1
VRespEffC	0.999 (0.7921, 1.208)	1.001	0.1635 (0.1161, 0.2598)	1
VKidC	0.999 (0.8263, 1.169)	1	0.1361 (0.09617, 0.2167)	1
VBldC	1.002 (0.8617, 1.141)	1	0.1096 (0.07755, 0.176)	1
lnPBC	0.8551 (0.6854, 1.065)	1.001	1.317 (1.232, 1.462)	1.001
lnPFatC	1.17 (0.8705, 1.595)	1.003	1.333 (1.247, 1.481)	1.001
lnPGutC	0.8197 (0.5649, 1.227)	1	1.362 (1.198, 1.895)	1
lnPLivC	1.046 (0.8886, 1.234)	1.001	1.152 (1.115, 1.214)	1
lnPRapC	1.021 (0.6239, 1.675)	1.002	1.373 (1.201, 1.988)	1
lnPRespC	0.993 (0.5964, 1.645)	1.001	1.356 (1.197, 1.948)	1
lnPKidC	0.9209 (0.6728, 1.281)	1	1.304 (1.201, 1.536)	1
lnPSlwC	1.258 (0.9228, 1.711)	1.001	1.364 (1.263, 1.544)	1
lnPRBCPlasTCAC	0.9763 (0.6761, 1.353)	1	1.276 (1.159, 1.634)	1
lnPBodTCAC	1.136 (0.6737, 1.953)	1.008	1.631 (1.364, 2.351)	1.003
lnPLivTCAC	1.283 (0.6425, 2.491)	1.008	1.651 (1.356, 2.658)	1
lnkDissocC	1.01 (0.5052, 2.017)	1.002	1.596 (1.315, 2.774)	1
lnBMaxkDC	0.9654 (0.5716, 1.733)	1.02	1.412 (1.234, 2.01)	1
lnPBodTCOHC	0.9454 (0.4533, 1.884)	1.045	1.734 (1.39, 3.151)	1.002
lnPLivTCOHC	0.926 (0.3916, 2.196)	1.013	1.785 (1.382, 4.142)	1.003
lnPBodTCOGC	1.968 (0.09185, 14.44)	1.031	1.414 (1.208, 2.571)	1
lnPLivTCOGC	7.484 (2.389, 26.92)	1.017	1.41 (1.208, 2.108)	1
lnkTSD	3.747 (0.2263, 62.58)	1.01	6.777 (2.844, 87.29)	1
lnkAS	2.474 (0.2542, 28.35)	1.004	10.16 (4.085, 143.7)	1
lnkAD	0.1731 (0.04001, 0.7841)	1.018	4.069 (2.373, 14.19)	1.009
lnkASTCA	1.513 (0.1401, 17.19)	1.002	4.376 (2.43, 22.83)	1
lnkASTCOH	0.6896 (0.01534, 25.81)	1.001	4.734 (2.444, 35.2)	1.001
lnV_{MAX}C	0.8948 (0.6377, 1.293)	1.028	1.646 (1.424, 2.146)	1.021

Table A-12. Posterior distributions for rat PBPK model population parameters (continued)

	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population GSD	
Sampled parameter	Median (2.5, 97.5%)	*R*	Median (2.5, 97.5%)	*R*
lnK_MC	0.0239 (0.01602, 0.04993)	1.001	2.402 (1.812, 4.056)	1.001
lnFracOtherC	0.344 (0.0206, 1.228)	1.442	3 (1.332, 10.04)	1.353
lnFracTCAC	0.2348 (0.122, 0.4616)	1.028	1.517 (1.264, 2.393)	1.001
lnV_{MAX}DCVGC	7.749 (0.2332, 458.8)	1.088	1.534 (1.262, 2.804)	1.001
lnClDCVGC	0.3556 (0.06631, 2.242)	1.018	1.509 (1.261, 2.553)	1
lnV_{MAX}KidDCVGC	0.2089 (0.04229, 1.14)	1.011	1.542 (1.263, 2.923)	1.001
lnClKidDCVGC	184 (26.29, 1312)	1.02	1.527 (1.265, 2.873)	1.001
lnV_{MAX}LungLivC	2.673 (0.4019, 14.16)	1.002	4.833 (1.599, 48.32)	1.002
lnK_MClara	0.02563 (0.005231, 0.197)	1.01	1.66 (1.279, 18.74)	1.002
lnFracLungSysC	2.729 (0.04124, 63.27)	1.027	1.536 (1.267, 2.868)	1.001
lnV_{MAX}TCOHC	1.832 (0.6673, 6.885)	1.041	1.667 (1.292, 3.148)	1.002
lnK_MTCOH	22.09 (3.075, 131.9)	1.186	1.629 (1.276, 3.773)	1.017
lnV_{MAX}GlucC	28.72 (10.02, 86.33)	1.225	2.331 (1.364, 5.891)	1.126
lnK_MGluc	6.579 (1.378, 23.57)	1.119	2.046 (1.309, 10.3)	1.125
lnkMetTCOHC	2.354 (0.3445, 15.83)	1.287	1.876 (1.283, 11.82)	1.182
lnkUrnTCAC	0.07112 (0.03934, 0.1329)	1.076	1.513 (1.27, 2.327)	1.003
lnkMetTCAC	0.3554 (0.1195, 0.8715)	1.036	1.528 (1.263, 2.444)	1.001
lnkBileC	8.7 (1.939, 26.71)	1.05	1.65 (1.282, 5.494)	1.017
lnkEHRC	1.396 (0.2711, 6.624)	1.091	1.647 (1.277, 5.582)	1.005
lnkUrnTCOGC	20.65 (2.437, 138)	1.041	1.595 (1.269, 5.257)	1.026
lnkNATC	0.002035 (0.0004799, 0.01019)	1.01	1.523 (1.261, 2.593)	1.001
lnkKidBioactC	0.006618 (0.0009409, 0.0367)	1.039	1.52 (1.261, 2.674)	1

Table A-13. Posterior distributions for rat residual errors

Measurement	Subject[a]	Residual error GSD	
		Median (2.5, 97.5%)	*R*
CInhPPM	Subject 3	1.124 (1.108, 1.147)	1
	Subject 16	1.106 (1.105, 1.111)	1
CMixExh	Subject 2	1.501 (1.398, 1.65)	1
Cart	Subject 2	1.174 (1.142, 1.222)	1
	Subject 6	1.523 (1.321, 1.918)	1.002
CVen	Subject 4	1.22 (1.111, 1.877)	1
	Subject 7	1.668 (1.489, 1.986)	1.001
	Subject 8	1.45 (1.234, 2.065)	1.014
	Subject 9	1.571 (1.426, 1.811)	1
	Subject 10	4.459 (2.754, 6.009)	1
	Subject 11	1.587 (1.347, 2.296)	1.002
	Subject 16	1.874 (1.466, 2.964)	1.011
	Subject 18	1.676 (1.188, 3.486)	1.003
CBldMix	Subject 12	1.498 (1.268, 2.189)	1
CFat	Subject 9	1.846 (1.635, 2.184)	1
	Subject 16	2.658 (1.861, 4.728)	1.001
CGut	Subject 9	1.855 (1.622, 2.243)	1
CKid	Subject 9	1.469 (1.354, 1.648)	1
CLiv	Subject 9	1.783 (1.554, 2.157)	1
	Subject 12	1.744 (1.401, 2.892)	1
	Subject 16	1.665 (1.376, 2.411)	1.001
CMus	Subject 9	1.653 (1.494, 1.919)	1
AExhpost	Subject 6	1.142 (1.108, 1.239)	1.003
	Subject 10	1.117 (1.106, 1.184)	1.004
	Subject 14	1.166 (1.107, 1.475)	1
	Subject 15	1.125 (1.106, 1.237)	1
CTCOH	Subject 6	1.635 (1.455, 1.983)	1.002
	Subject 10	1.259 (1.122, 1.868)	1.009
	Subject 11	1.497 (1.299, 1.923)	1.01
	Subject 13	1.611 (1.216, 3.556)	1.001
	Subject 17	1.45 (1.213, 2.208)	1.004
	Subject 18	1.142 (1.107, 1.268)	1
CPlasTCA	Subject 4	1.134 (1.106, 1.254)	1
	Subject 5	1.141 (1.107, 1.291)	1
	Subject 11	1.213 (1.136, 1.381)	1
	Subject 19	1.201 (1.145, 1.305)	1

Table A-13. Posterior distributions for rat residual errors (continued)

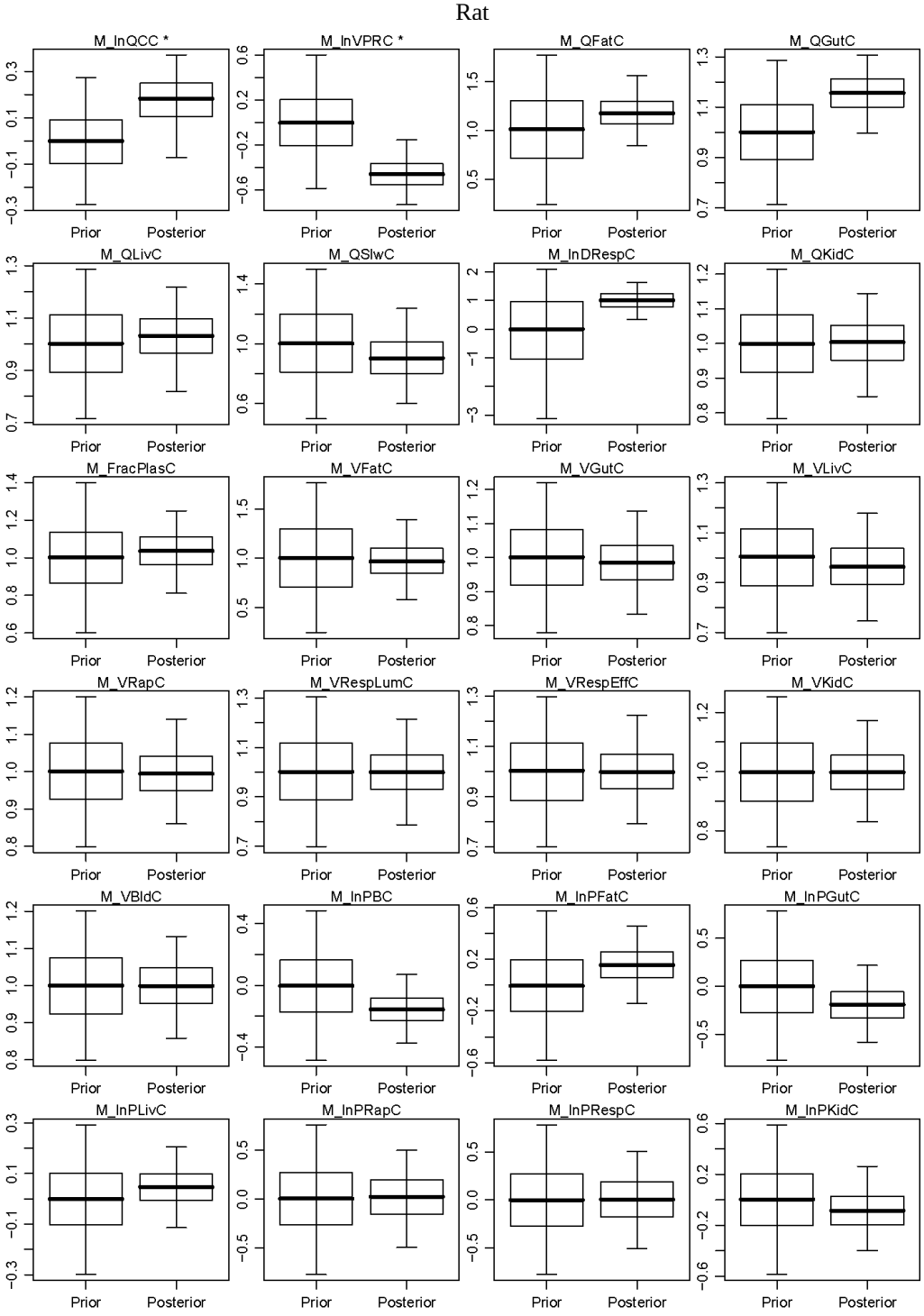
Measurement	Subject[a]	Residual error GSD	
		Median (2.5, 97.5%)	*R*
CBldTCA	Subject 4	1.134 (1.106, 1.258)	1
	Subject 5	1.14 (1.107, 1.289)	1
	Subject 6	1.59 (1.431, 1.878)	1.001
	Subject 11	1.429 (1.292, 1.701)	1.001
	Subject 17	1.432 (1.282, 1.675)	1.03
	Subject 18	1.193 (1.12, 1.358)	1.004
	Subject 19	1.214 (1.153, 1.327)	1
CLivTCA	Subject 19	1.666 (1.443, 2.104)	1
AUrnTCA	Subject 1	1.498 (1.125, 2.18)	1.135
	Subject 6	1.95 (1.124, 5.264)	1.003
	Subject 8	1.221 (1.146, 1.375)	1.003
	Subject 10	1.18 (1.108, 1.444)	1.007
	Subject 17	1.753 (1.163, 4.337)	1.001
	Subject 19	1.333 (1.201, 1.707)	1
ABileTCOG	Subject 6	2.129 (1.128, 5.363)	1.003
CTCOG	Subject 17	2.758 (1.664, 5.734)	1.028
AUrnTCOGTCOH	Subject 1	1.129 (1.106, 1.232)	1.004
	Subject 6	1.483 (1.113, 4.791)	1.002
	Subject 8	1.115 (1.106, 1.162)	1
	Subject 10	1.145 (1.107, 1.305)	1
	Subject 17	2.27 (1.53, 4.956)	1.009
AUrnNDCVC	Subject 1	1.168 (1.11, 1.33)	1.002
AUrnTCTotMole	Subject 6	1.538 (1.182, 3.868)	1.002
	Subject 7	1.117 (1.106, 1.153)	1.001
	Subject 14	1.121 (1.106, 1.207)	1
	Subject 15	1.162 (1.108, 1.358)	1
TotCTCOH	Subject 17	1.488 (1.172, 2.366)	1.015

[a]The nineteen subjects are: (1) Bernauer et al. (1996); (2) Dallas et al. (1991); (3) Fisher et al. (1989) females; (4) Fisher et al. (1991) females; (5) Fisher et al. (1991) males; (6) Green and Prout (1985), Prout et al. (1985), male OA rats; (7) Hissink et al. (2002); (8) Kaneko et al. (1994) (9) Keys et al. (2003); (10) Kimmerle and Eben (1973b); (11) Larson and Bull (1992b, a); (12) Lee et al. (2000a); (13) Merdink et al. (1999); (14) Prout et al. (1985) AP rats; (15) Prout et al. (1985) OM rats; (16) Simmons et al. (2002); (17) Stenner et al. (1997); (18) Templin et al. (1995b); and (19) Yu et al. (2000).

Table A-14. Posterior correlations for rat population mean parameters

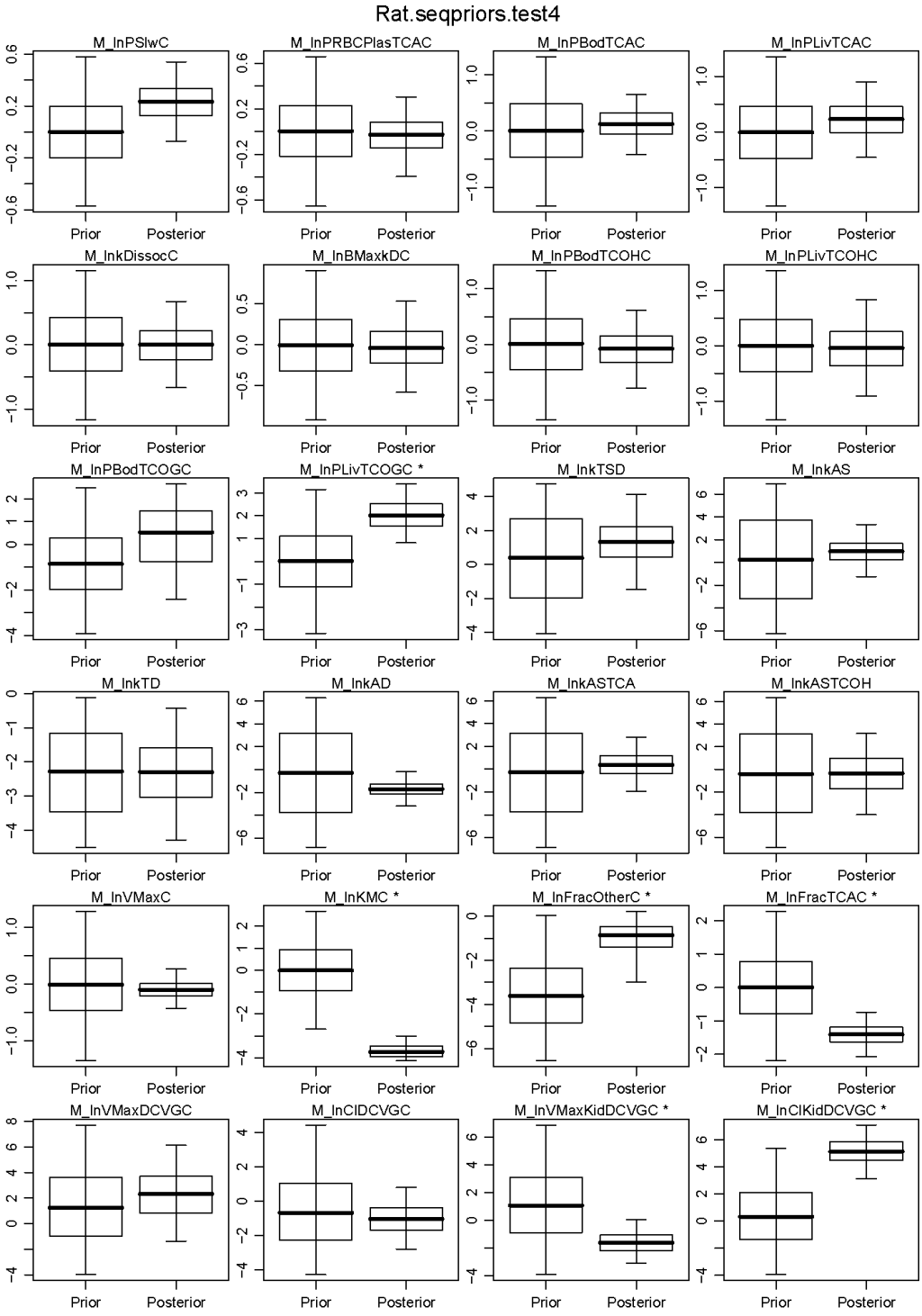
Rat		
Parameter 1	**Parameter 2**	**Correlation coefficient**
lnkNATC	lnV_{MAX}KidDCVGC	-0.599
lnkBileC	lnPLivTCOGC	-0.587
lnKMTCOH	lnV_{MAX}TCOHC	0.567
lnKMGluc	lnV_{MAX}GlucC	0.506
lnClKidDCVGC	lnkNATC	-0.497
lnkUrnTCAC	lnPBodTCAC	0.421
lnV_{MAX}C	VLivC	-0.417
lnBMaxkDC	lnkUrnTCAC	0.397
lnkUrnTCOGC	lnPBodTCOGC	-0.389
lnPFatC	VFatC	-0.385
lnClKidDCVGC	lnV_{MAX}KidDCVGC	0.384
lnKMGluc	lnKMTCOH	0.383
lnPLivTCOGC	lnV_{MAX}GlucC	0.358
lnBMaxkDC	lnPBodTCAC	0.352
lnClDCVGC	lnClKidDCVGC	0.343
FracPlasC	lnPRBCPlasTCAC	-0.337
lnClDCVGC	lnkNATC	-0.331
lnkEHRC	lnV_{MAX}GlucC	0.322
lnkBileC	lnkUrnTCOGC	0.307
lnFracLungSysC	lnFracOtherC	0.304
lnFracOtherC	lnkMetTCOHC	-0.296
lnFracLungSysC	lnKMTCOH	-0.271
lnkMetTCAC	lnPBodTCAC	0.264
lnkMetTCAC	VLivC	-0.261
lnKMTCOH	lnPBodTCOGC	-0.260
lnFracTCAC	lnKMTCOH	0.258
lnDRespC	lnVPRC	0.254
lnFracOtherC	lnKMTCOH	-0.252

Note: only parameter pairs with correlation coefficient ≥0.25 are listed.



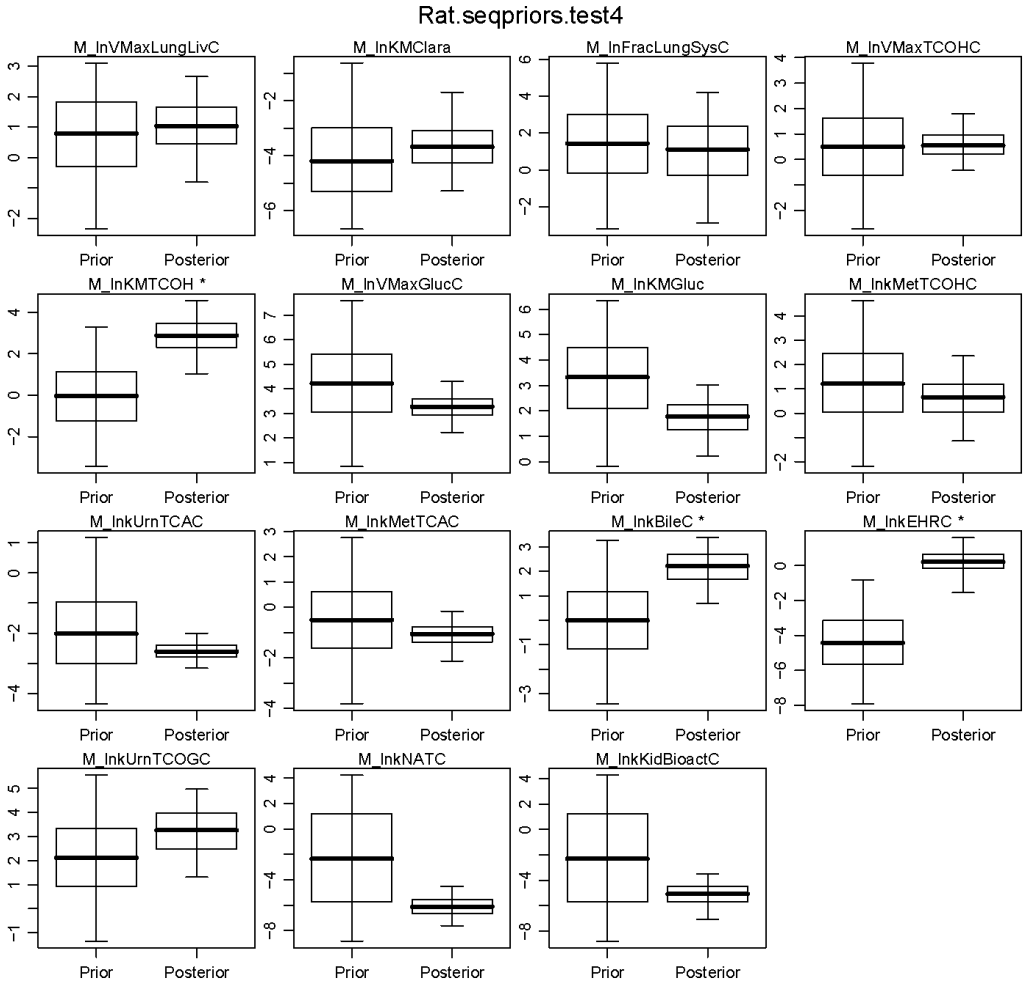
Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-19. Prior and posterior rat population mean parameters (Part 1).



Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-20. Prior and posterior rat population mean parameters (Part 2).



Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-21. Prior and posterior rat population mean parameters (Part 3).

Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

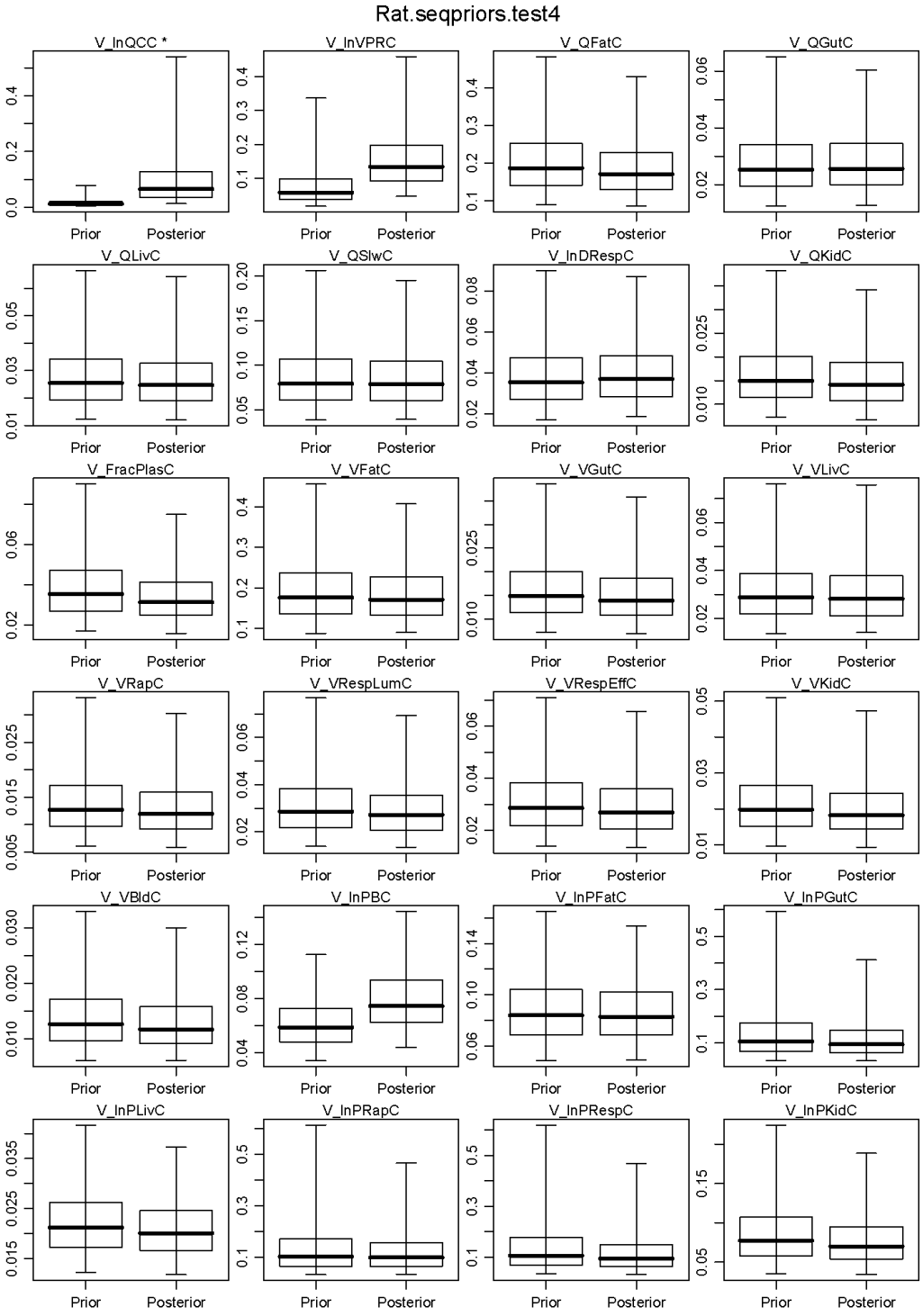
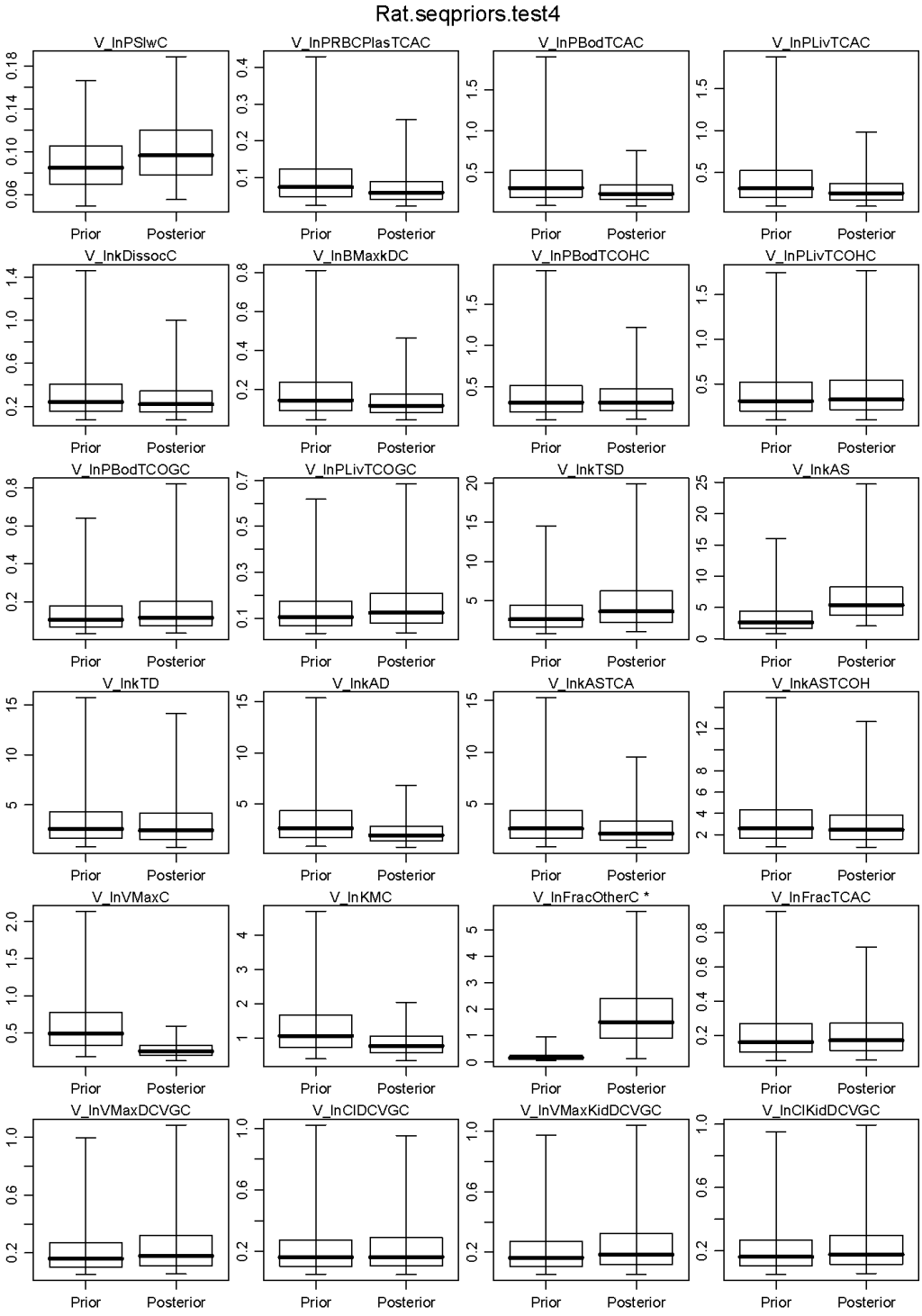
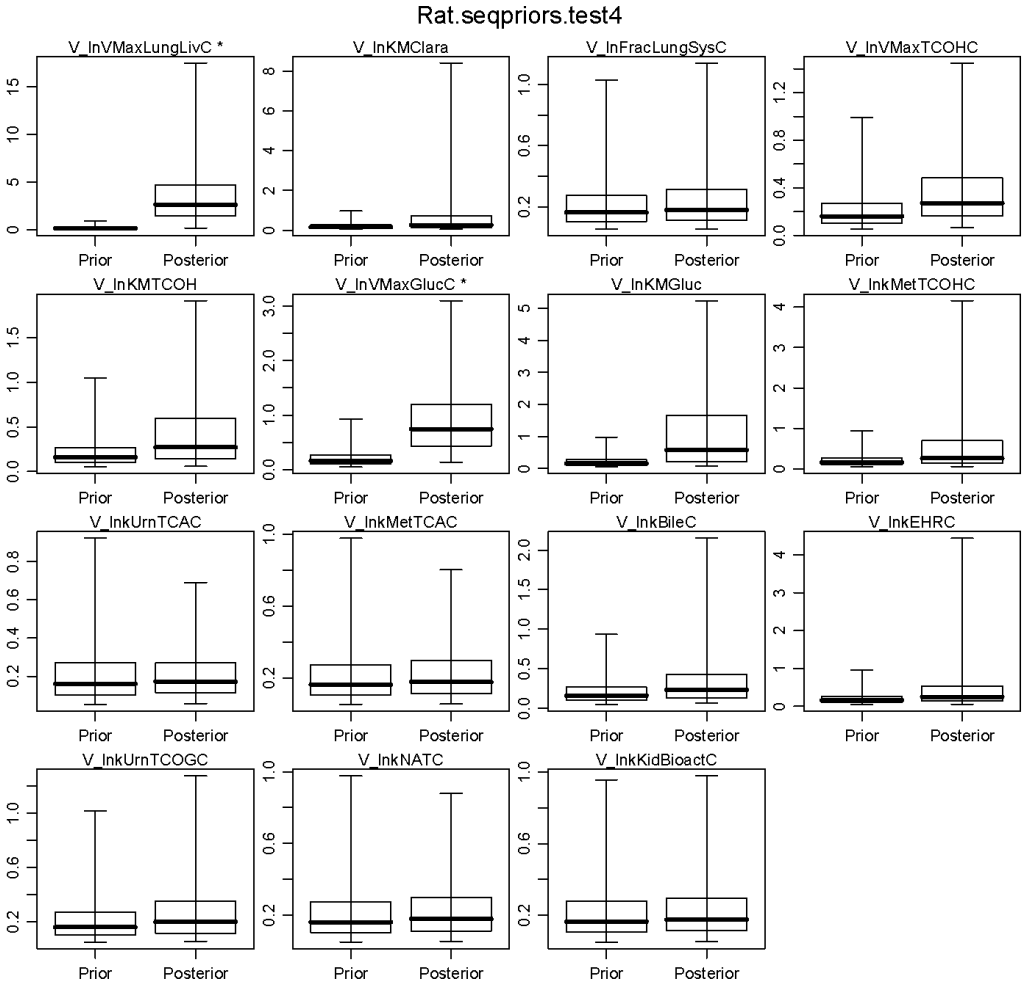


Figure A-22. Prior and posterior rat population variance parameters (Part 1).



Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-23. Prior and posterior rat population variance parameters (Part 2).



Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-24. Prior and posterior rat population variance parameters (Part 3).

Table A-15. Posterior distributions for human PBPK model population parameters

Sampled parameter	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population GSD	
	Median (2.5, 97.5%)	*R*	Median (2.5, 97.5%)	*R*
lnQCC	0.837 (0.6761, 1.022)	1.038	1.457 (1.271, 1.996)	1.036
lnVPRC	1.519 (1.261, 1.884)	1.007	1.497 (1.317, 1.851)	1.008
QFatC	0.7781 (0.405, 1.143)	1.014	0.6272 (0.4431, 0.9773)	1
QGutC	0.7917 (0.6631, 0.925)	1.017	0.1693 (0.1199, 0.2559)	1.019
QLivC	0.5099 (0.1737, 0.8386)	1.031	0.4167 (0.2943, 0.6324)	1.009
QSlwC	0.7261 (0.4864, 0.9234)	1.011	0.3166 (0.2254, 0.4802)	1.005
lnDRespC	0.626 (0.3063, 1.013)	1.197	1.291 (1.158, 2.006)	1.083
QKidC	1.007 (0.9137, 1.103)	1.009	0.1004 (0.07307, 0.1545)	1
FracPlasC	1.001 (0.9544, 1.047)	1.01	0.04275 (0.03155, 0.06305)	1
VFatC	0.788 (0.48, 1.056)	1.005	0.3666 (0.2696, 0.5542)	1
VGutC	1 (0.937, 1.067)	1.007	0.06745 (0.04923, 0.1038)	1
VLivC	1.043 (0.8683, 1.23)	1.047	0.1959 (0.1424, 0.3017)	1.003
VRapC	0.9959 (0.9311, 1.06)	1.006	0.06692 (0.04843, 0.1027)	1
VRespLumC	1.003 (0.8461, 1.164)	1.001	0.1671 (0.1209, 0.255)	1
VRespEffC	1 (0.8383, 1.159)	1.001	0.1672 (0.1215, 0.259)	1
VKidC	0.9965 (0.8551, 1.14)	1.007	0.1425 (0.1037, 0.2183)	1
VBldC	1.013 (0.9177, 1.108)	1.003	0.1005 (0.07265, 0.1564)	1
lnPBC	0.9704 (0.8529, 1.101)	1.001	1.216 (1.161, 1.307)	1.002
lnPFatC	0.8498 (0.7334, 0.9976)	1.002	1.188 (1.113, 1.366)	1.002
lnPGutC	1.095 (0.7377, 1.585)	1.029	1.413 (1.214, 2.05)	1.002
lnPLivC	0.9907 (0.6679, 1.441)	1.01	1.338 (1.203, 1.683)	1
lnPRapC	0.93 (0.6589, 1.28)	1.003	1.528 (1.248, 2.472)	1.001
lnPRespC	1.018 (0.6773, 1.5)	1.015	1.32 (1.192, 1.656)	1
lnPKidC	0.9993 (0.8236, 1.219)	1.003	1.155 (1.097, 1.287)	1
lnPSlwC	1.157 (0.8468, 1.59)	1.018	1.69 (1.383, 3.157)	1.008
lnPRBCPlasTCAC	0.3223 (0.04876, 0.8378)	1.007	5.507 (3.047, 19.88)	1.003
lnPBodTCAC	1.194 (0.929, 1.481)	1.043	1.327 (1.185, 1.67)	1.018
lnPLivTCAC	1.202 (0.8429, 1.634)	1.046	1.285 (1.162, 1.648)	1.007
lnkDissocC	0.9932 (0.9387, 1.053)	1.012	1.043 (1.026, 1.076)	1.003
lnBMaxkDC	0.8806 (0.7492, 1.047)	1.038	1.157 (1.085, 1.37)	1.012
lnPBodTCOHC	1.703 (1.439, 2.172)	1.019	1.409 (1.267, 1.678)	1.011
lnPLivTCOHC	1.069 (0.7643, 1.485)	1.028	1.288 (1.165, 1.629)	1.002
lnPBodTCOGC	0.7264 (0.1237, 2.54)	1.003	11.98 (5.037, 185.3)	1.017
lnPLivTCOGC	6.671 (1.545, 24.87)	1.225	5.954 (2.653, 23.68)	1.052
lnPeffDCVG	0.01007 (0.003264, 0.03264)	1.004	1.385 (1.201, 2.03)	1.001
lnkASTCA	4.511 (0.04731, 465.7)	1	5.467 (2.523, 71.06)	1
lnkASTCOH	8.262 (0.0677, 347.9)	1	5.481 (2.513, 67.86)	1
lnV_{MAX}C	0.3759 (0.2218, 0.5882)	1.026	2.21 (1.862, 2.848)	1.003
lnClC	12.64 (5.207, 39.96)	1.028	4.325 (2.672, 9.003)	1.016

Table A-15. Posterior distributions for human PBPK model population parameters (continued)

Sampled parameter	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population GSD	
	Median (2.5, 97.5%)	*R*	Median (2.5, 97.5%)	*R*
lnFracOtherC	0.1186 (0.02298, 0.2989)	1.061	3.449 (1.392, 9.146)	1.102
lnFracTCAC	0.1315 (0.07115, 0.197)	1.026	2.467 (1.916, 3.778)	1.01
lnClDCVGC	2.786 (1.326, 5.769)	1.08	2.789 (1.867, 4.877)	1.02
lnK_MDCVGC	1.213 (0.3908, 4.707)	1.029	4.43 (2.396, 18.56)	1.035
lnClKidDCVGC	0.04538 (0.001311, 0.1945)	1.204	3.338 (1.295, 30.46)	1.095
lnK_MKidDCVGC	0.2802 (0.1096, 1.778)	1.097	1.496 (1.263, 2.317)	1.001
lnV_{MAX}LungLivC	3.772 (0.8319, 9.157)	1.035	2.228 (1.335, 21.89)	1.014
lnK_MClara	0.2726 (0.02144, 1.411)	1.041	11.63 (1.877, 682.7)	1.041
lnFracLungSysC	24.08 (6.276, 81.14)	1.016	1.496 (1.263, 2.439)	1.001
lnClTCOHC	0.1767 (0.1374, 0.2257)	1.011	1.888 (1.624, 2.307)	1.01
lnK_MTCOH	2.221 (1.296, 4.575)	1.02	2.578 (1.782, 4.584)	1.015
lnClGlucC	0.2796 (0.2132, 0.3807)	1.056	1.955 (1.583, 2.418)	1.079
lnK_MGluc	133.4 (51.56, 277.2)	1.02	1.573 (1.266, 4.968)	1.011
lnkMetTCOHC	0.7546 (0.1427, 2.13)	1.007	5.011 (2.668, 15.71)	1.002
lnkUrnTCAC	0.04565 (0.0324, 0.06029)	1.005	1.878 (1.589, 2.48)	1.006
lnkMetTCAC	0.2812 (0.1293, 0.5359)	1.004	2.529 (1.78, 4.211)	1.002
lnkBileC	6.855 (3.016, 20.69)	1.464	1.589 (1.27, 3.358)	1.015
lnkEHRC	0.1561 (0.09511, 0.2608)	1.1	1.699 (1.348, 2.498)	1.015
lnkUrnTCOGC	15.78 (6.135, 72.5)	1.007	9.351 (4.93, 29.96)	1.003
lnkDCVGC	7.123 (5.429, 9.702)	1.026	1.507 (1.311, 1.897)	1.008
lnkNATC	0.0003157 (0.0001087, 0.002305)	1.008	1.54 (1.261, 3.306)	1
lnkKidBioactC	0.06516 (0.01763, 0.1743)	1.001	1.523 (1.262, 2.987)	1

Table A-16. Posterior distributions for human residual errors

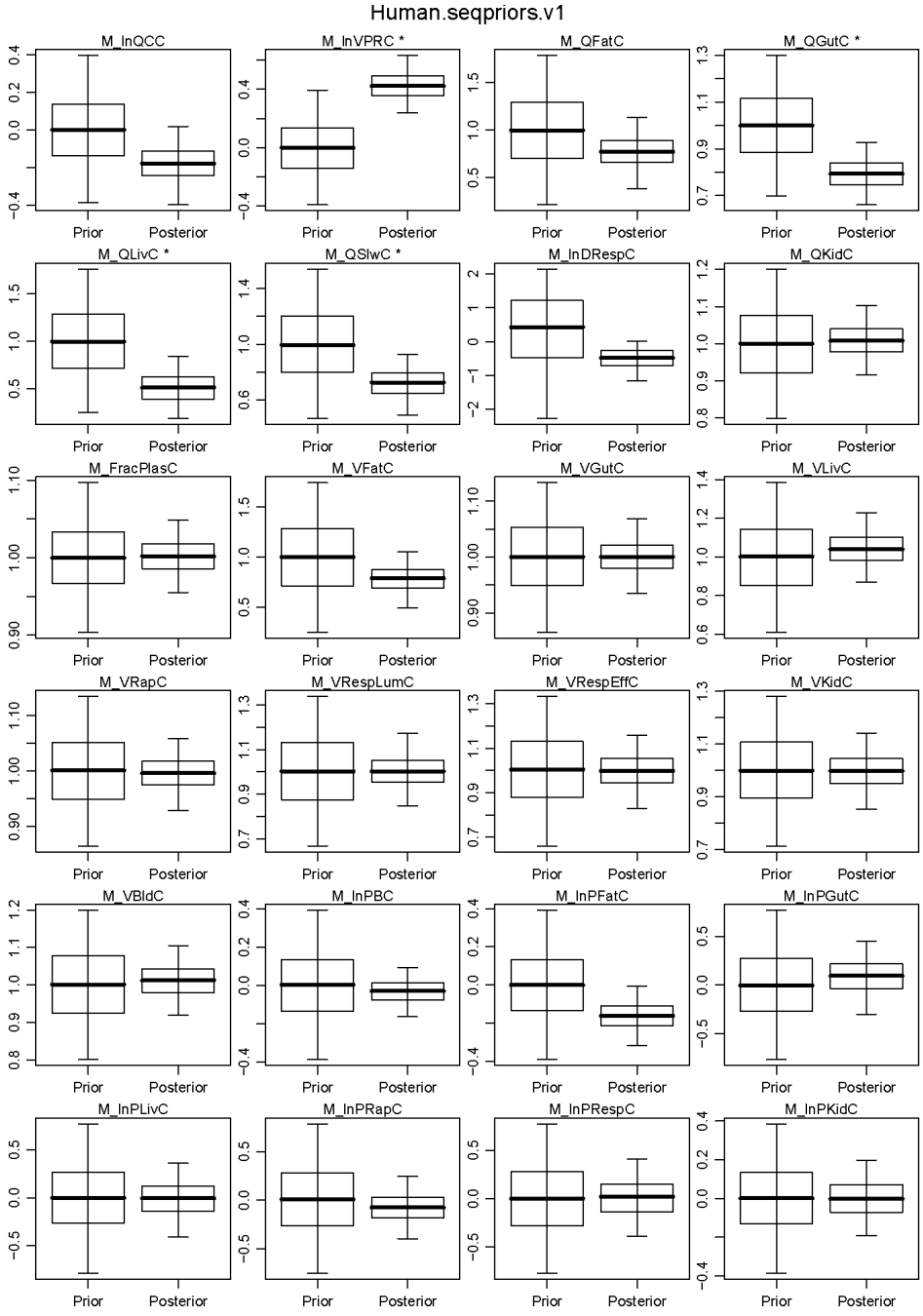
Measurement	Subject[a]	Residual error GSD	
		Median (2.5, 97.5%)	*R*
RetDose	Subject 4	1.131 (1.106, 1.25)	1.001
CAlvPPM	Subject 1	1.832 (1.509, 2.376)	1.015
	Subject 4	1.515 (1.378, 1.738)	1
	Subject 5	1.44 (1.413, 1.471)	1
CVen	Subject 1	1.875 (1.683, 2.129)	1.018
	Subject 3	1.618 (1.462, 1.862)	1
	Subject 4	1.716 (1.513, 2.057)	1.001
	Subject 5	2.948 (2.423, 3.8)	1.007
CTCOH	Subject 1	1.205 (1.185, 1.227)	1.012
	Subject 3	1.213 (1.187, 1.247)	1
	Subject 5	2.101 (1.826, 2.571)	1.001
	Subject 7	1.144 (1.106, 2.887)	1.123
CPlasTCA	Subject 2	1.117 (1.106, 1.17)	1.001
	Subject 7	1.168 (1.123, 1.242)	1
CBldTCA	Subject 1	1.138 (1.126, 1.152)	1.003
	Subject 2	1.119 (1.106, 1.178)	1
	Subject 4	1.488 (1.351, 1.646)	1.018
	Subject 5	1.438 (1.367, 1.537)	1.002
zAUrnTCA	Subject 1	1.448 (1.414, 1.485)	1.001
	Subject 2	1.113 (1.105, 1.149)	1.001
	Subject 3	1.242 (1.197, 1.301)	1.001
	Subject 4	1.538 (1.441, 1.67)	1
	Subject 6	1.158 (1.118, 1.228)	1
	Subject 7	1.119 (1.106, 1.181)	1
zAUrnTCA_collect	Subject 3	1.999 (1.178, 3.903)	1.003
	Subject 5	2.787 (2.134, 4.23)	1.001
AUrnTCOGTCOH	Subject 1	1.106 (1.105, 1.112)	1.001
	Subject 3	1.11 (1.105, 1.125)	1
	Subject 4	1.124 (1.107, 1.151)	1.001
	Subject 6	1.117 (1.106, 1.157)	1.001
	Subject 7	1.134 (1.106, 1.348)	1.003
AUrnTCOGTCOH_collect	Subject 3	1.3 (1.111, 2.333)	1.004
	Subject 5	1.626 (1.524, 1.767)	1
CDCVGmol	Subject 1	1.53 (1.436, 1.656)	1.009
zAUrnNDCVC	Subject 6	1.167 (1.124, 1.244)	1
TotCTCOH	Subject 1	1.204 (1.185, 1.226)	1.011
	Subject 4	1.247 (1.177, 1.366)	1.009
	Subject 5	1.689 (1.552, 1.9)	1.001

[a]The seven subjects are: (1) Fisher et al. (1998); (2) Paycok and Powell (1945); (3) Kimmerle and Eben (1973a); (4) Monster et al. (1976); (5) Chiu et al. (2007); (6) Bernauer et al. (1996); and (7) Muller et al. (1974).

Table A-17. Posterior correlations for human population mean parameters

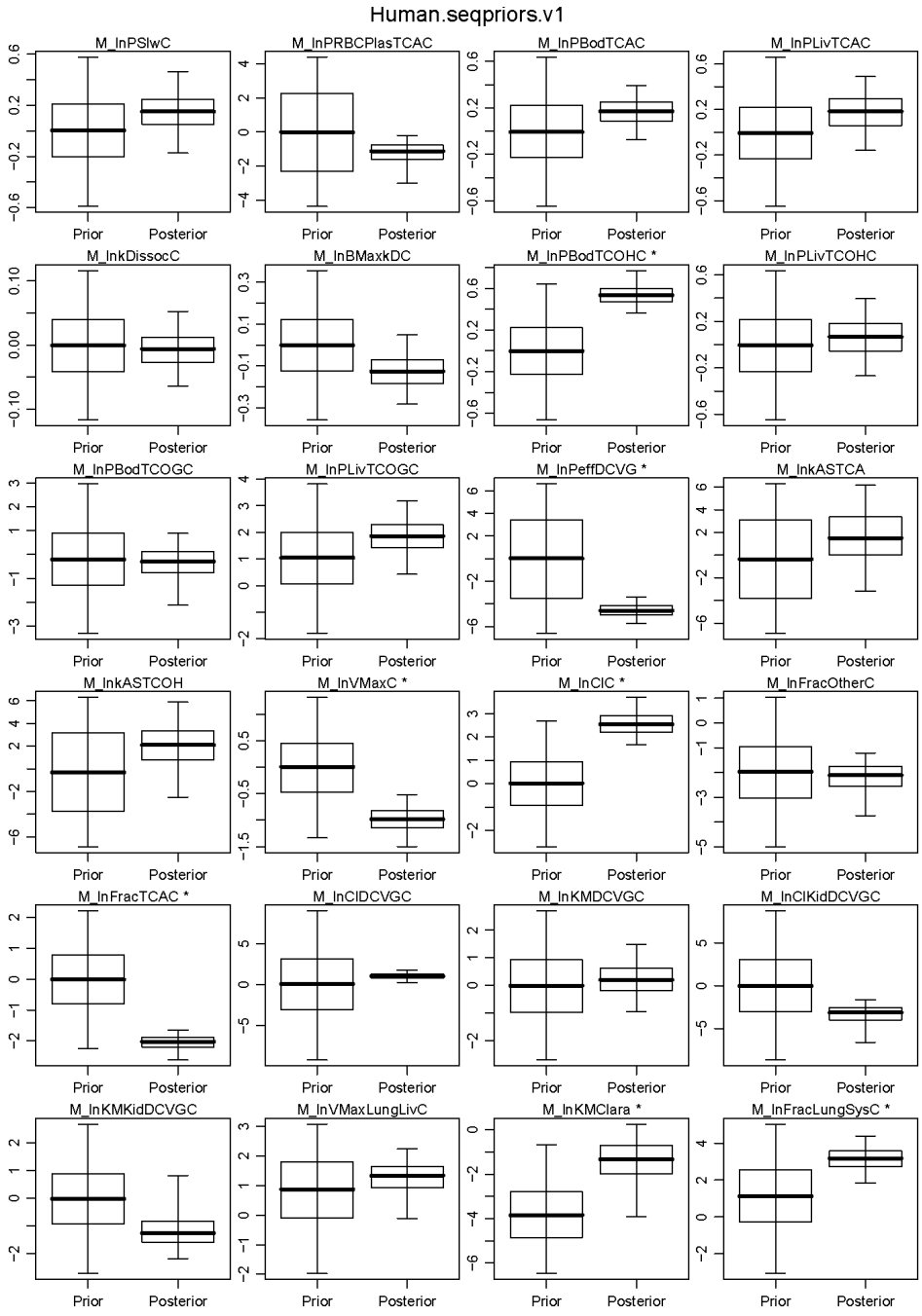
Human		
Parameter 1	**Parameter 2**	**Correlation coefficient**
lnkBileC	lnPLivTCOGC	-0.649
lnClKidDCVGC	lnKMKidDCVGC	-0.567
lnClGlucC	lnkEHRC	0.438
lnkMetTCAC	lnPLivTCAC	-0.392
lnClKidDCVGC	lnDRespC	-0.324
lnClKidDCVGC	lnkEHRC	-0.301
lnKMTCOH	lnPBodTCAC	0.289
lnkMetTCAC	lnPBodTCAC	0.283
lnClKidDCVGC	lnkBileC	-0.277
lnkEHRC	lnPBodTCOHC	-0.277
lnClDCVGC	lnkDCVGC	0.269
lnBMaxkDC	lnPBodTCAC	0.267
lnFracOtherC	lnQCC	0.260
lnFracOtherC	lnkDCVGC	-0.258
lnFracOtherC	VLivC	0.257
lnFracOtherC	lnPLivTCOGC	-0.256
lnClDCVGC	lnFracOtherC	-0.256
lnClDCVGC	VLivC	-0.252

Note: only parameter pairs with correlation coefficient ≥0.25 are listed.



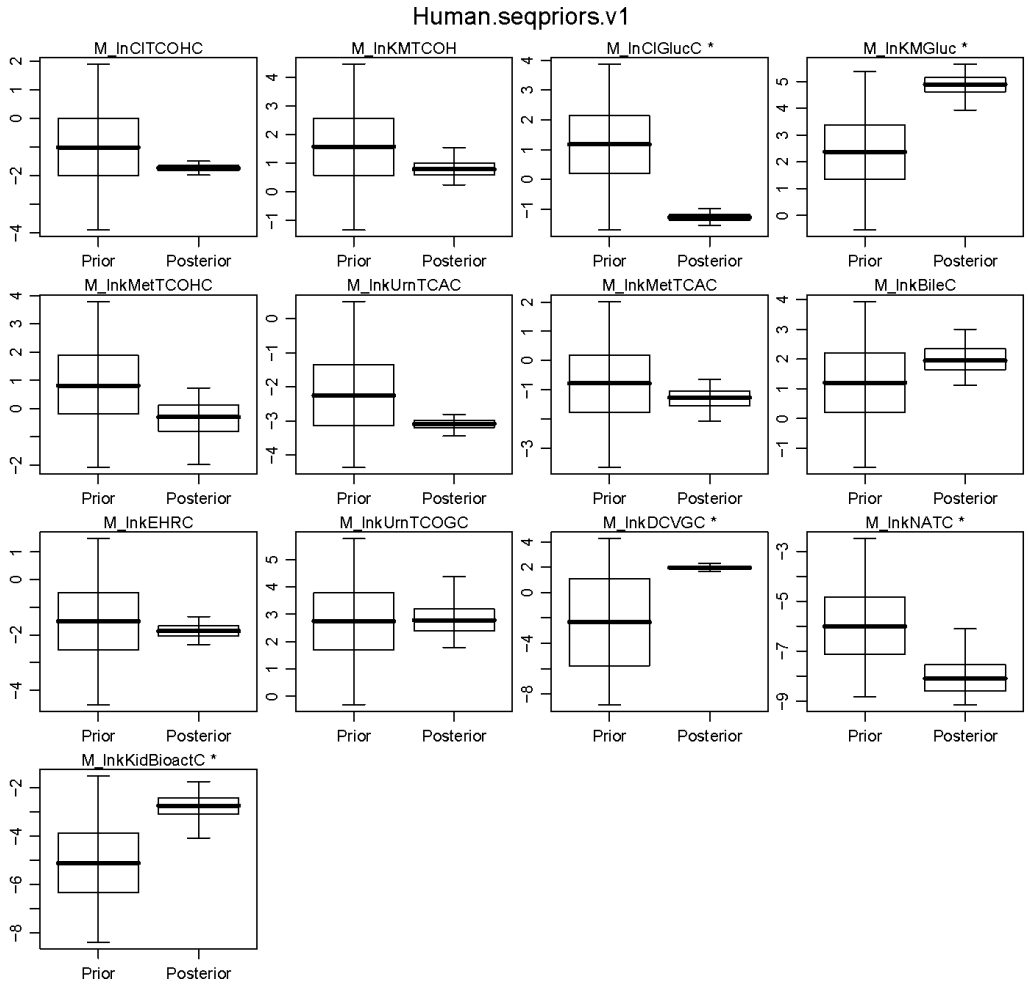
Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-25. Prior and posterior human population mean parameters (Part 1).



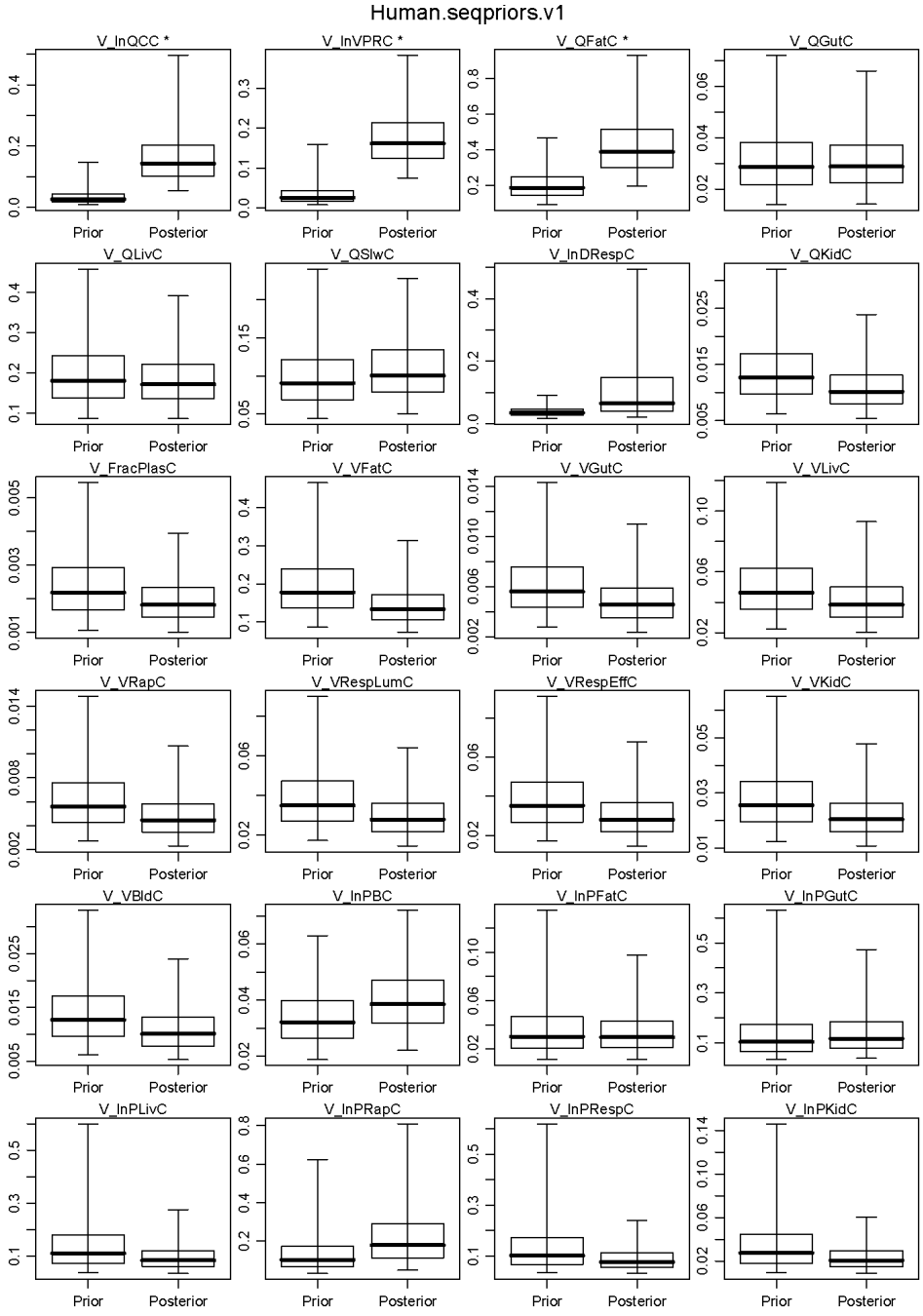
Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-26. Prior and posterior human population mean parameters (Part 2).



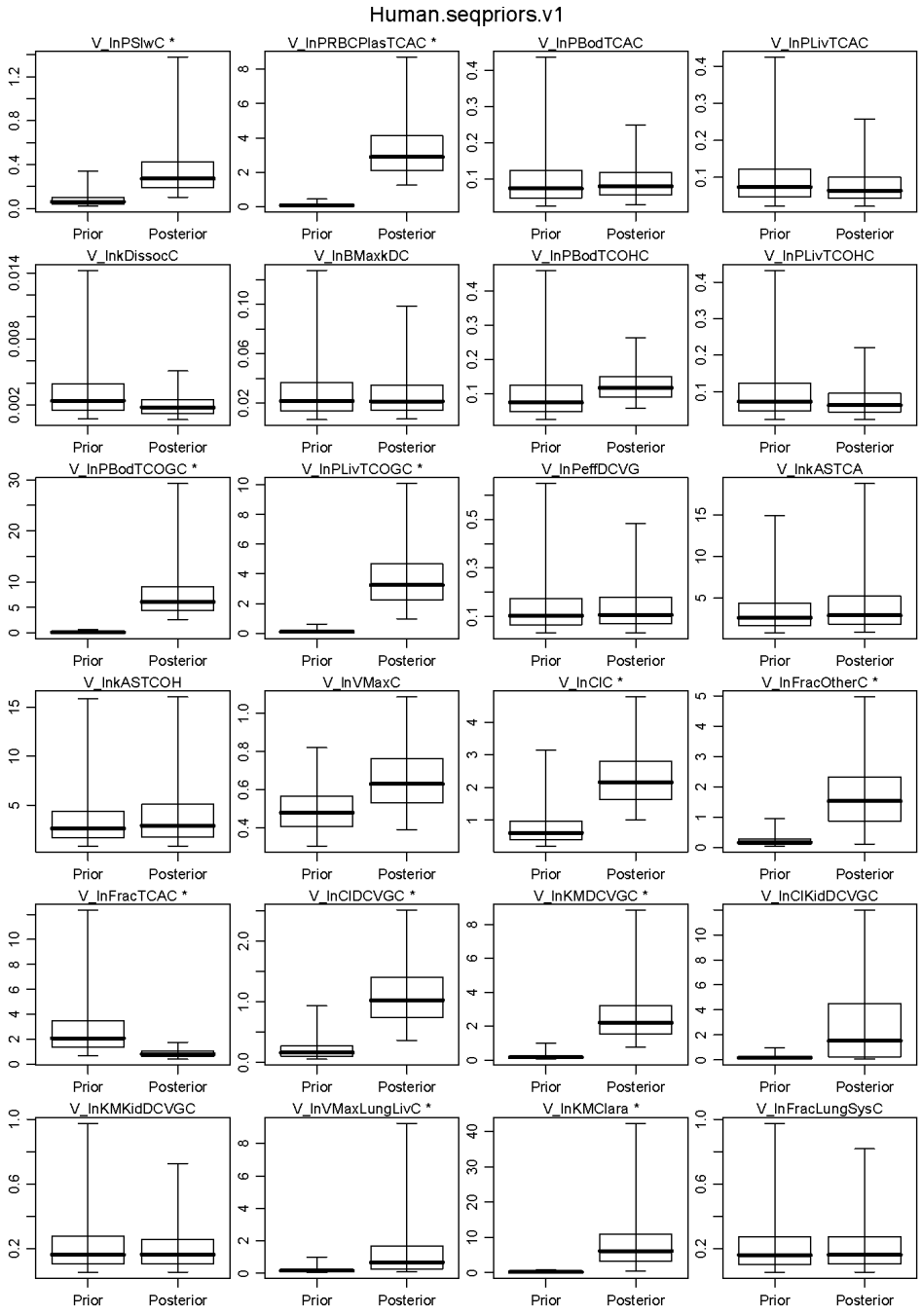
Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-27. Prior and posterior human population mean parameters (Part 3).



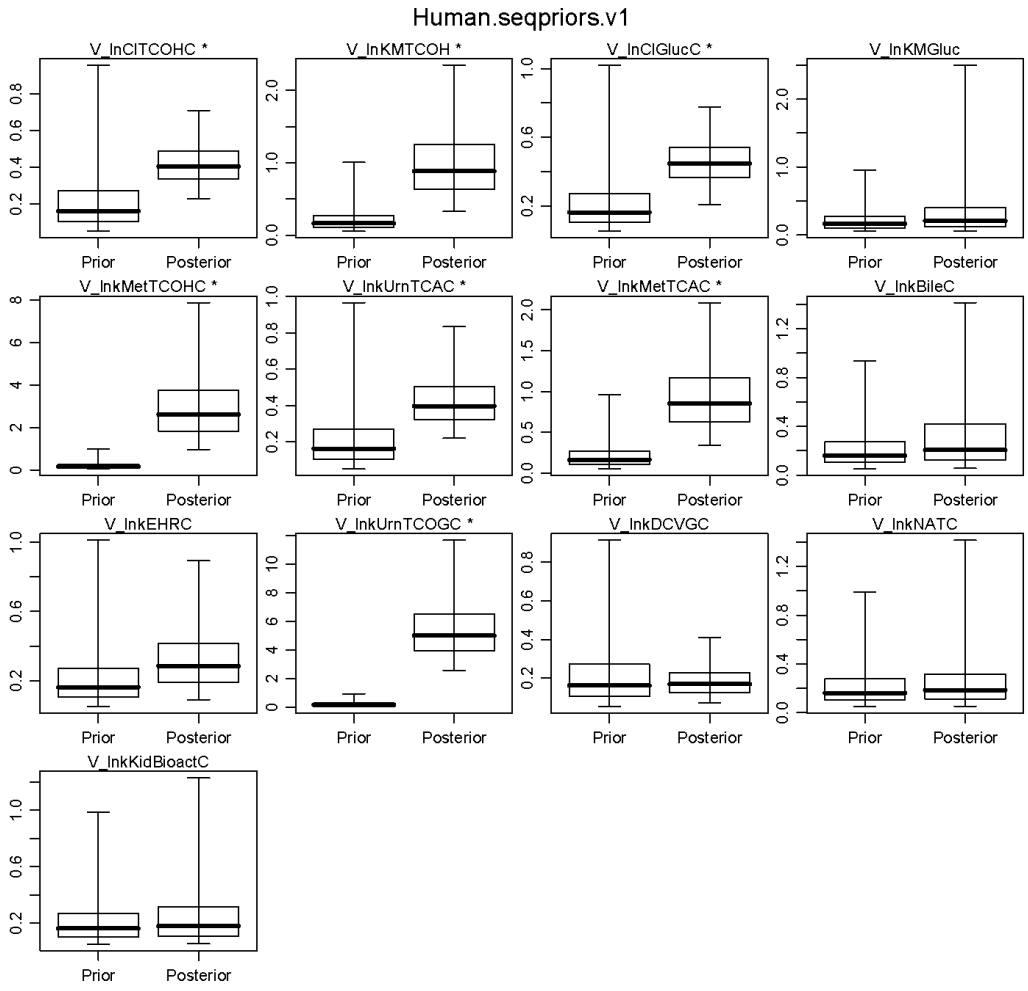
Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-28. Prior and posterior human population variance parameters (Part 1).



Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-29. Prior and posterior human population variance parameters (Part 2).



Thick lines are medians, boxes are interquartile regions, and error bars are (2.5, 97.5%) CIs. Parameters labeled with "*" have nonoverlapping interquartile regions.

Figure A-30. Prior and posterior human population variance parameters (Part 3).

A.5.2. Comparison of Model Predictions with Data

Time-course graphs of calibration and evaluation data compared to posterior predictions are shown in Figures A-31 to A-35. For each panel, the boxes are the experimental data, the solid red line is the prediction using the posterior mean of the subject-specific parameters (only shown for calibration data), and the shaded regions (or + with error bars, for single data points) are bounded by the 2.5, 25, 50, 75, and 97.5% population-based predictions.

A.5.2.1. Mouse Data and Model Predictions

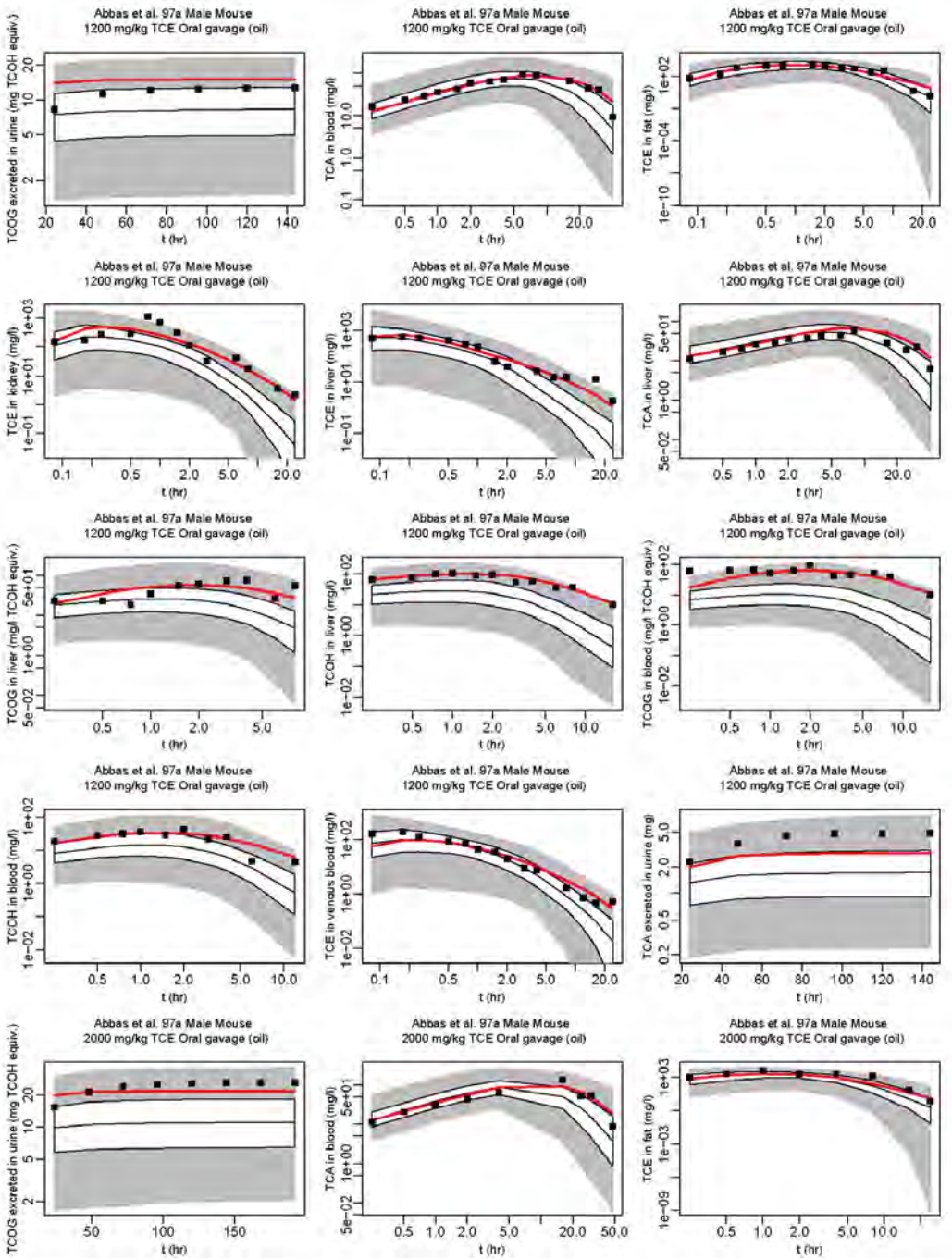


Figure A-31. Comparison of mouse calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions).

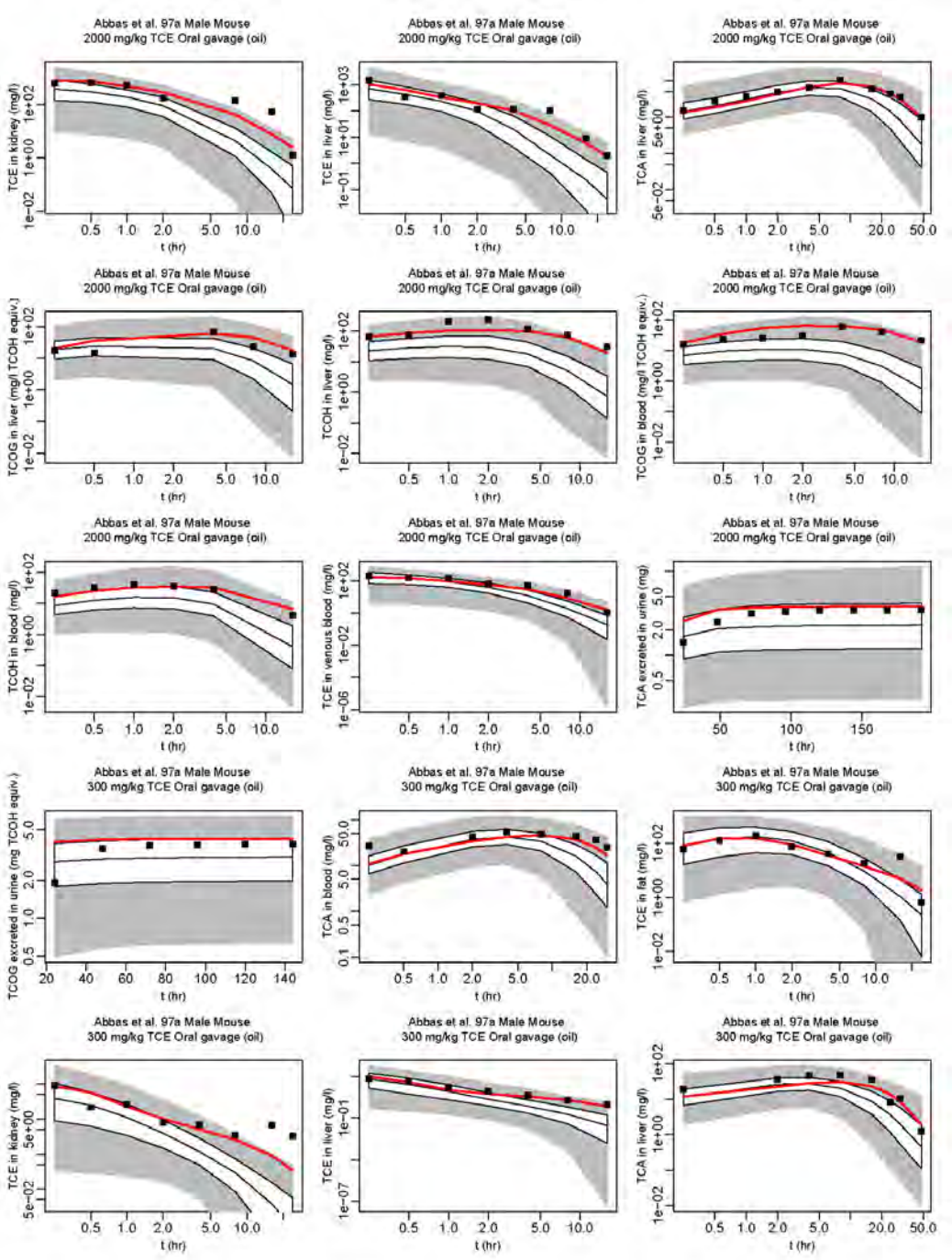


Figure A-31. Comparison of mouse calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

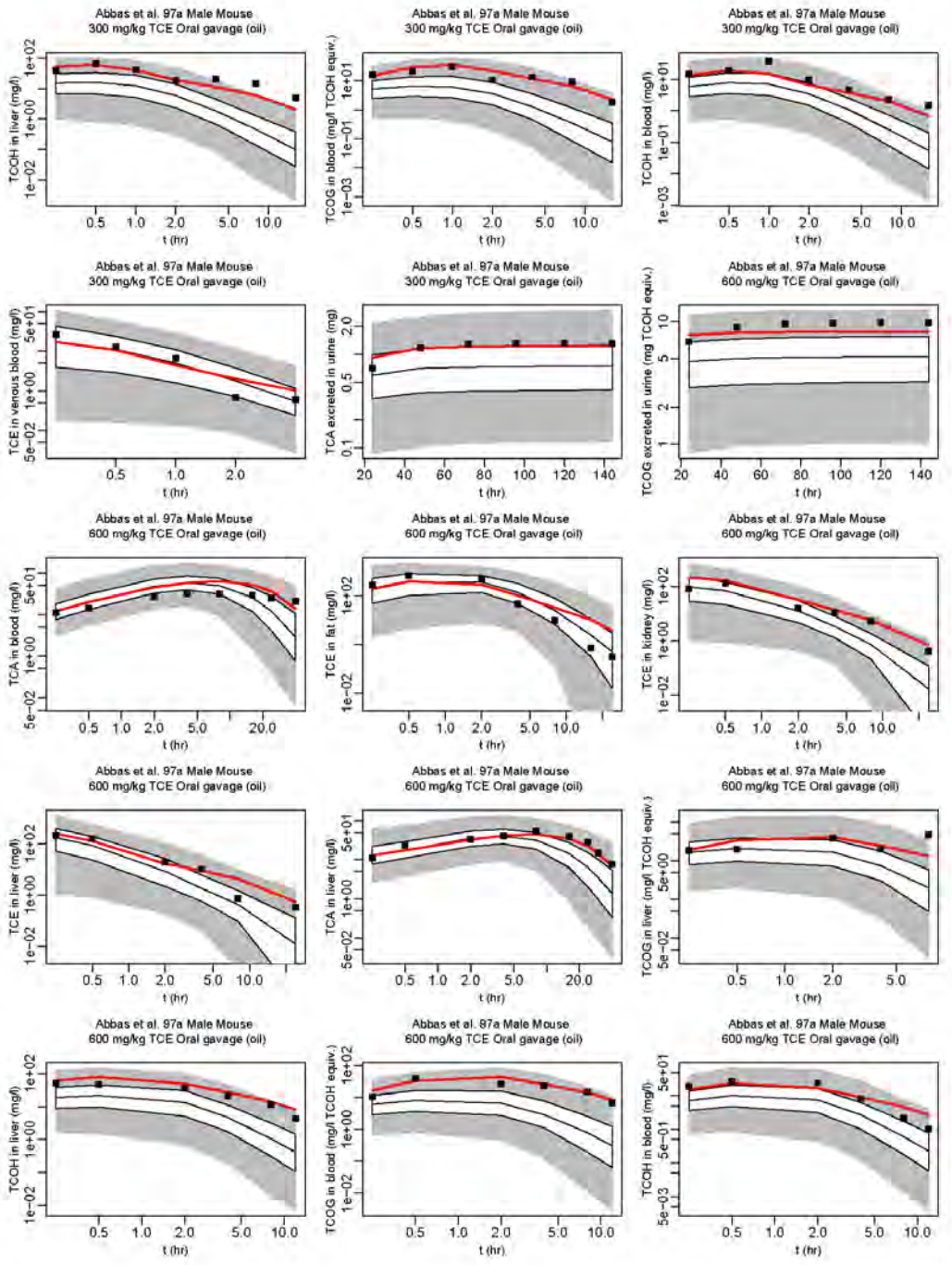


Figure A-31. Comparison of mouse calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

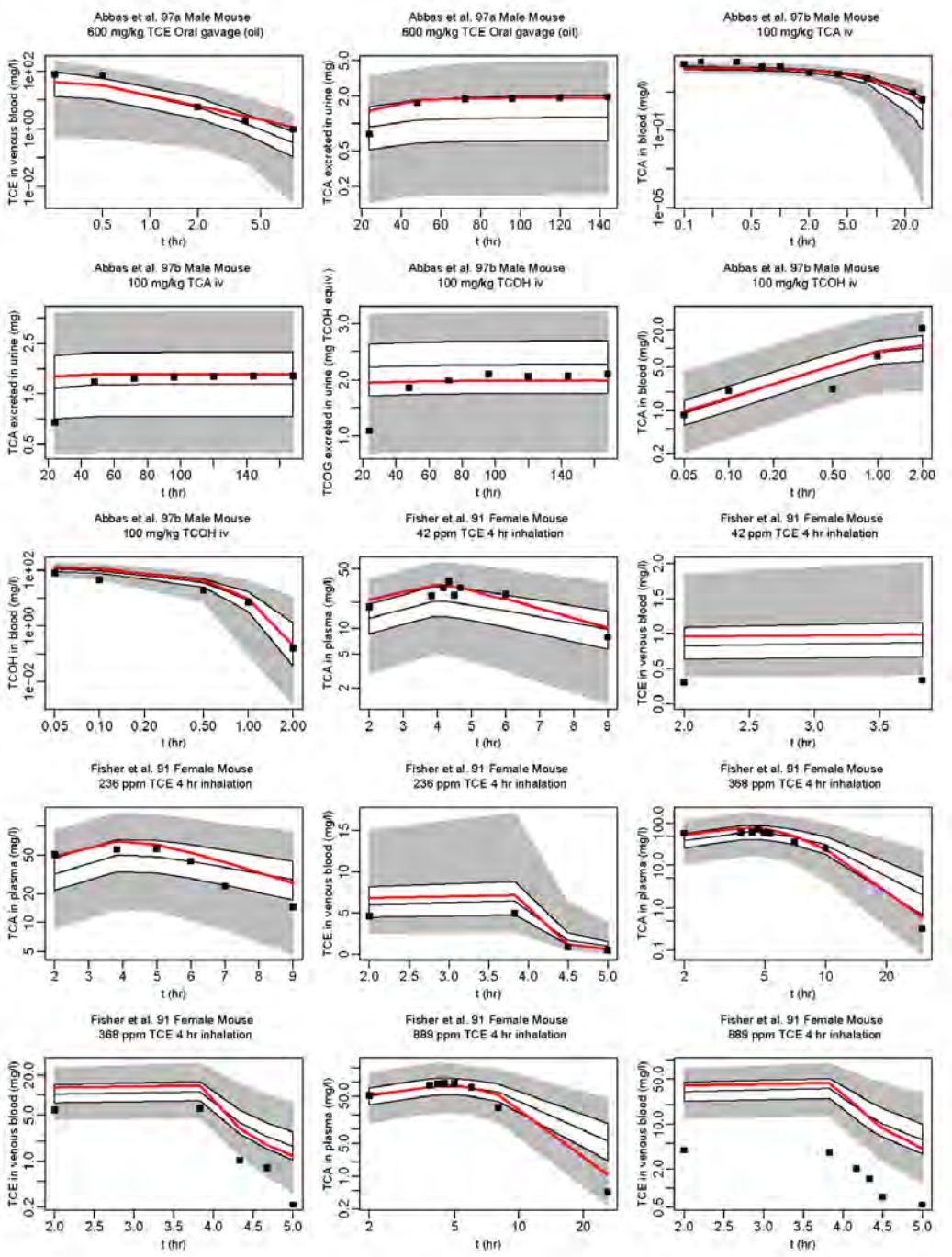


Figure A-31. Comparison of mouse calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

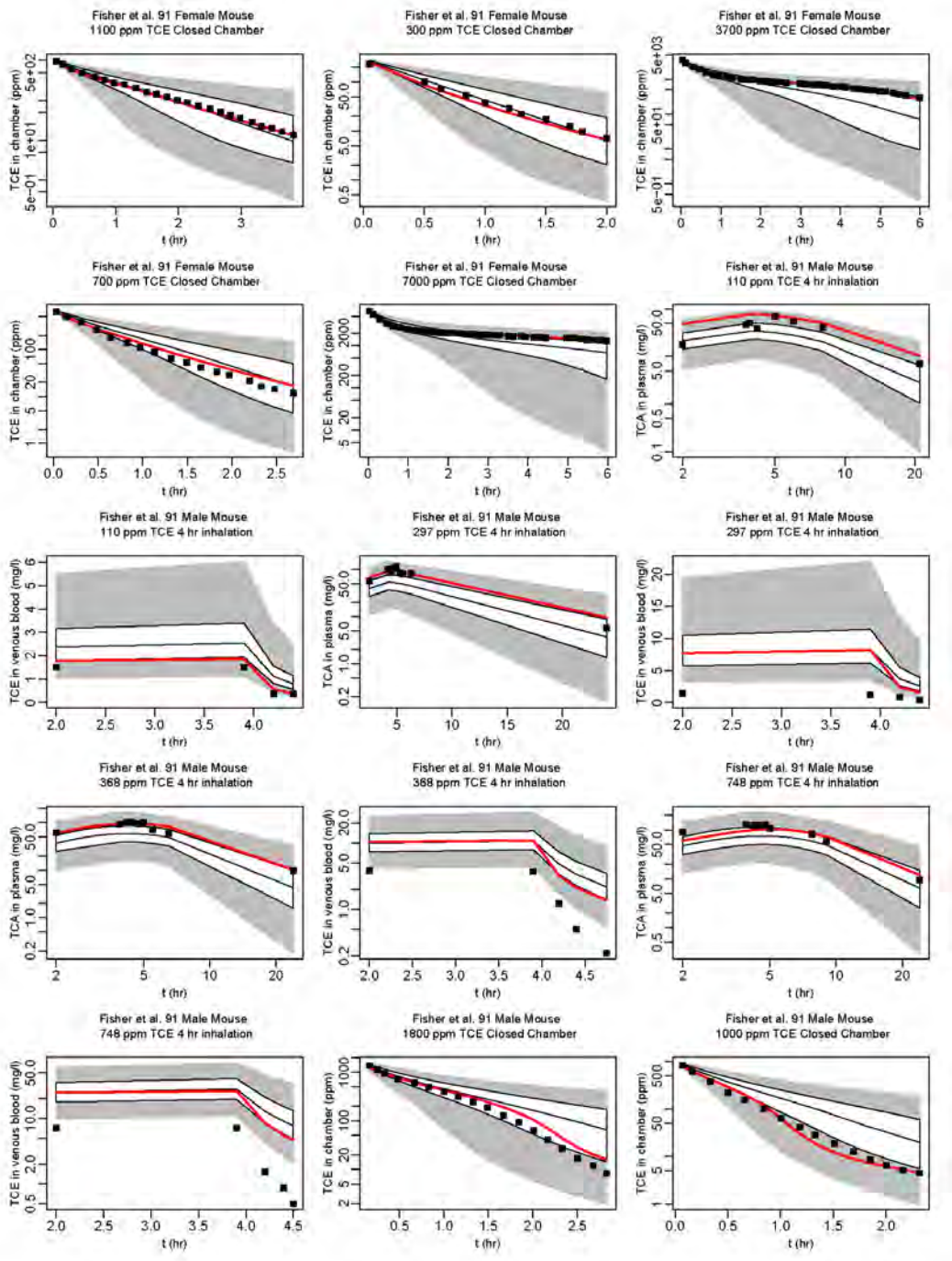


Figure A-31. Comparison of mouse calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

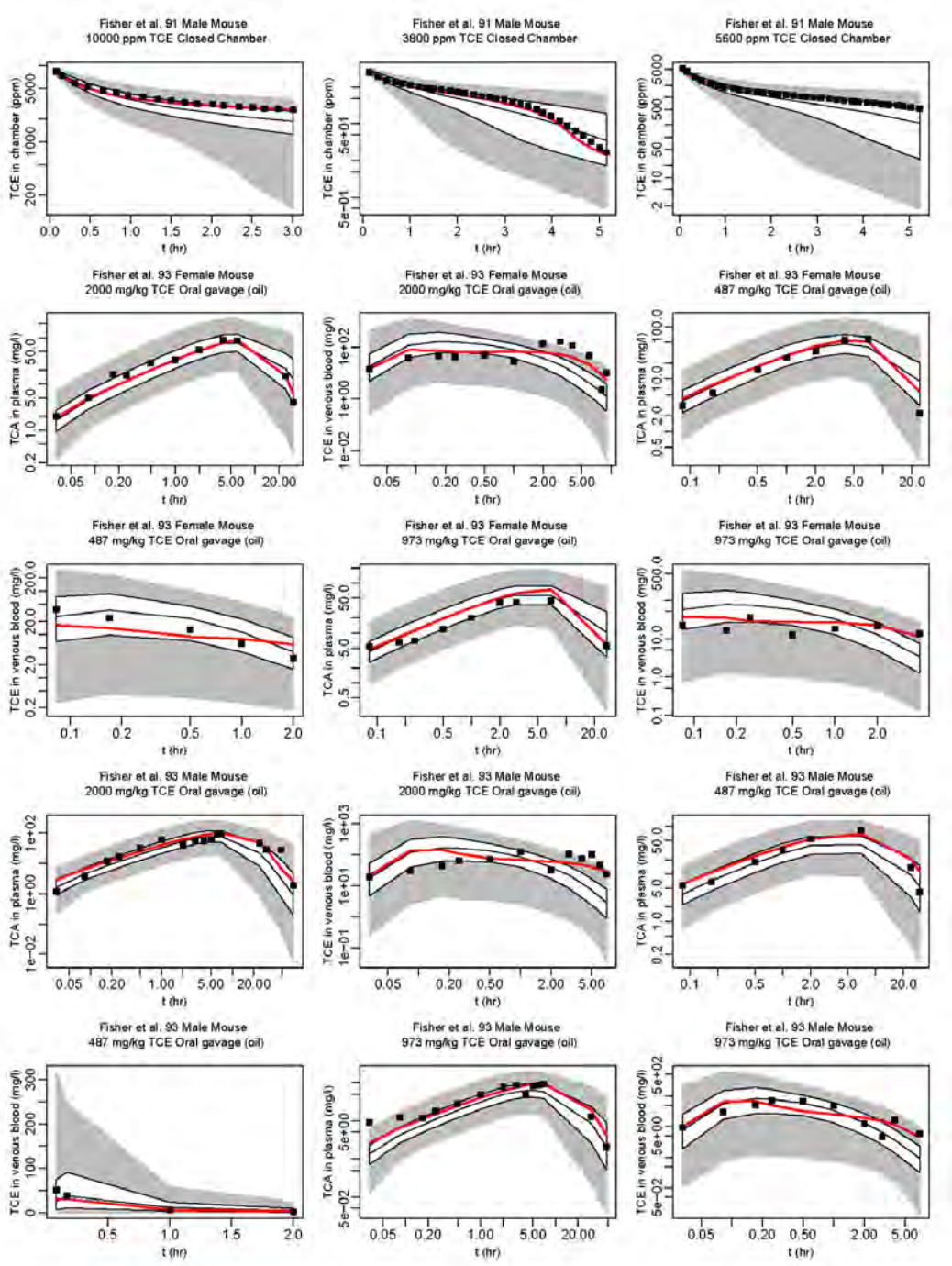


Figure A-31. Comparison of mouse calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

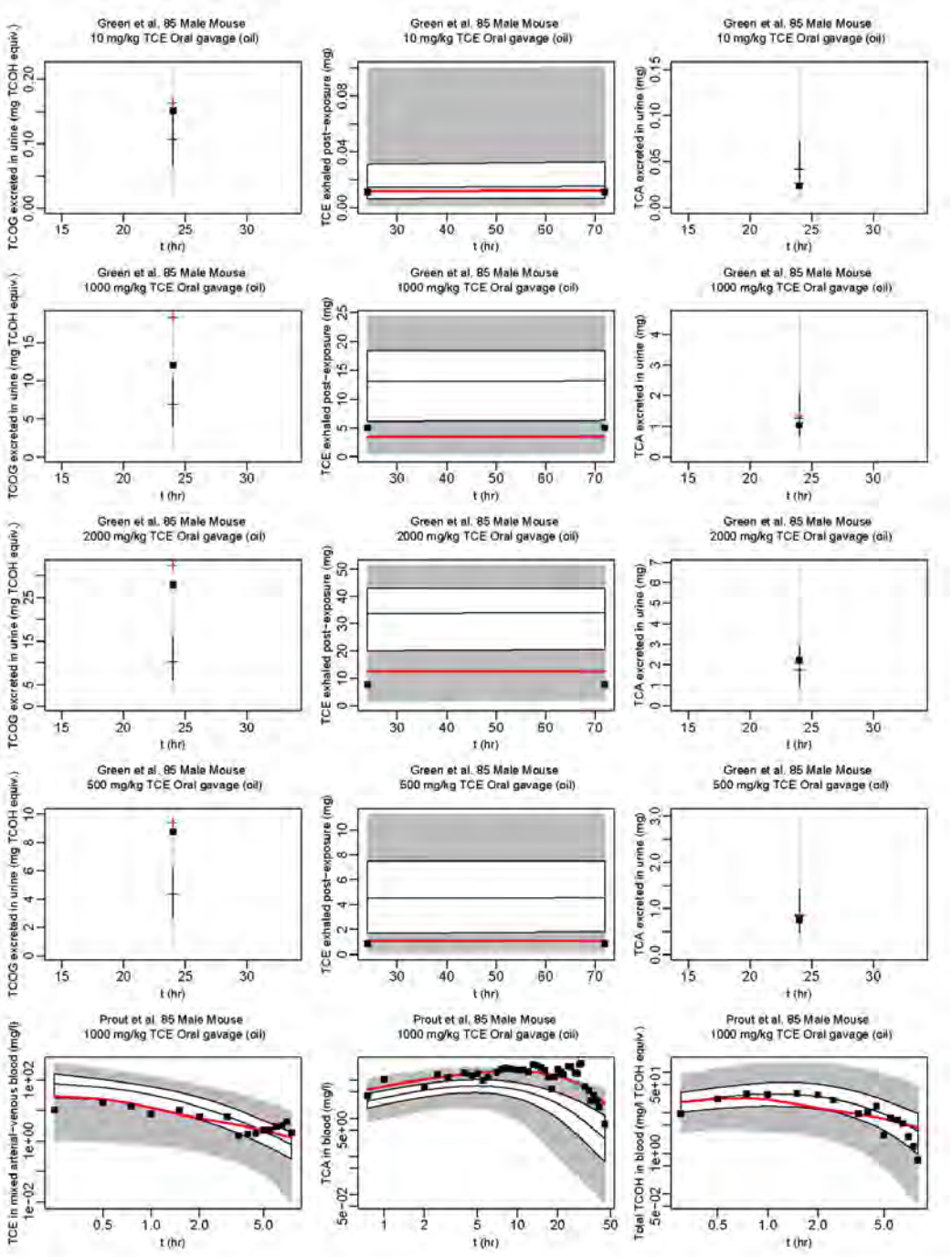


Figure A-31. Comparison of mouse calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

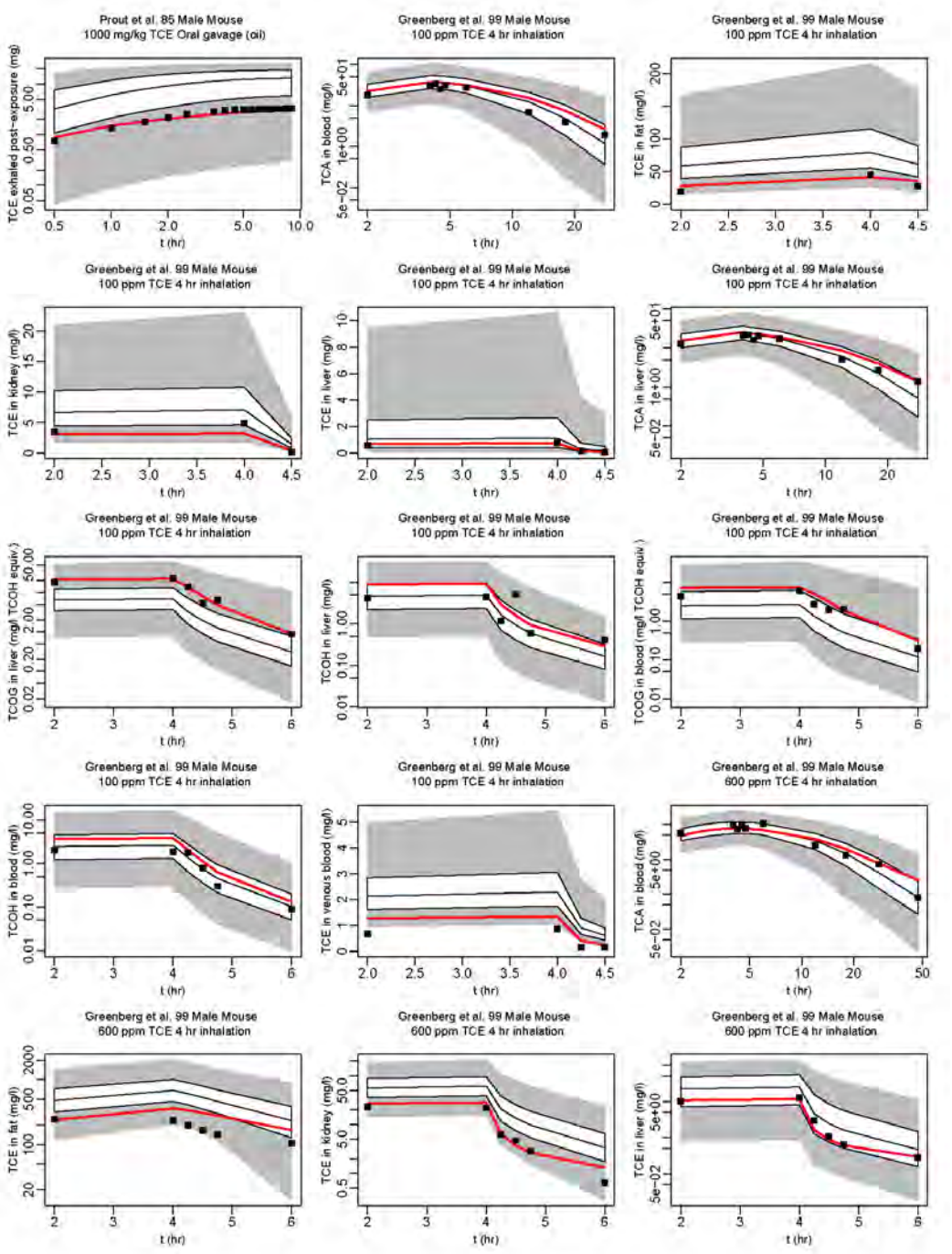


Figure A-31. Comparison of mouse calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

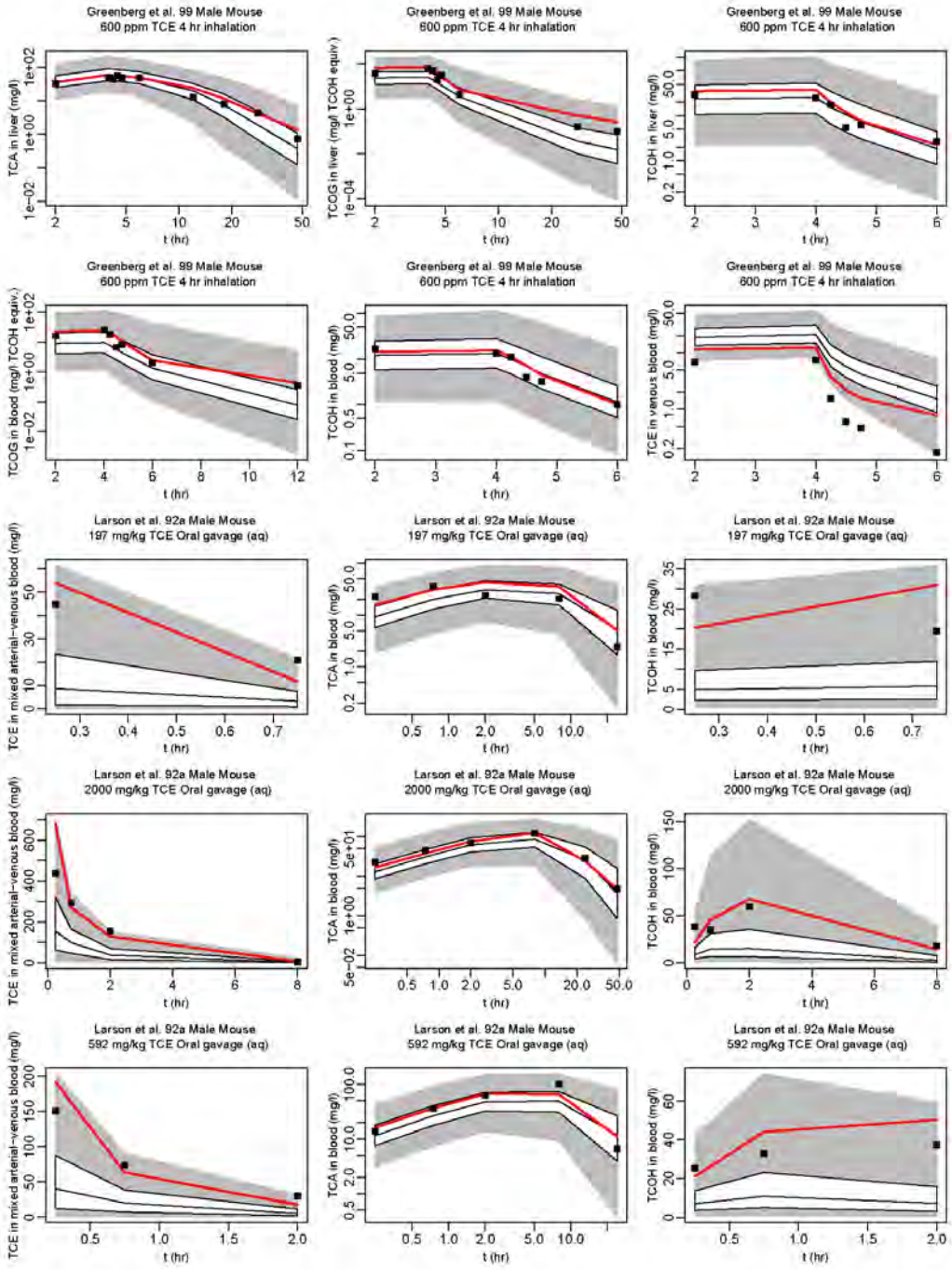


Figure A-31. Comparison of mouse calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

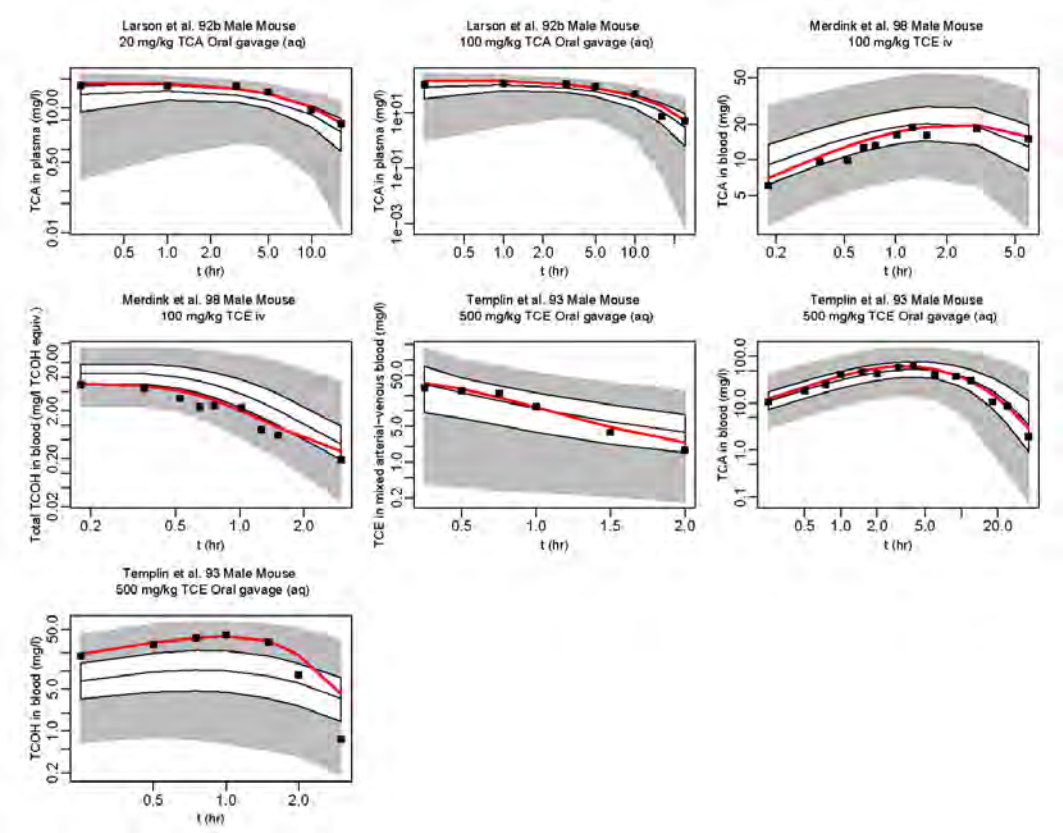


Figure A-31. Comparison of mouse calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

A.5.2.2. Rat Data and Model Predictions

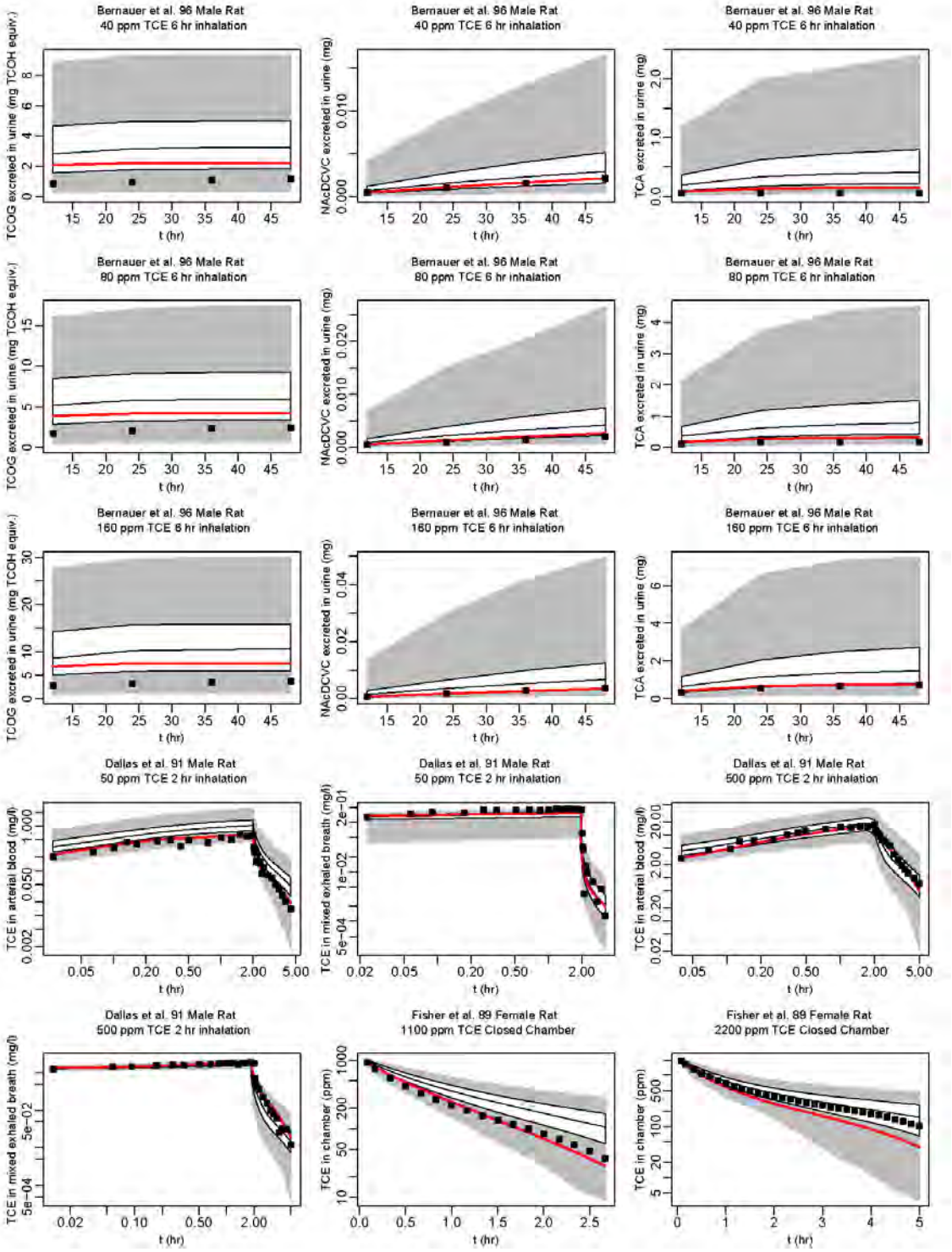


Figure A-32. Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions).

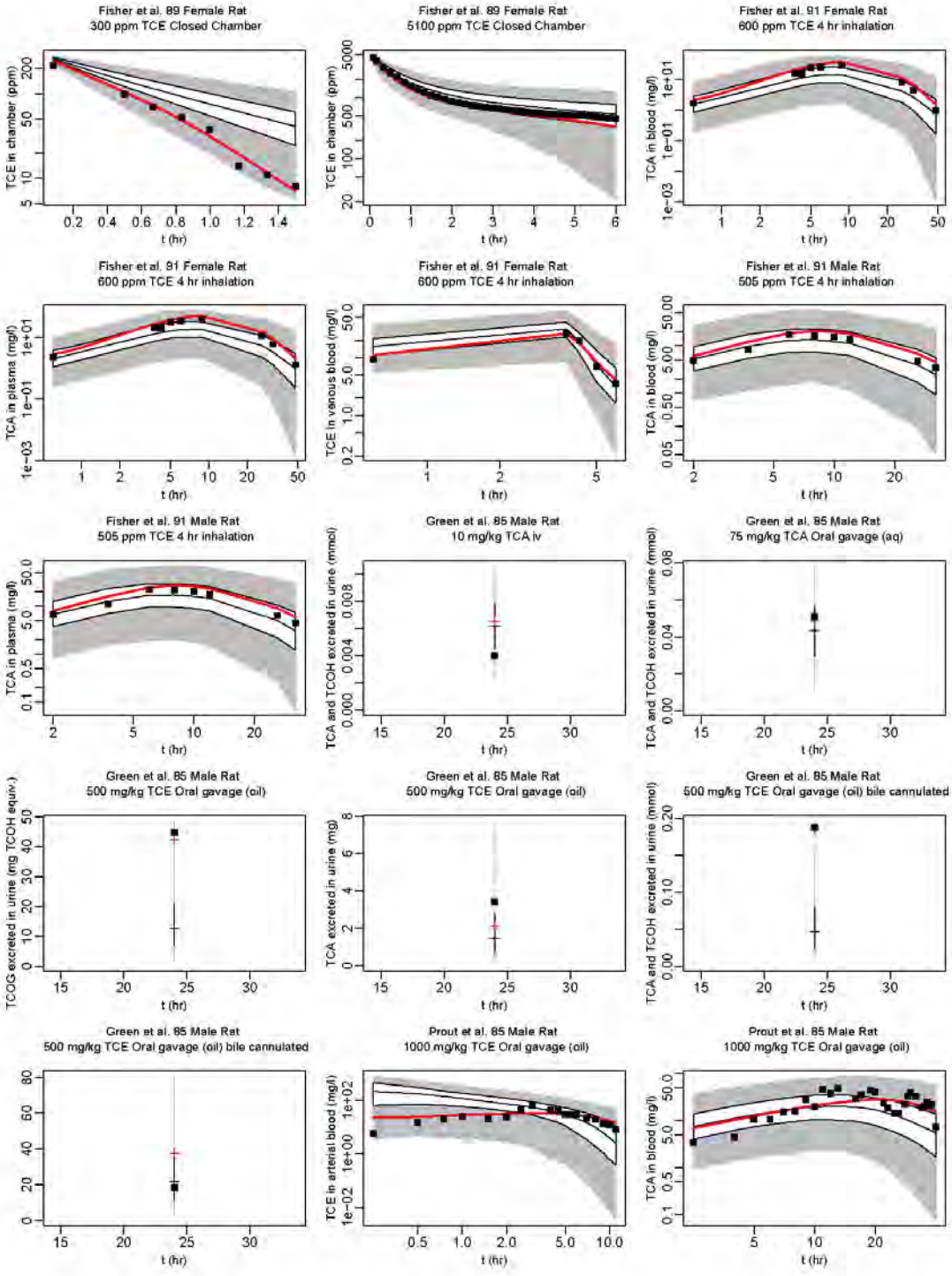


Figure A-32. Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

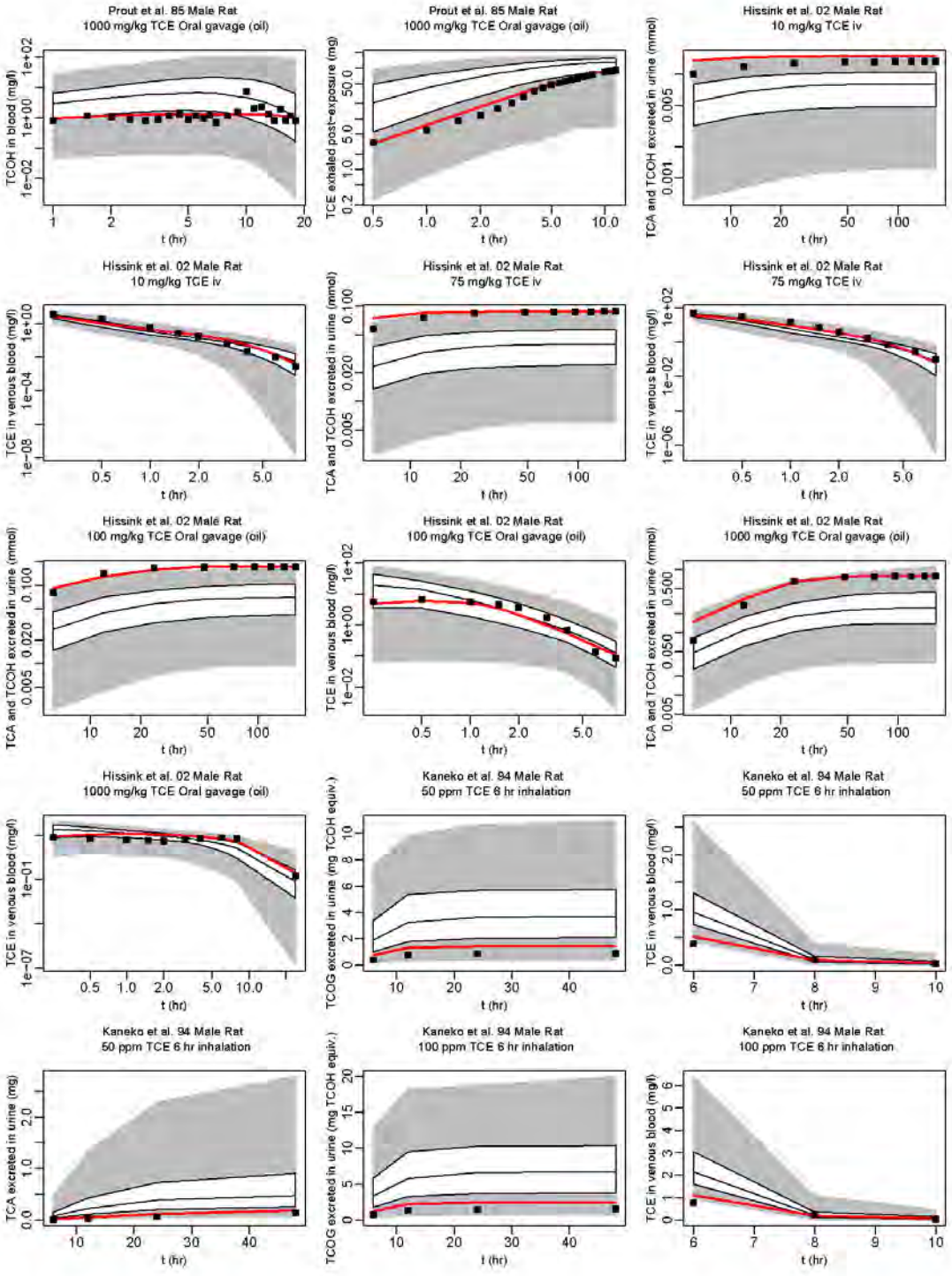


Figure A-32. Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

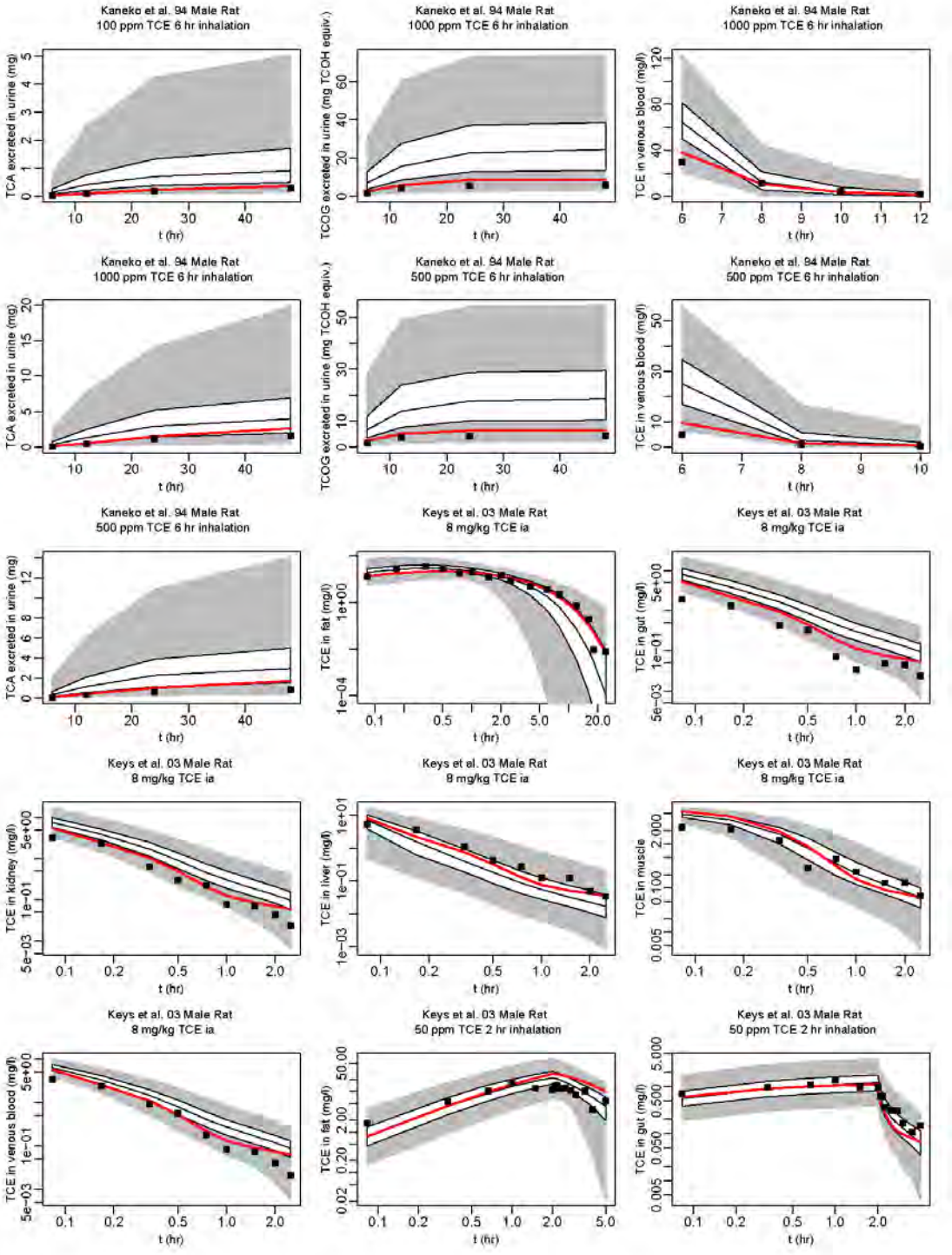


Figure A-32. (Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25 50 75, and 97.5% population-based predictions) (continued).

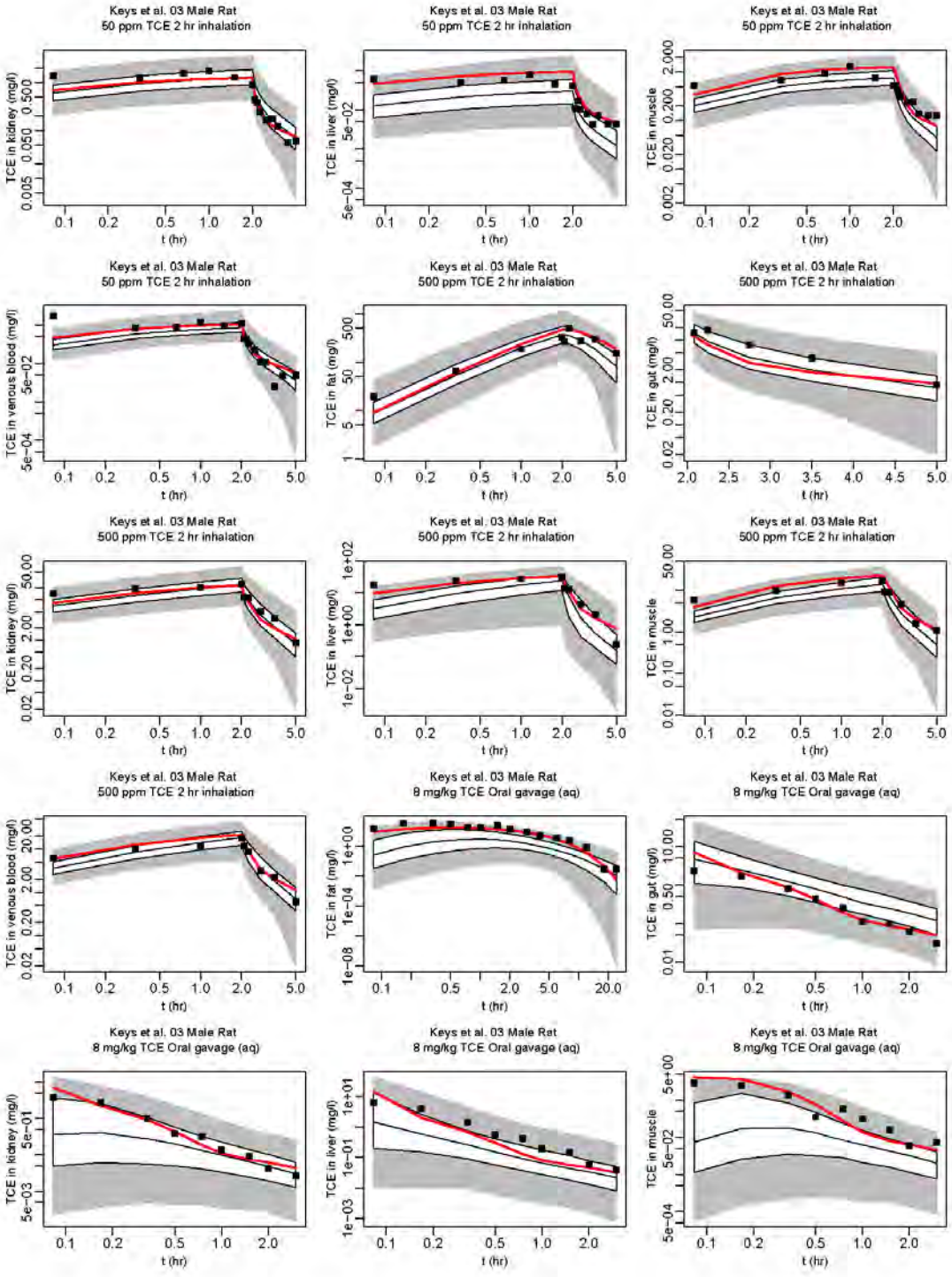


Figure A-32. Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

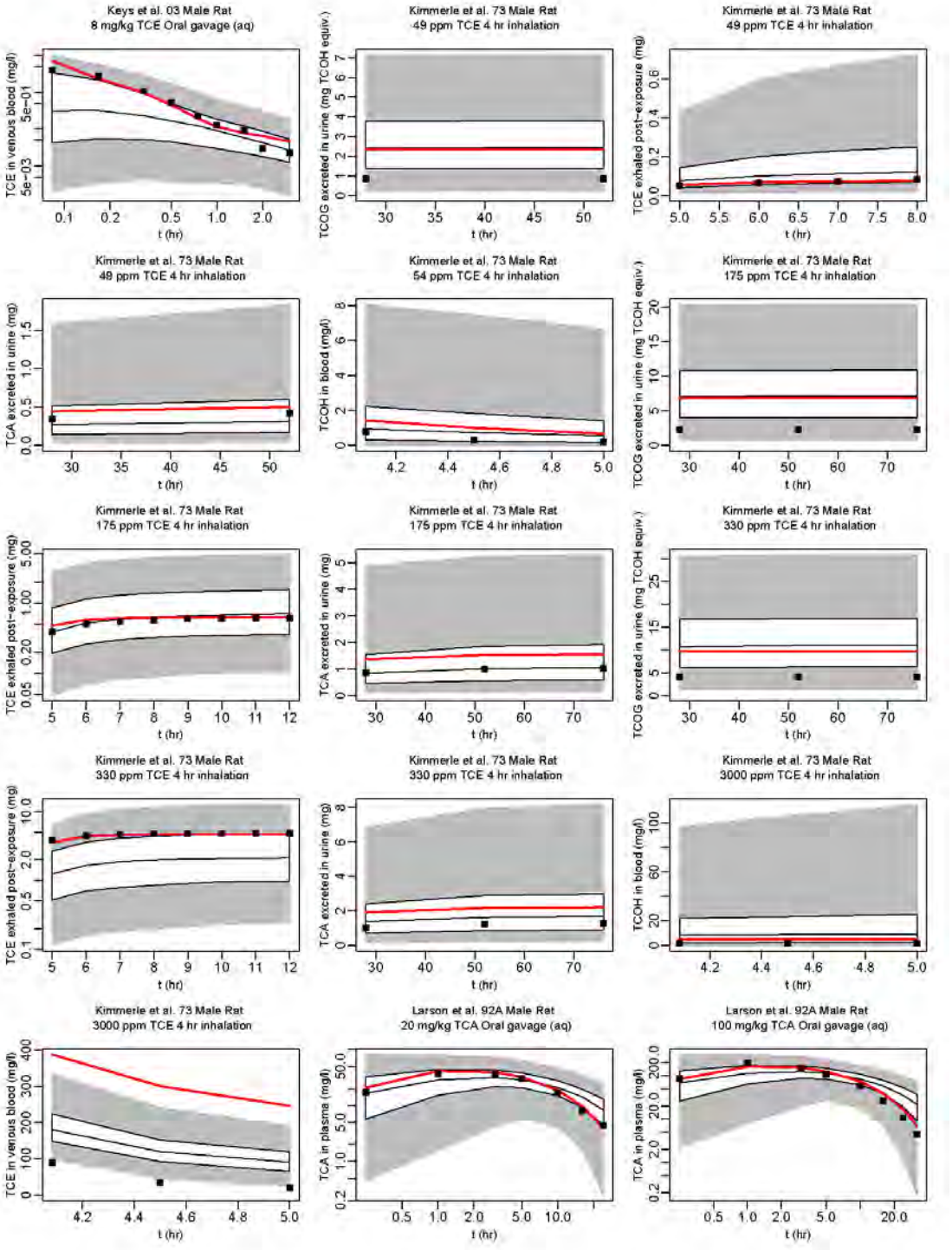


Figure A-32. Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

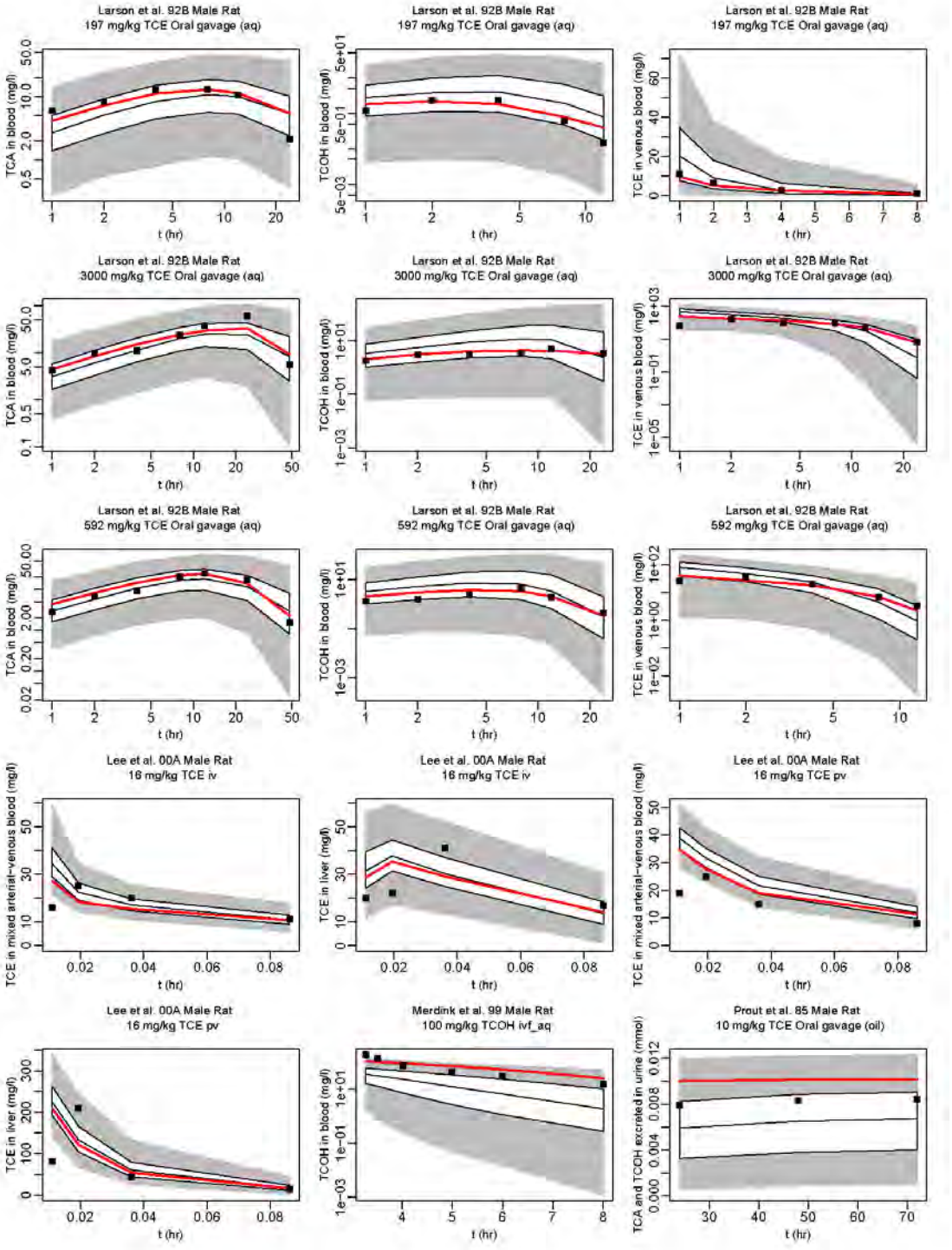


Figure A-32 Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

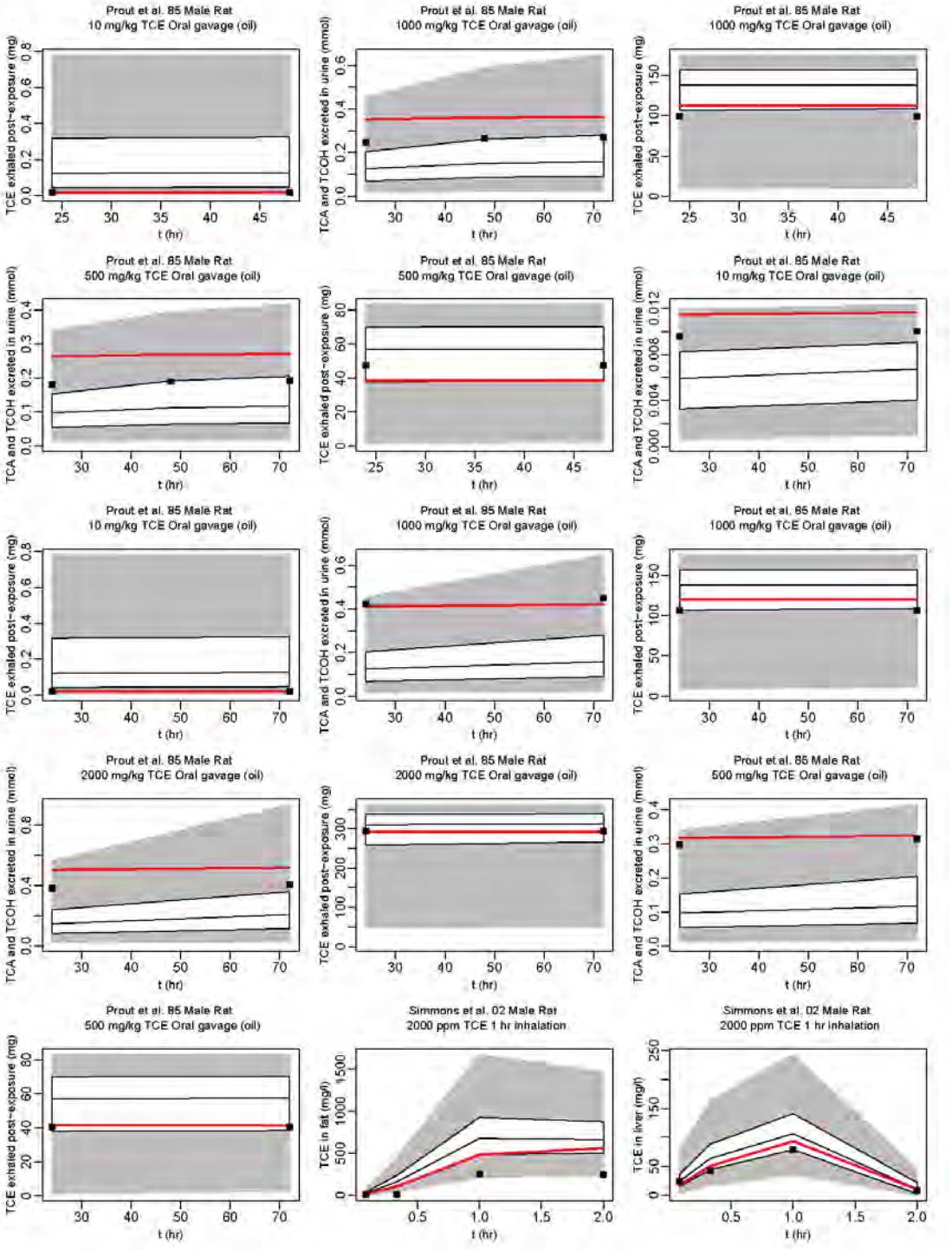


Figure A-32 Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

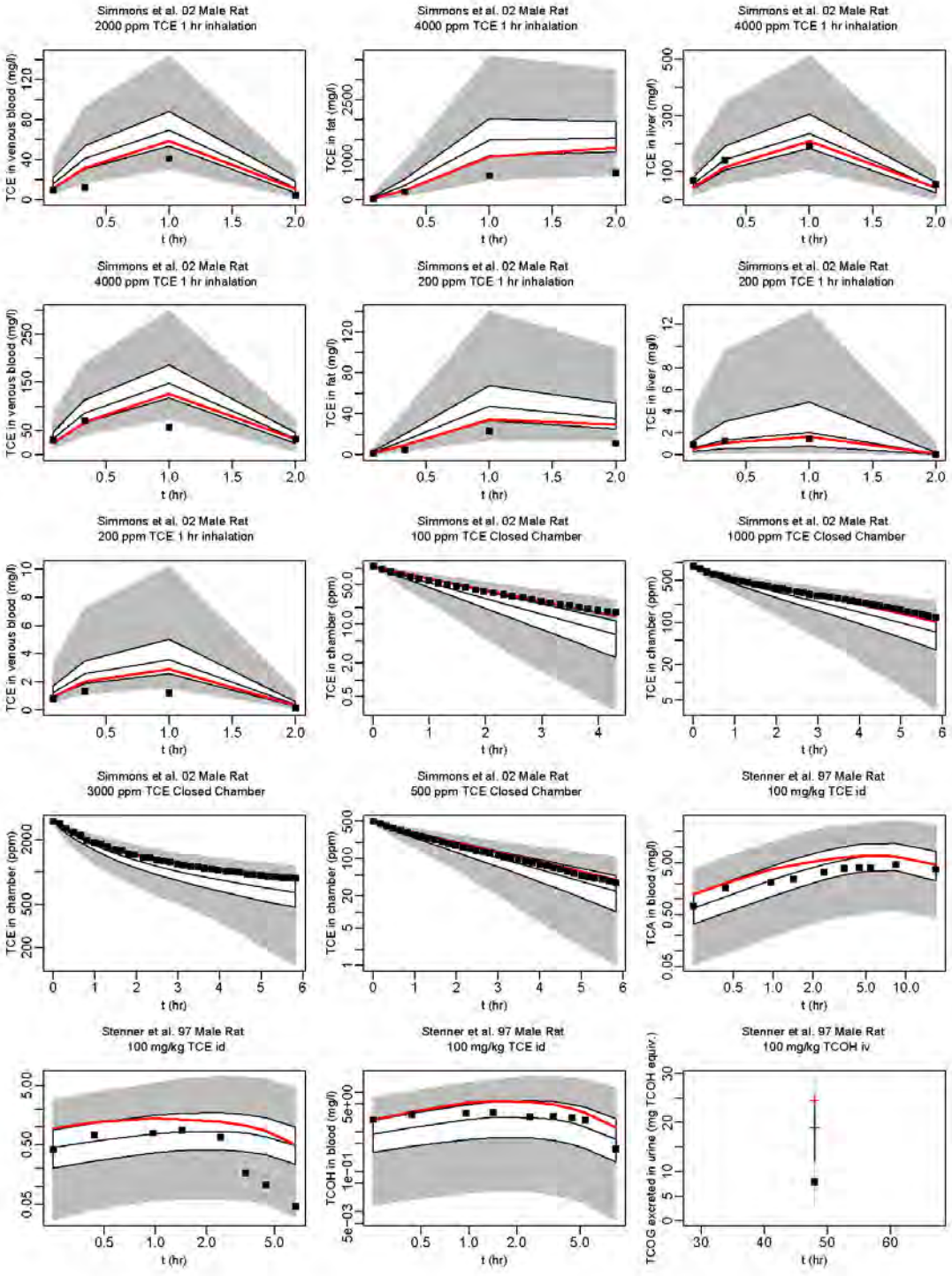


Figure A-32 Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

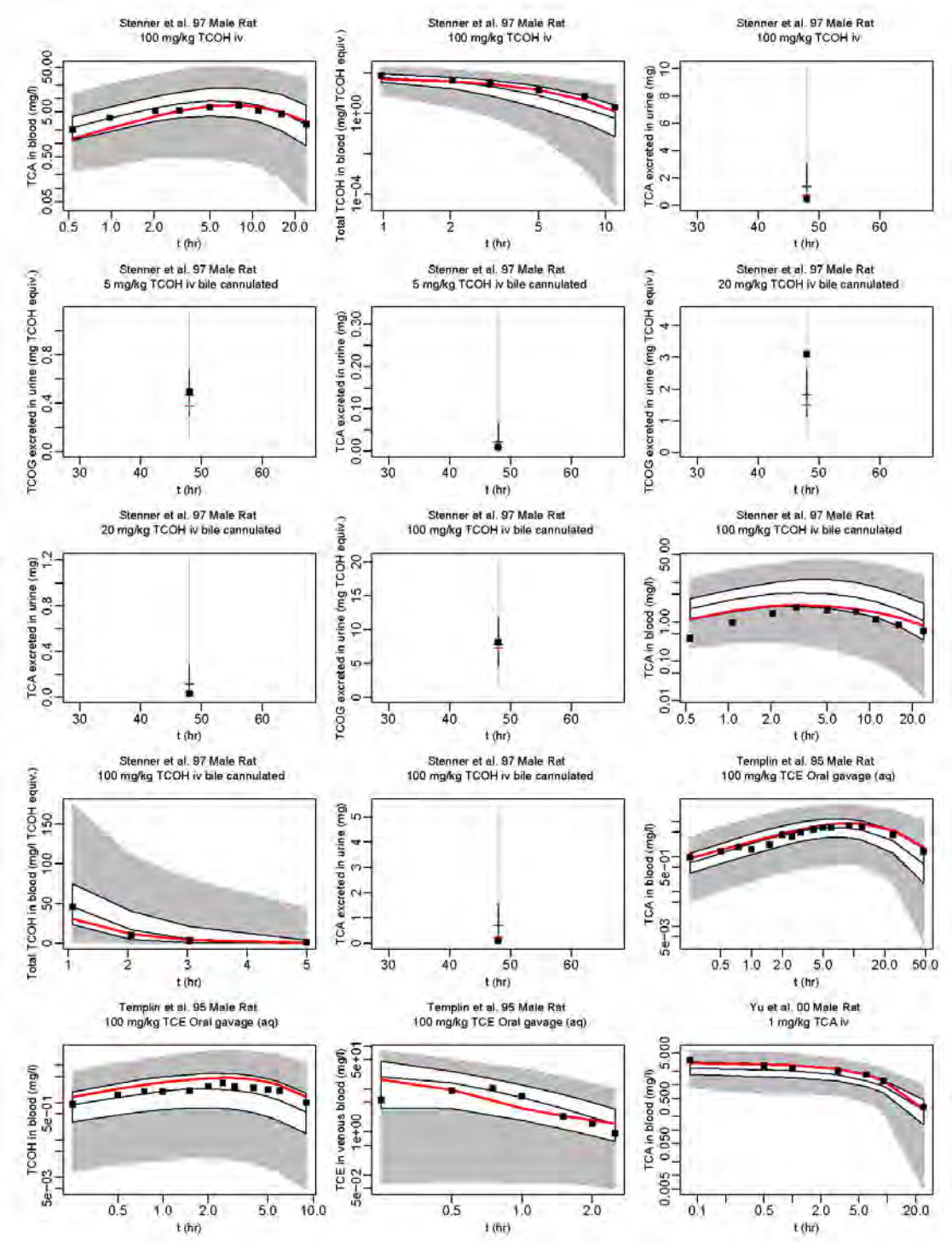


Figure A-32. Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

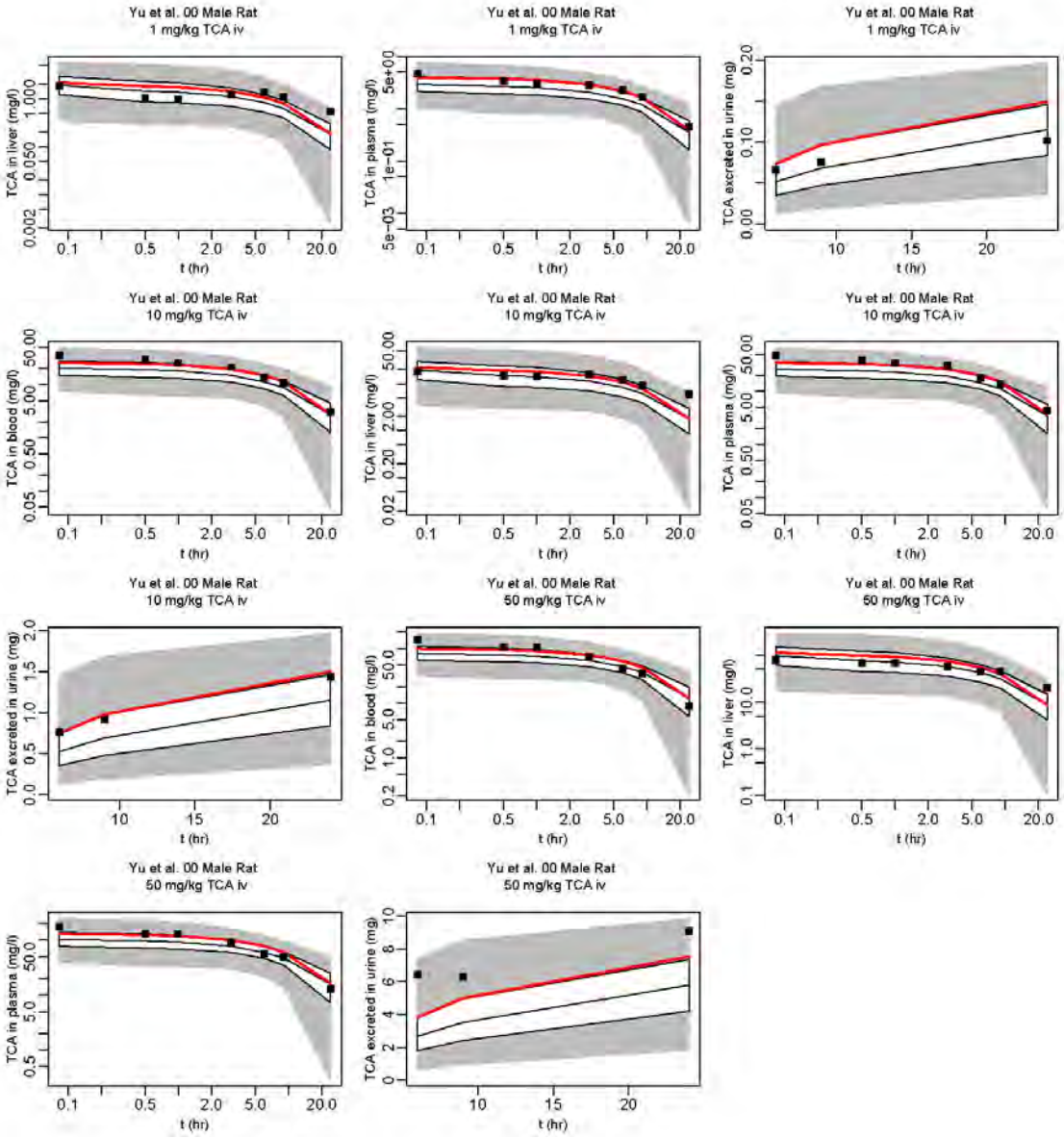


Figure A-32. Comparison of rat calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

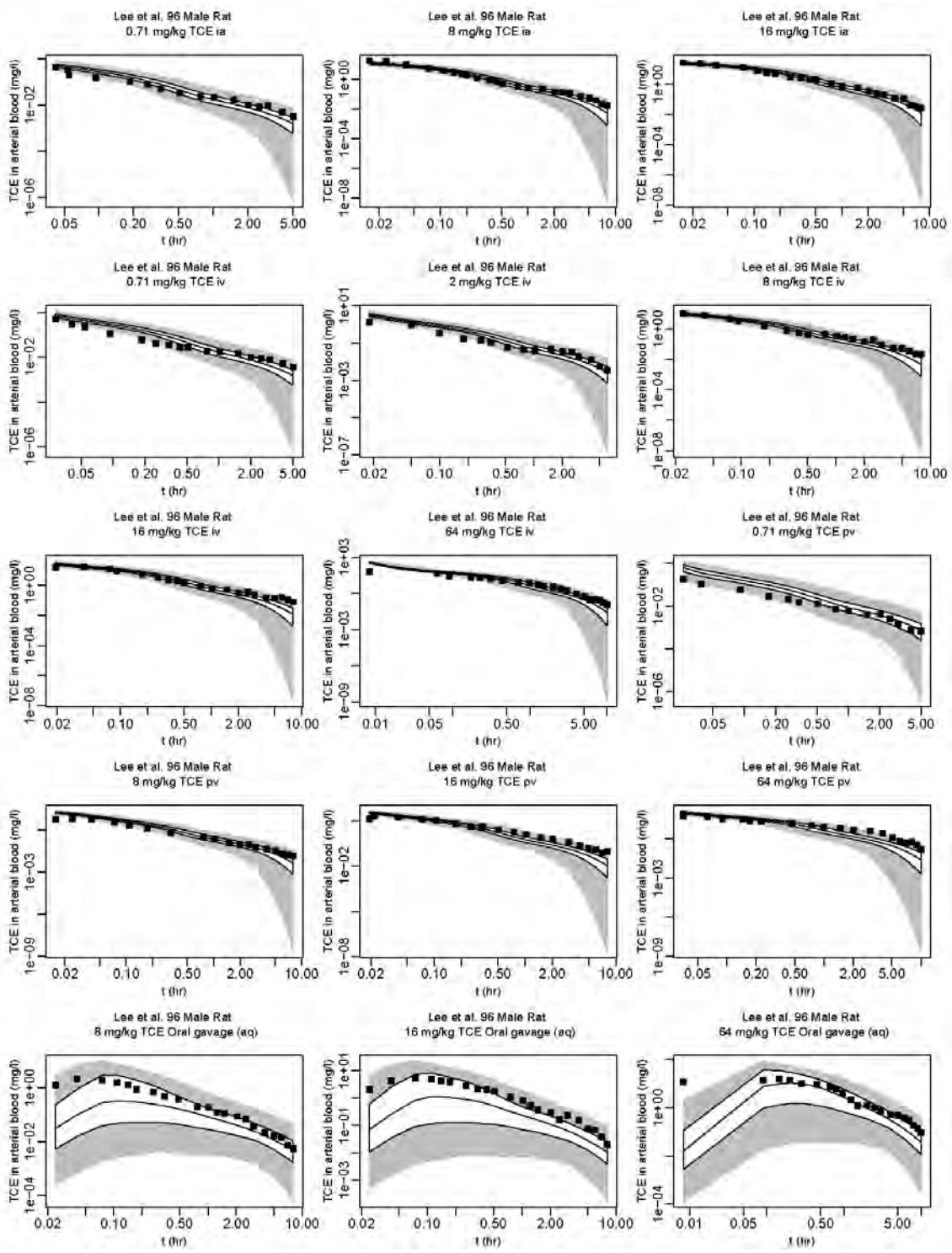


Figure A-33. Comparison of rat evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions).

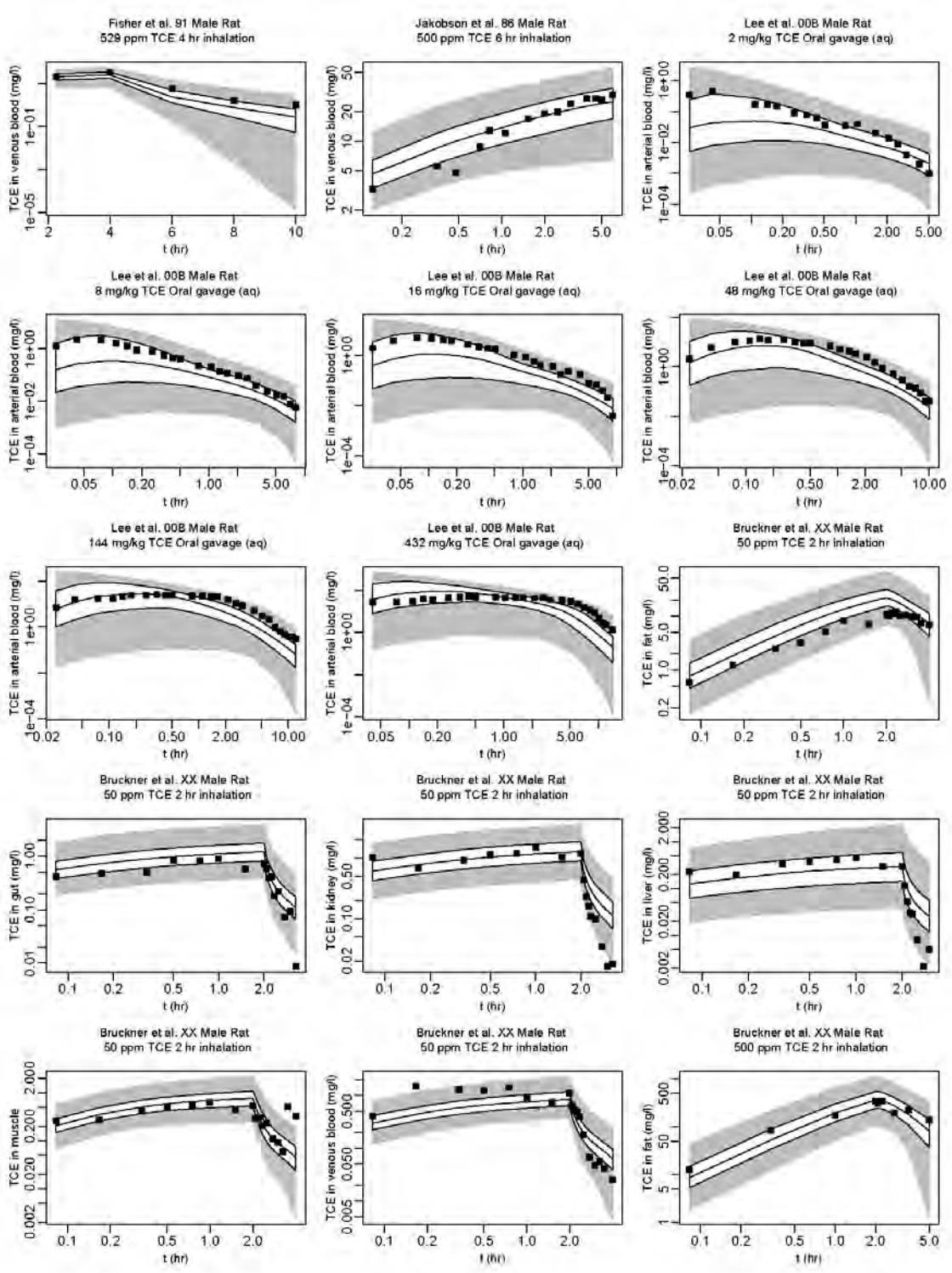


Figure A-33. Comparison of rat evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

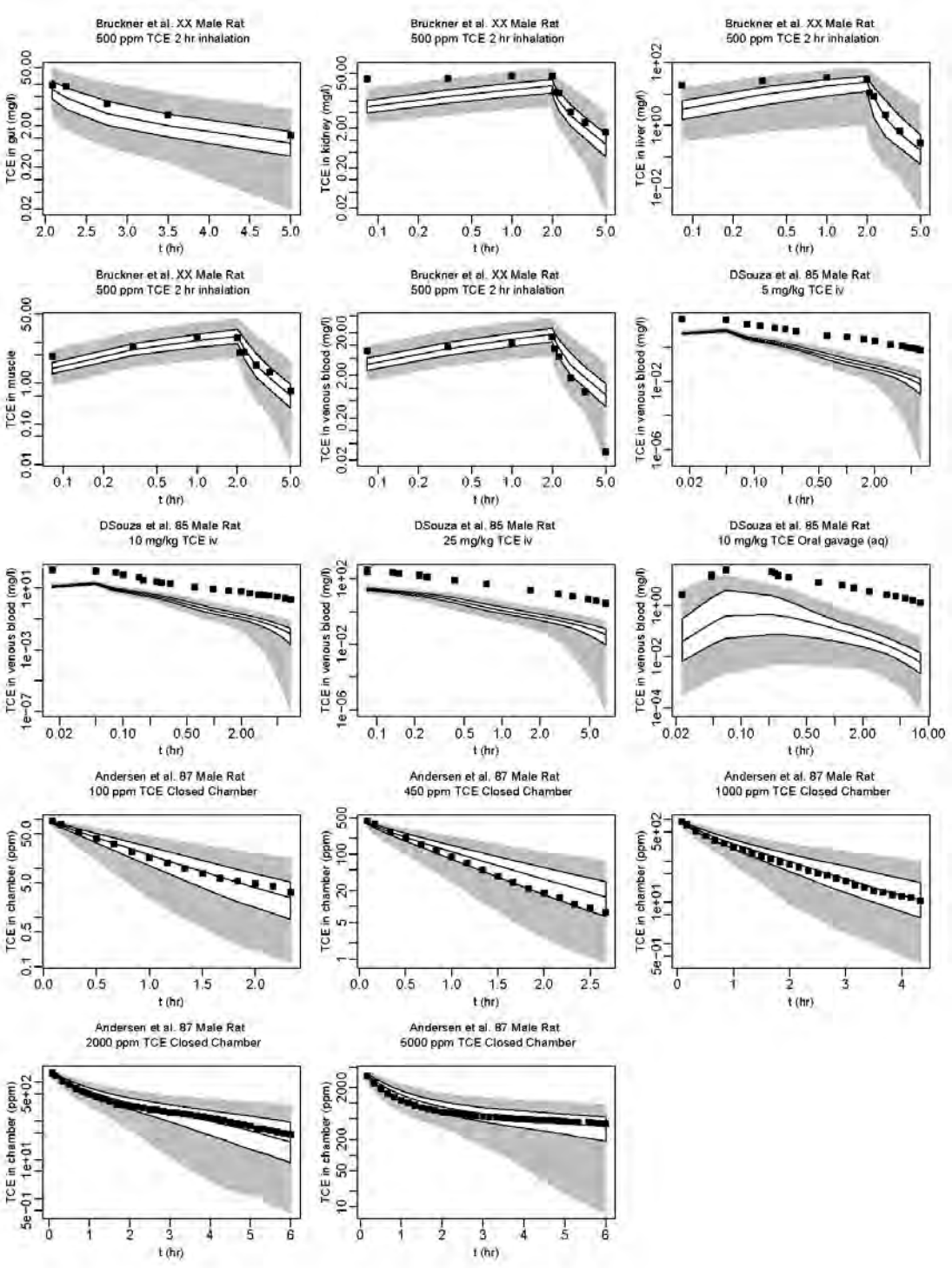


Figure A-33. Comparison of rat evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

A.5.2.3. Human Data and Model Predictions

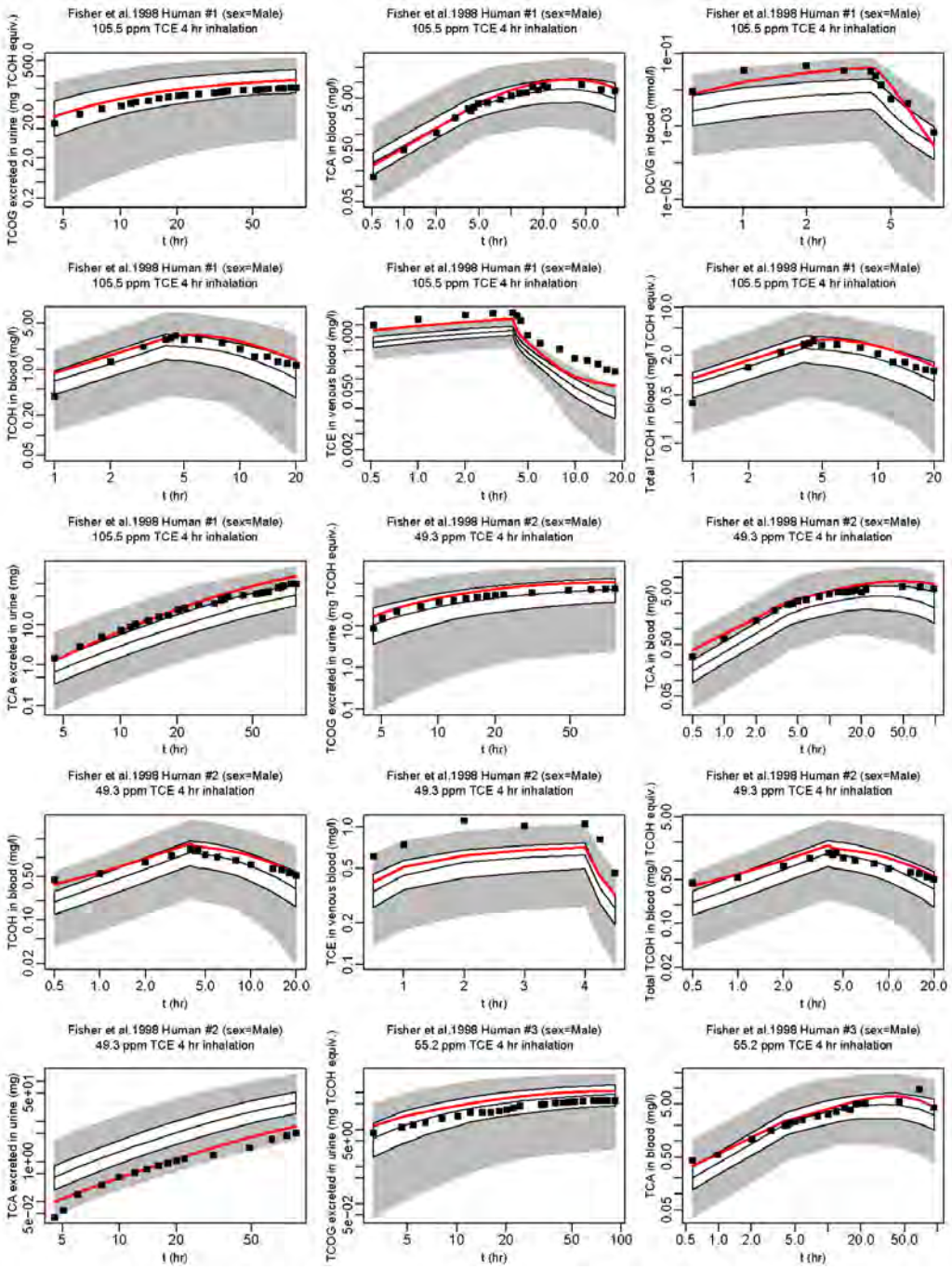


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions).

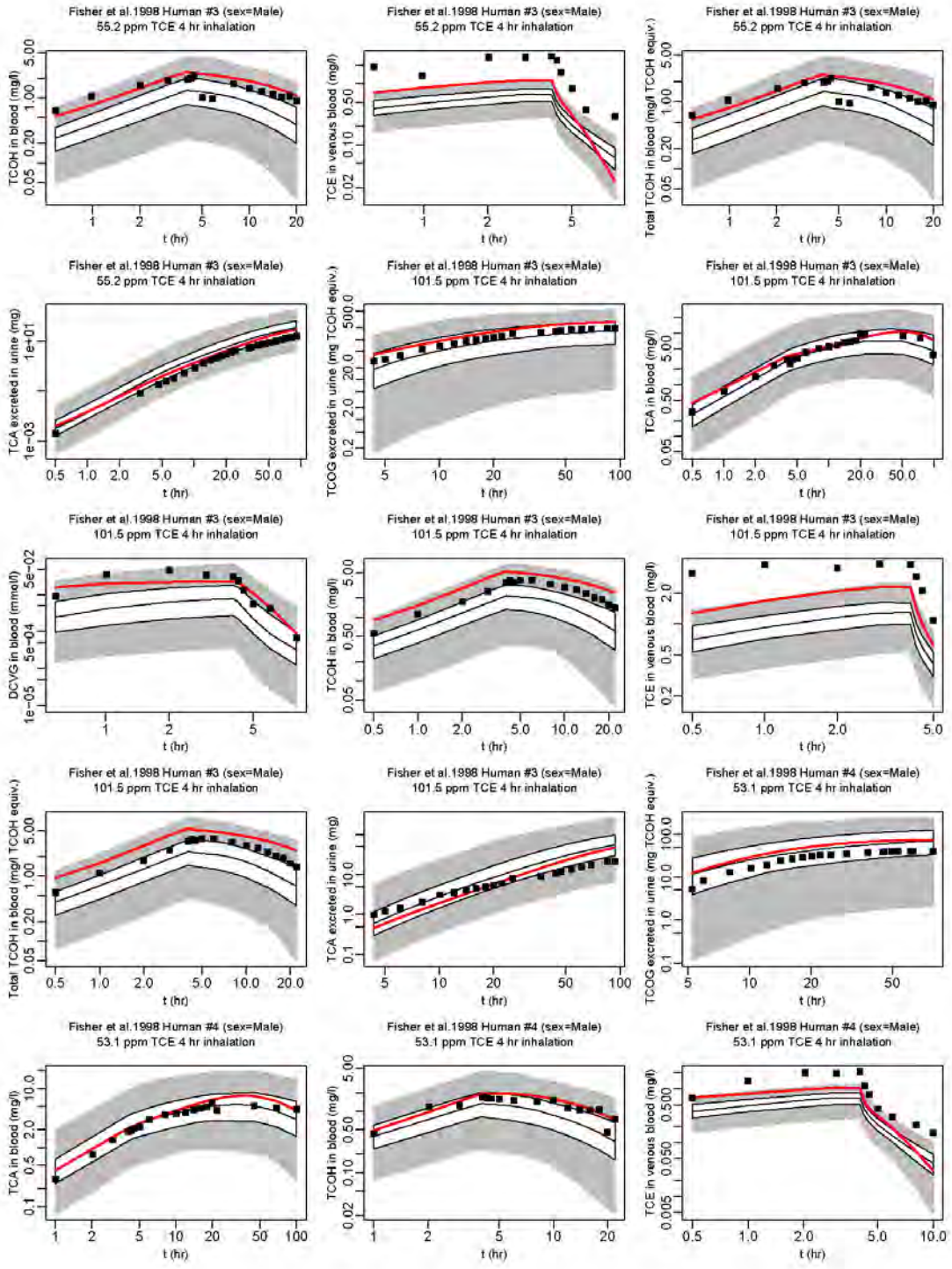


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

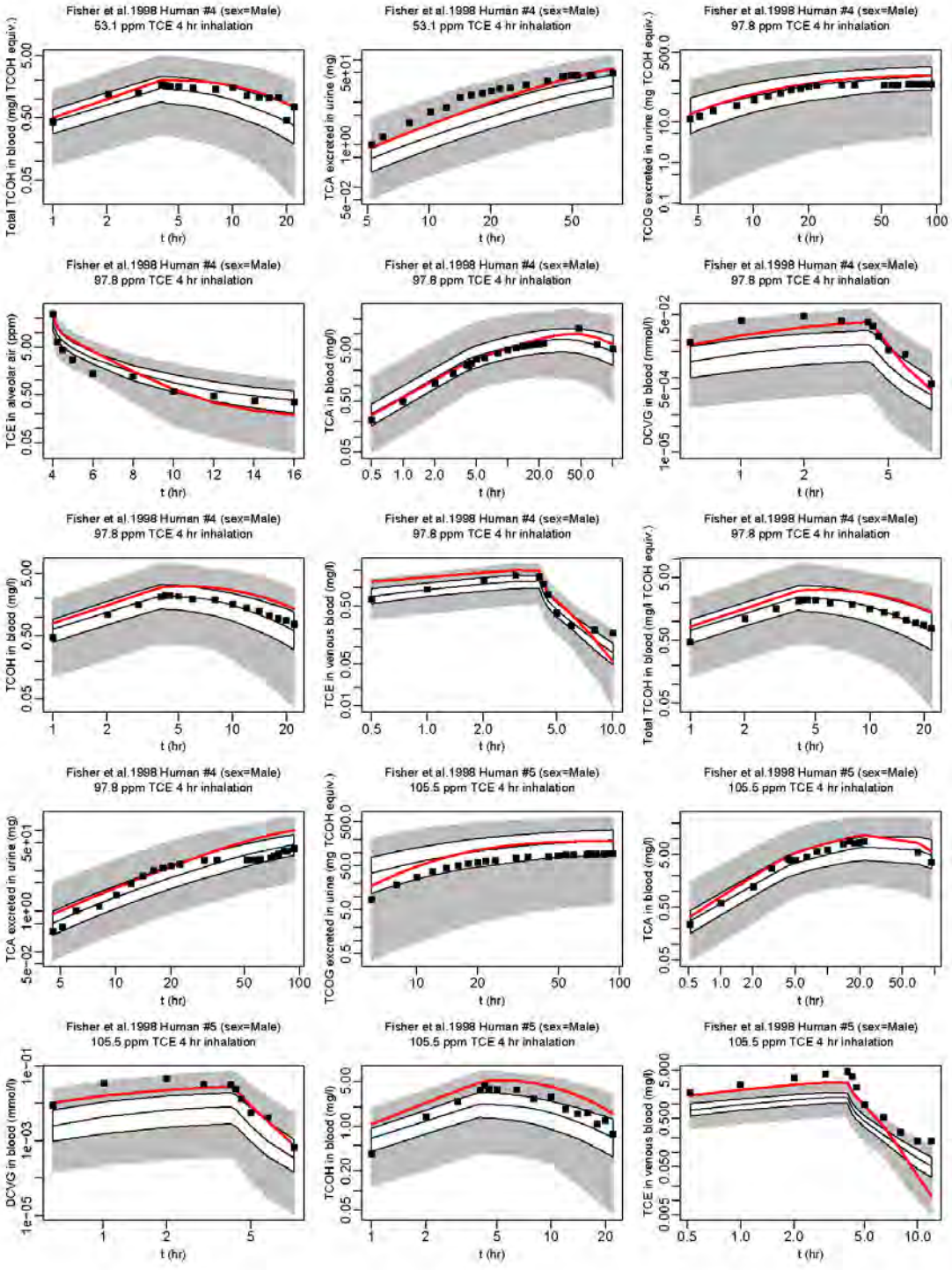


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

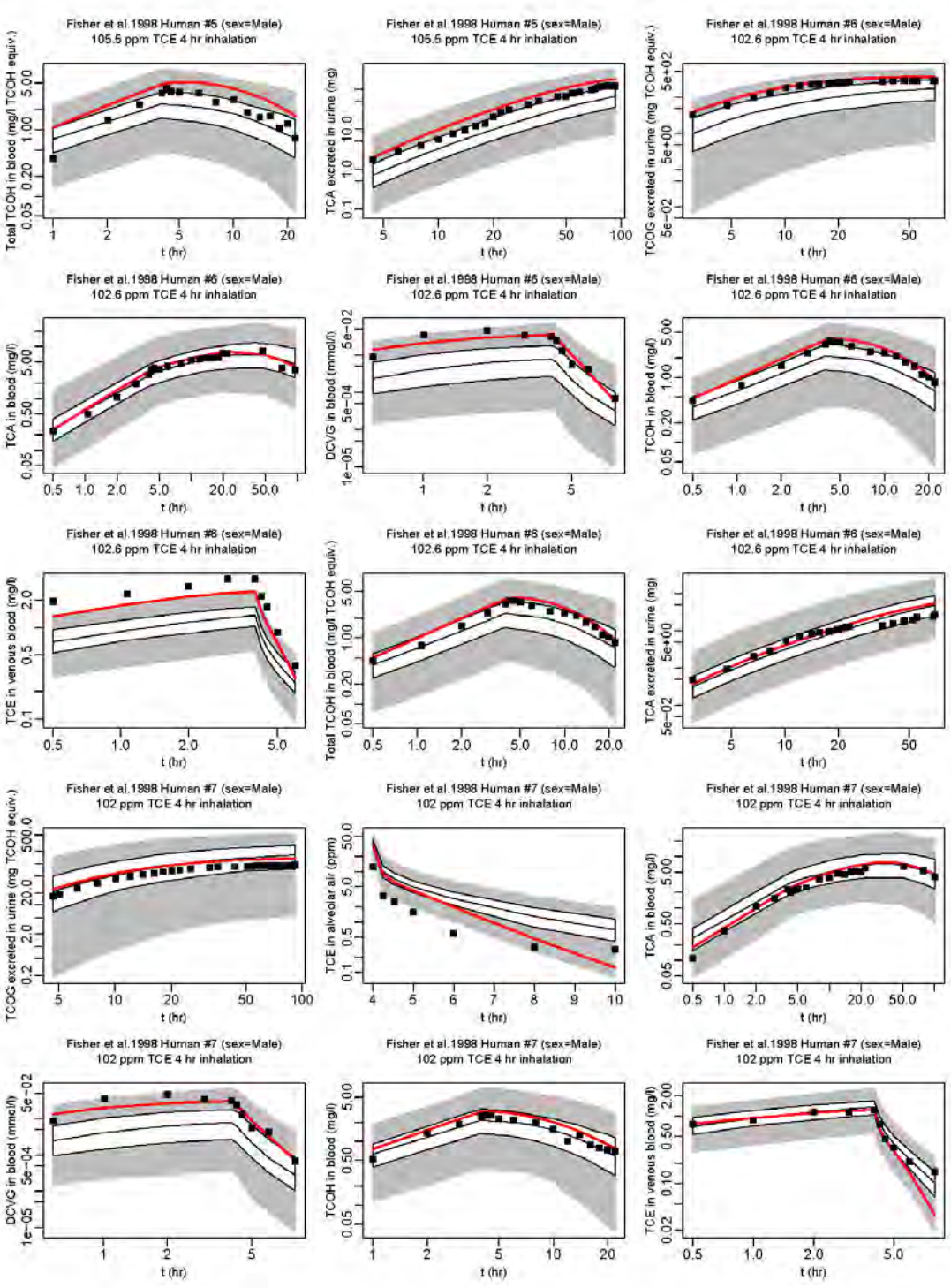


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

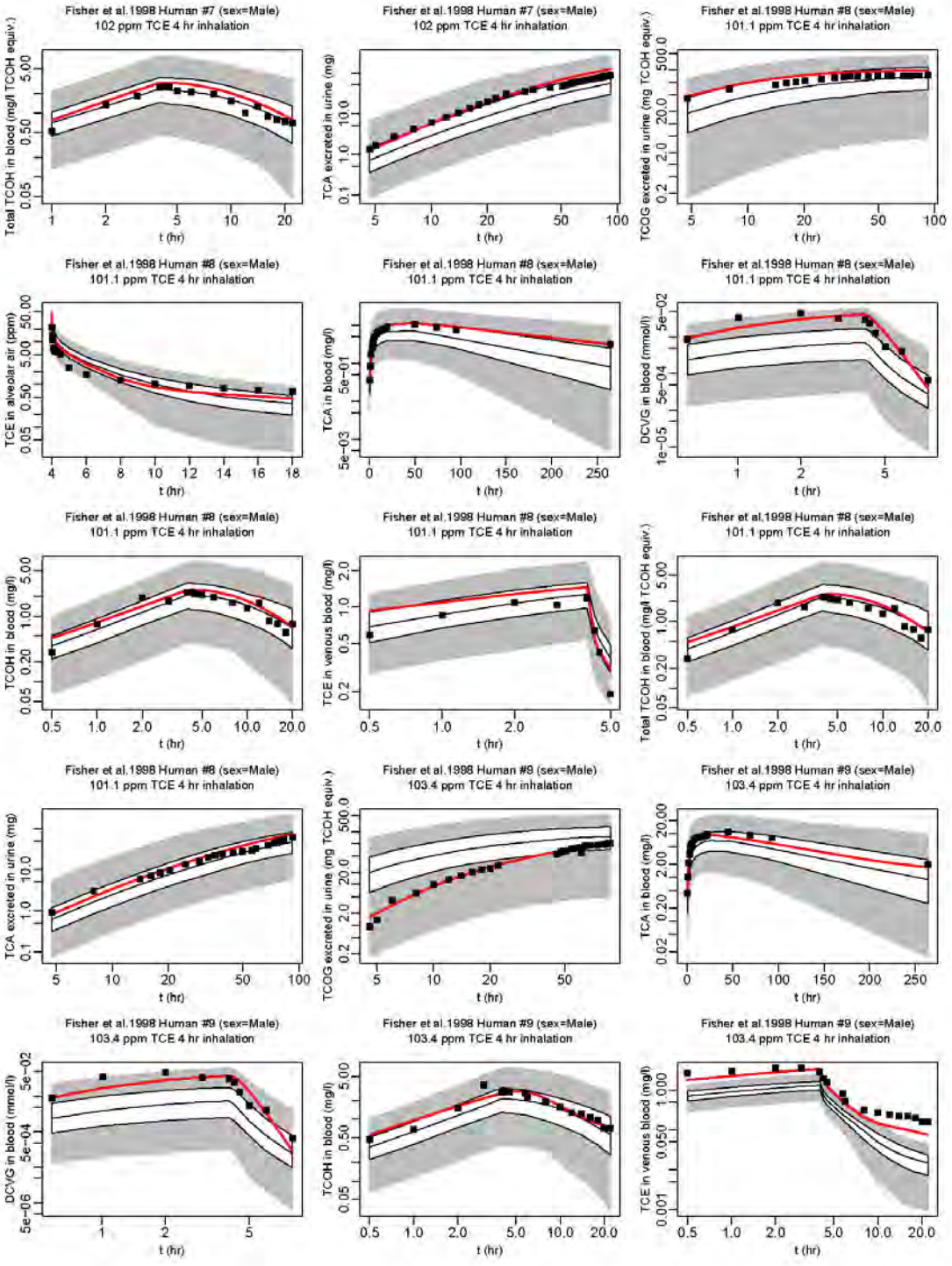


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

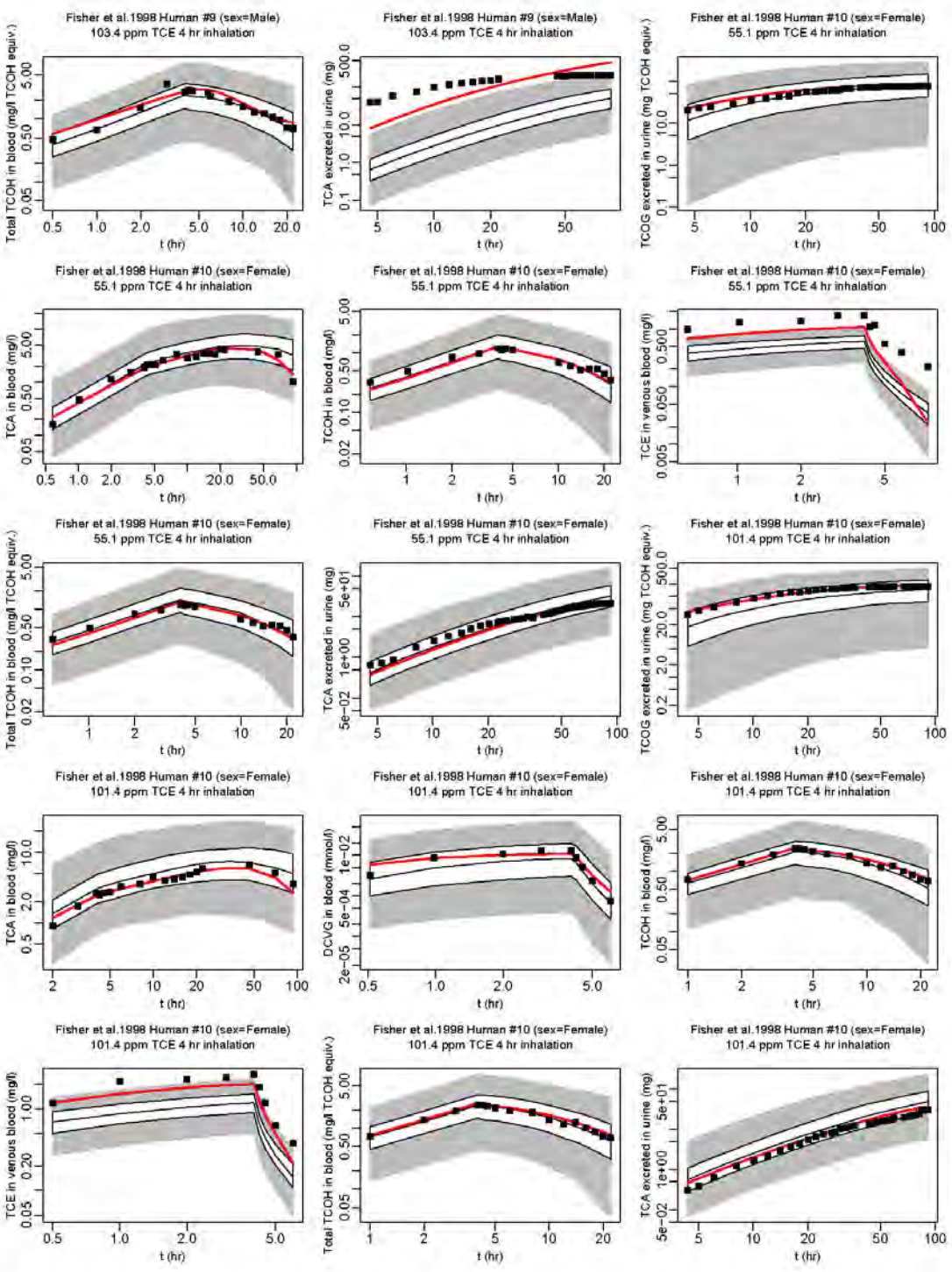


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

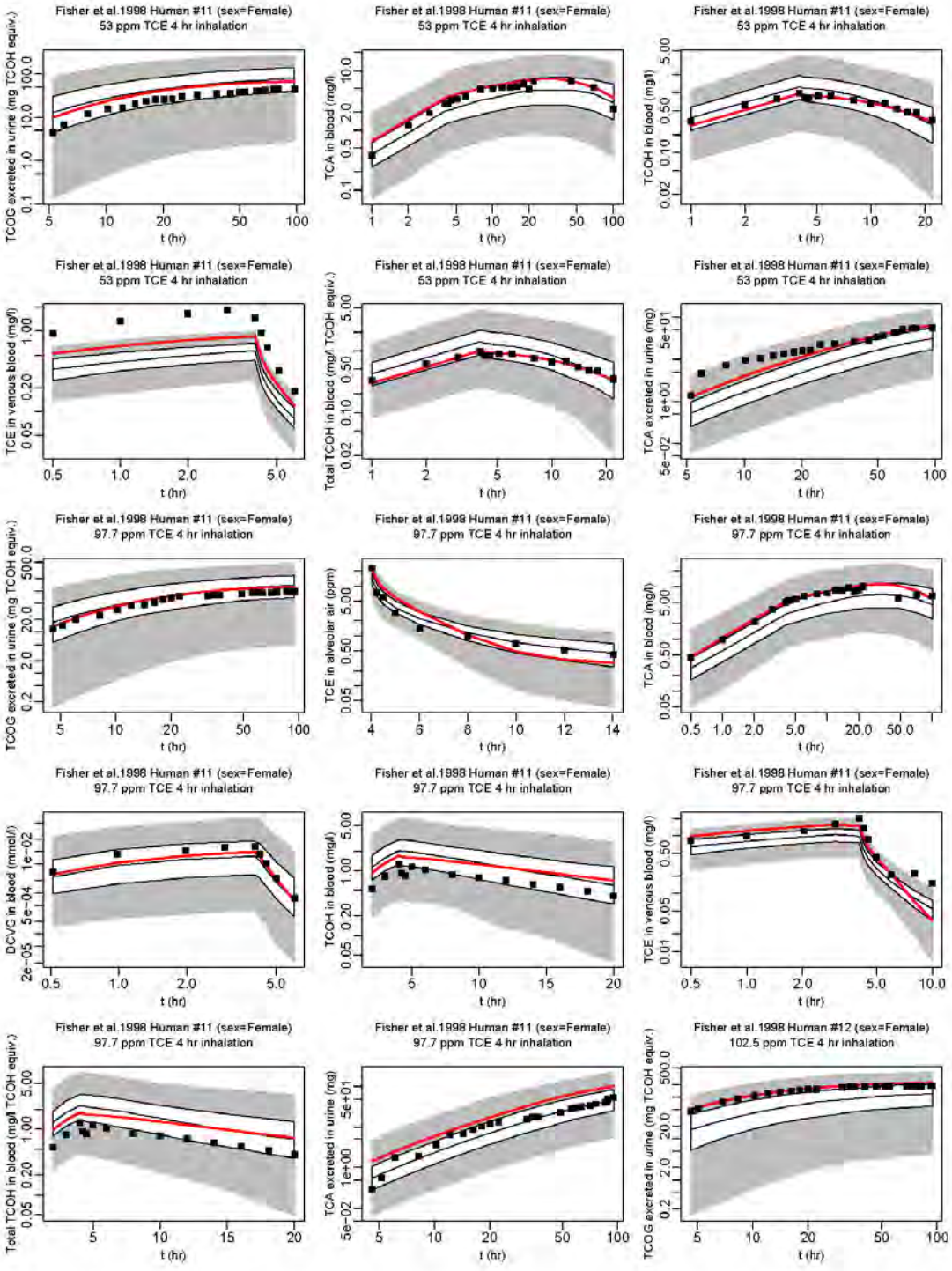


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

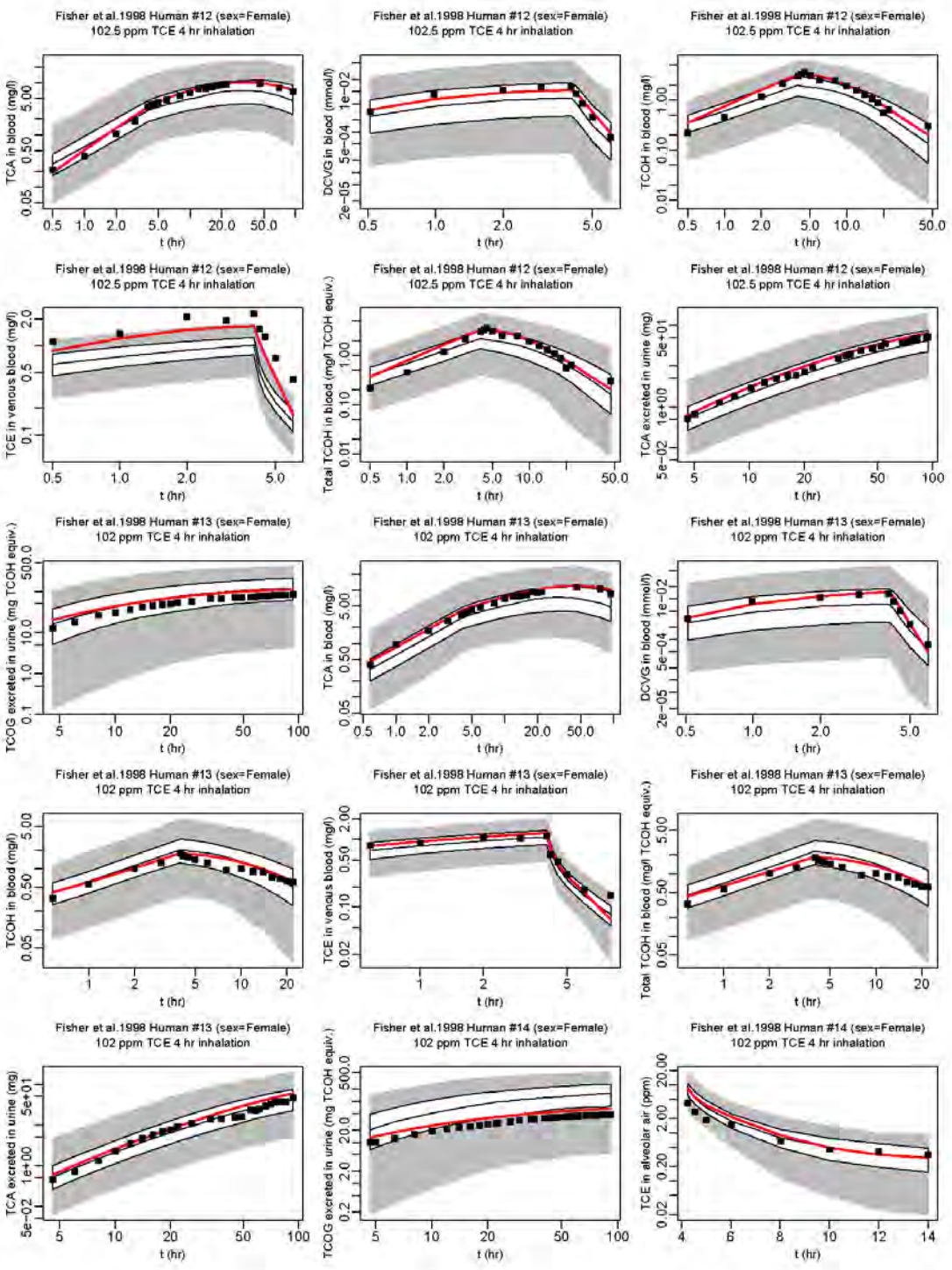


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

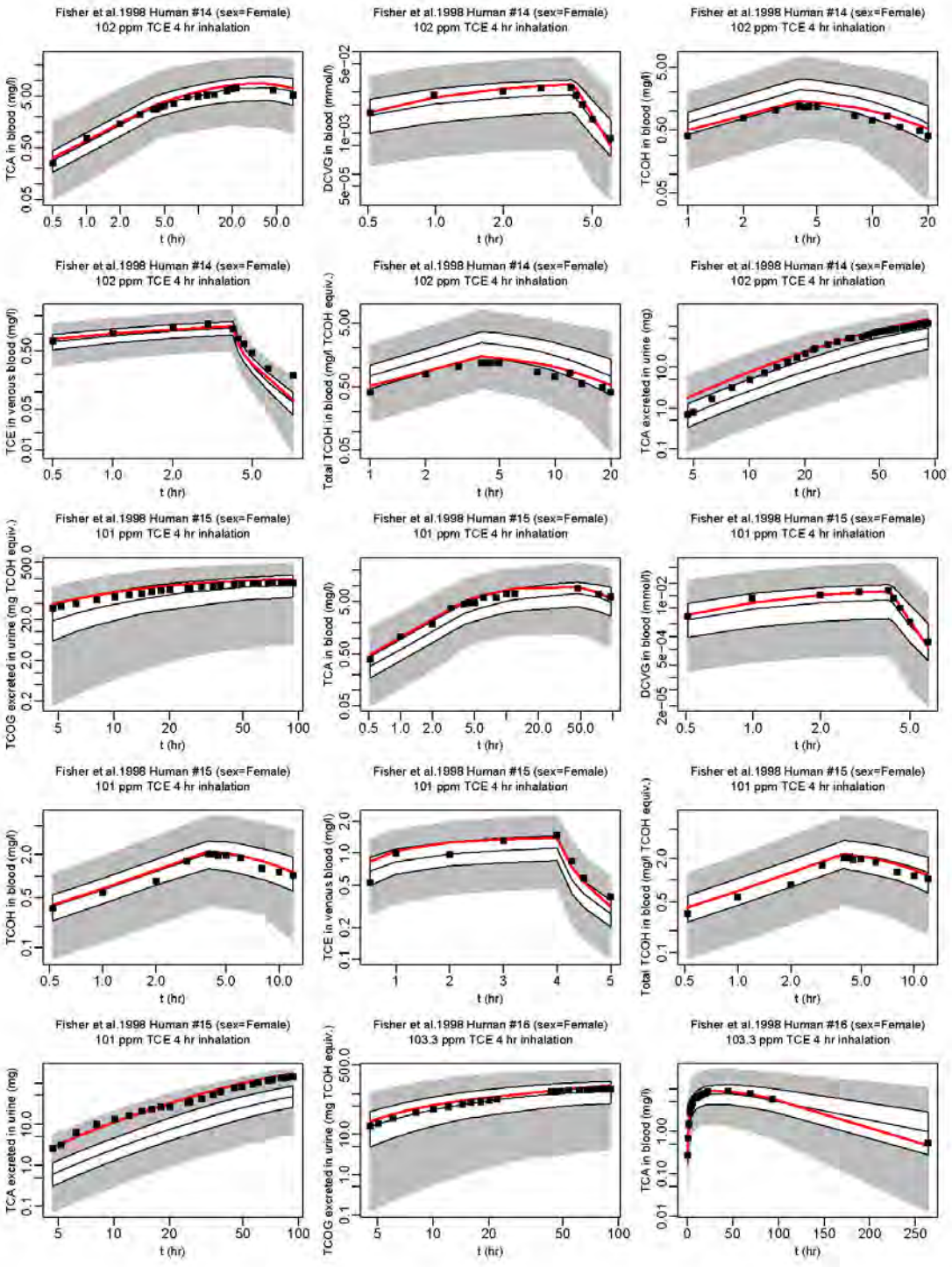


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

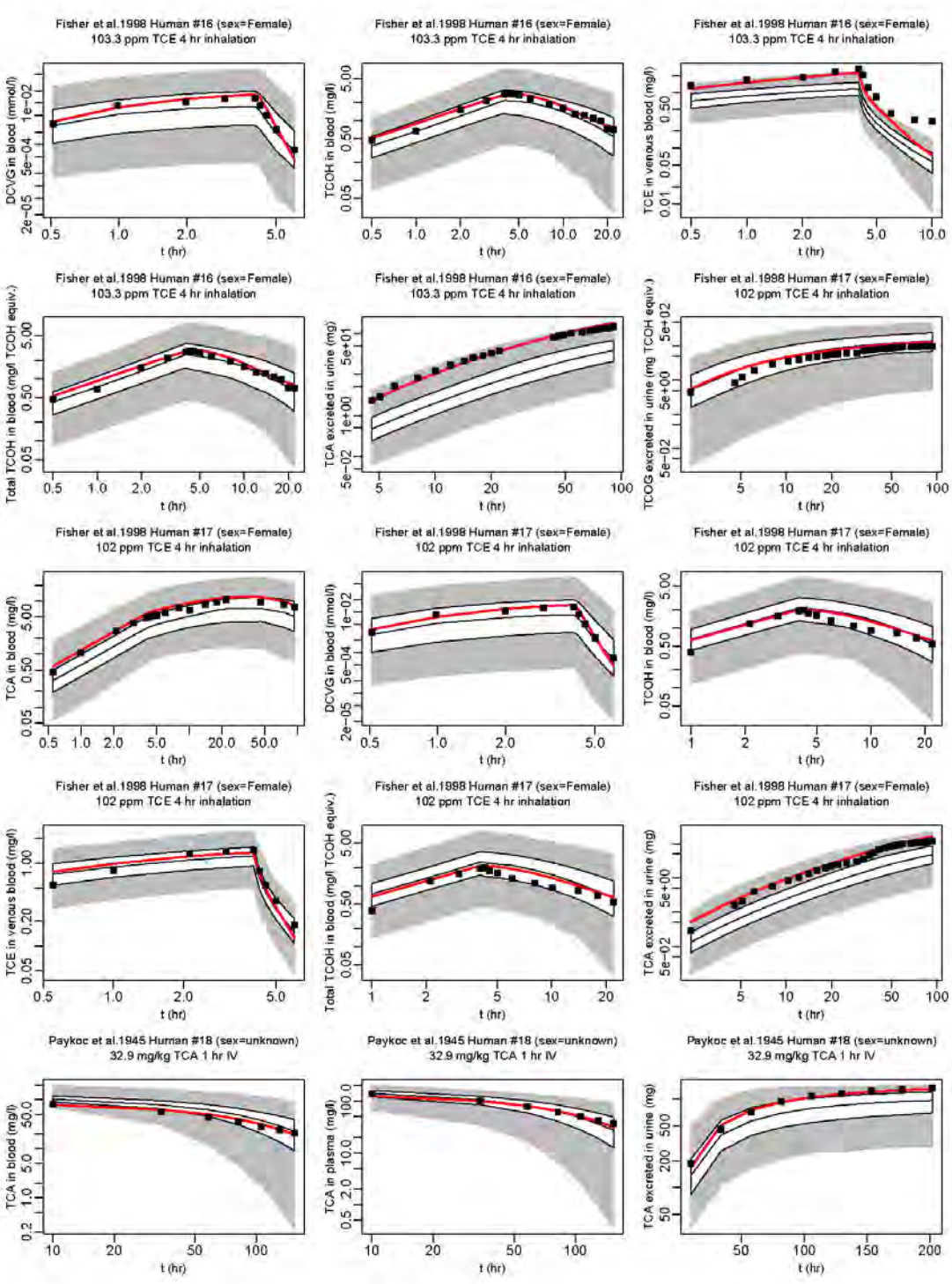


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

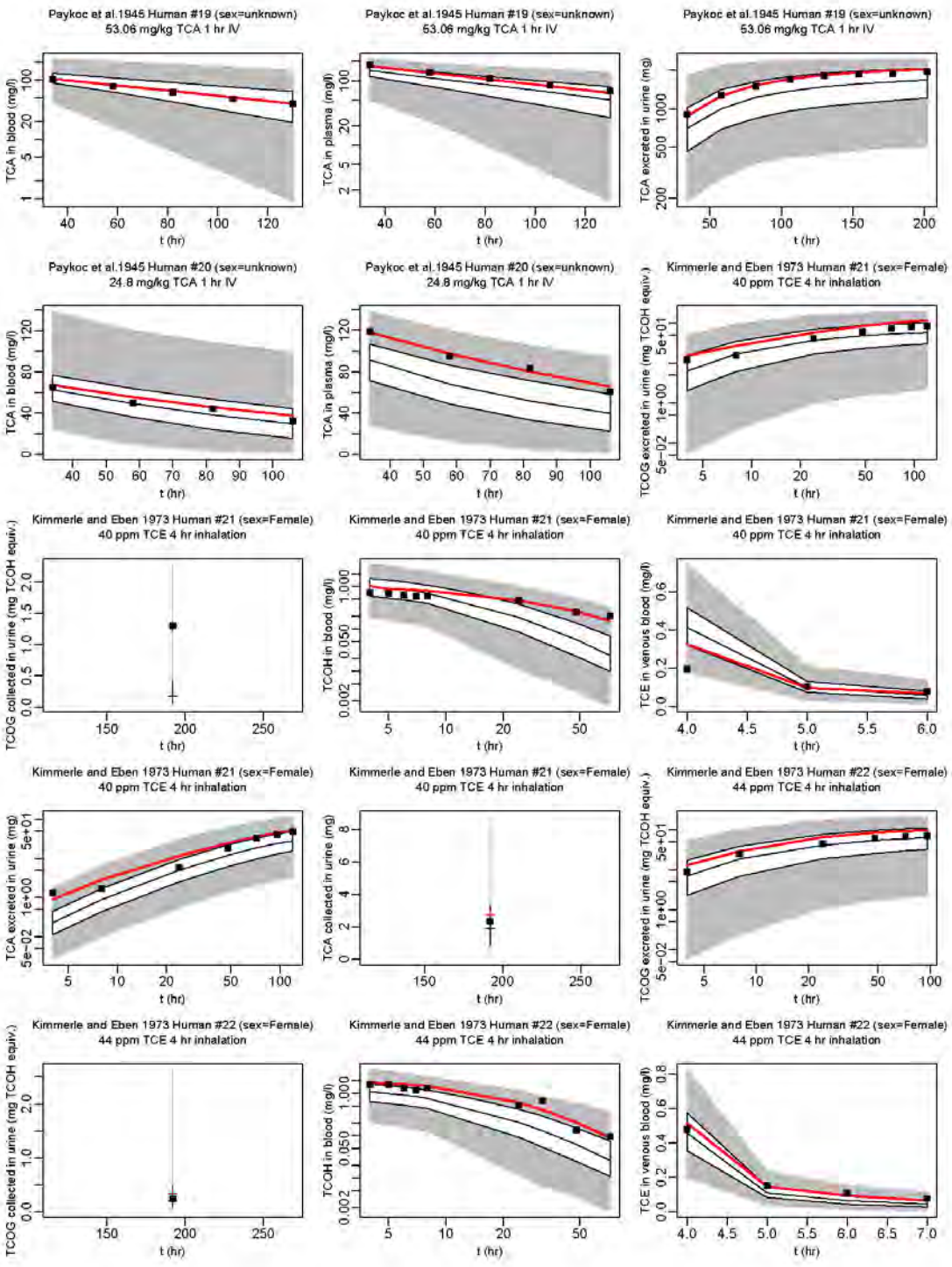
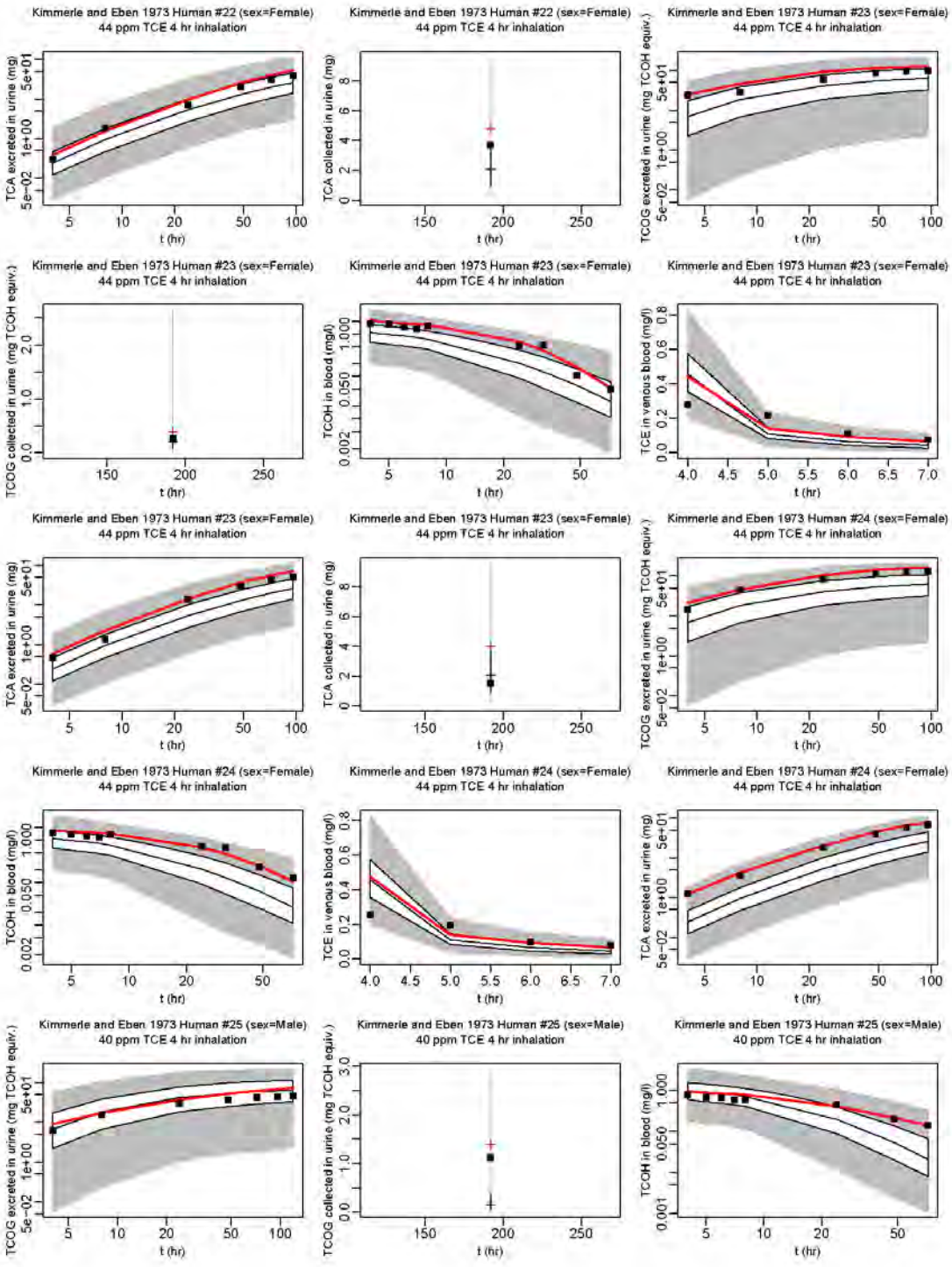


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).



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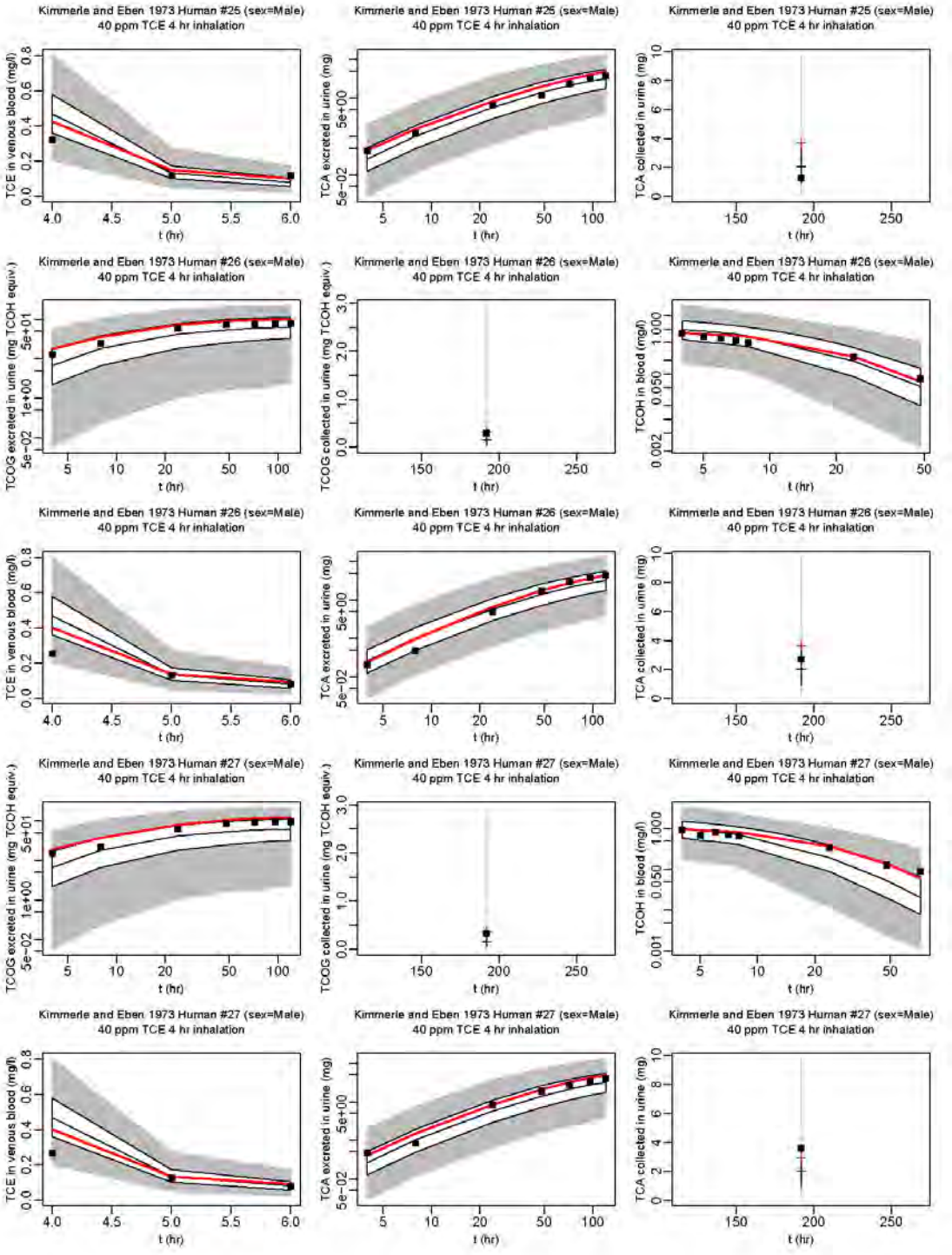

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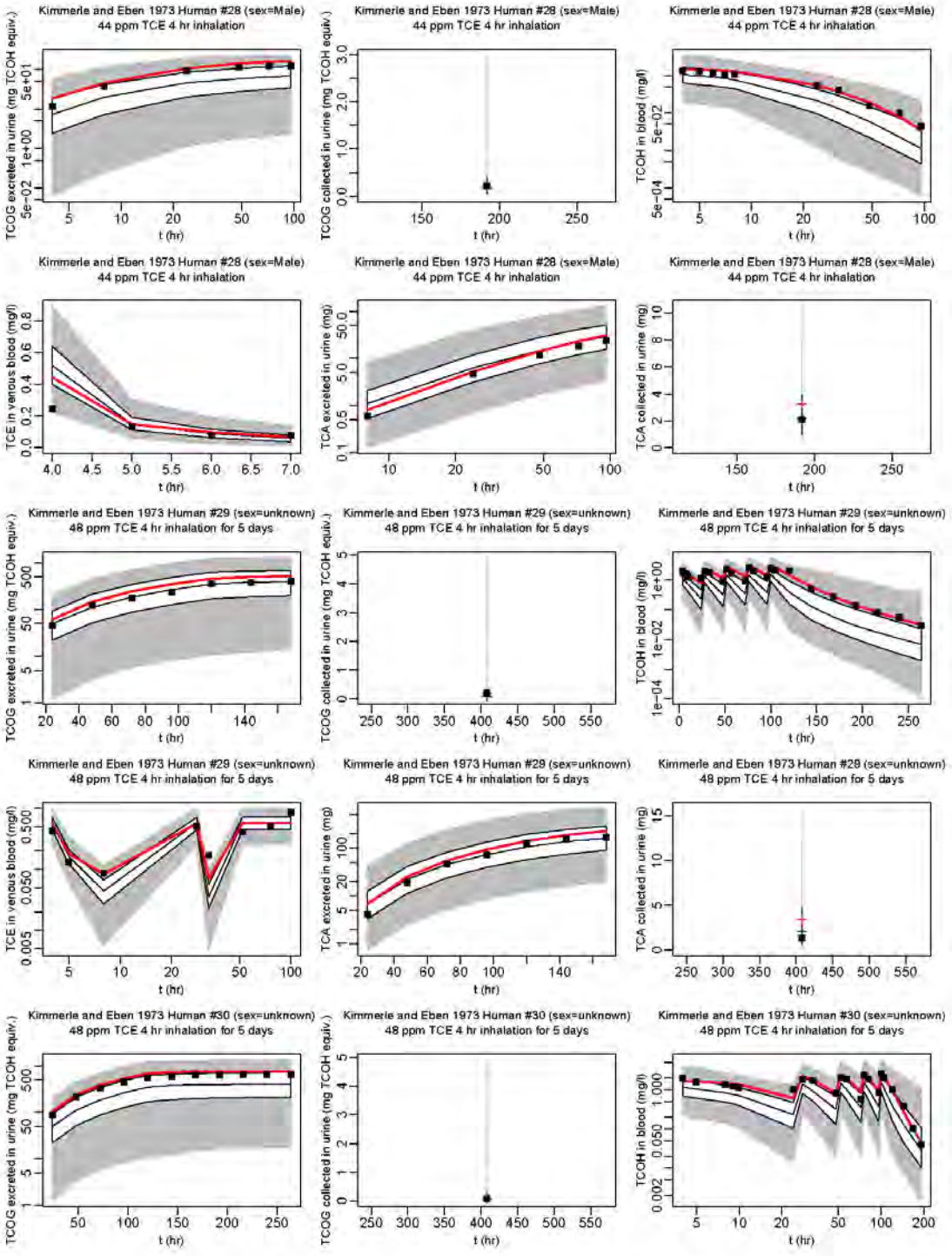


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

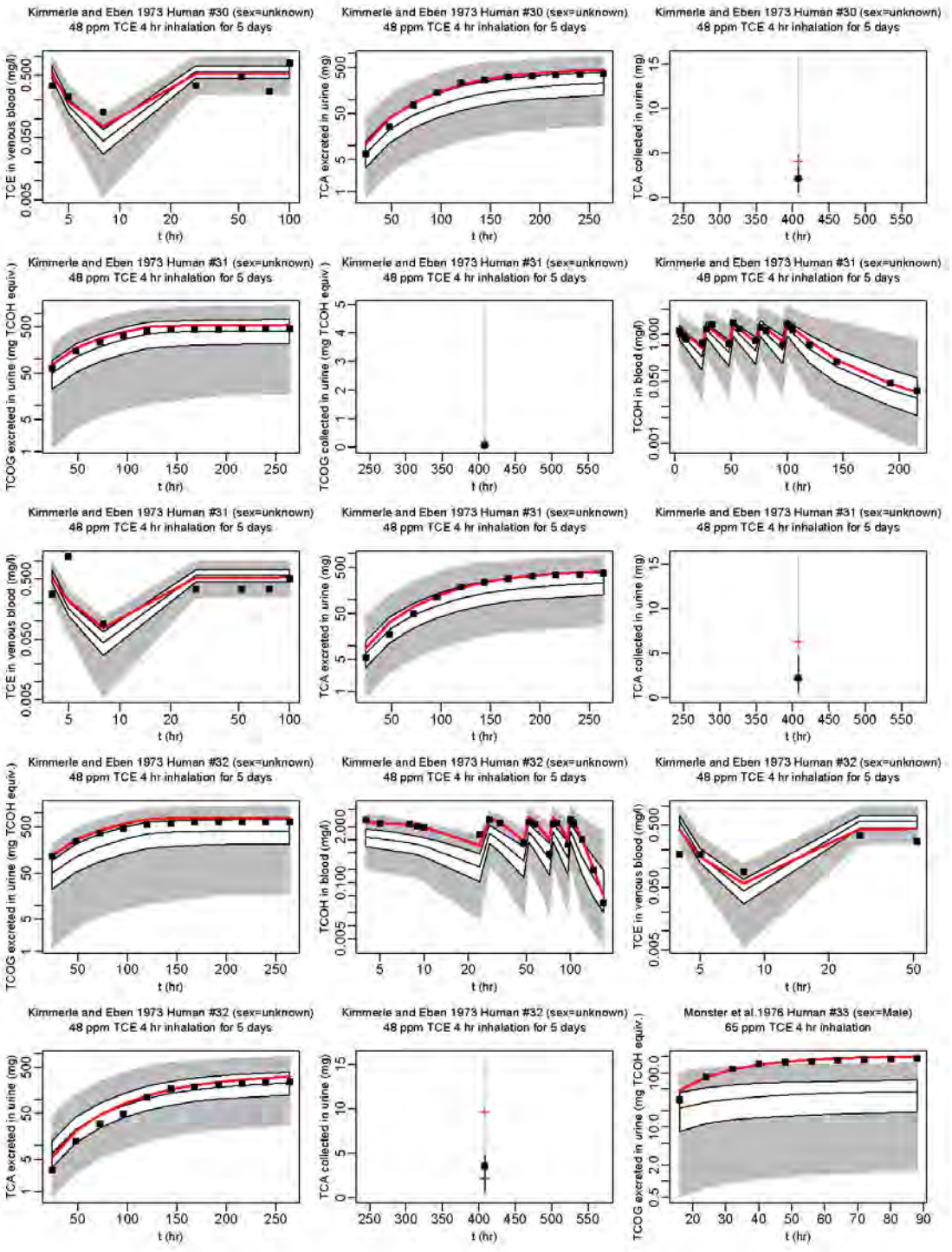


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

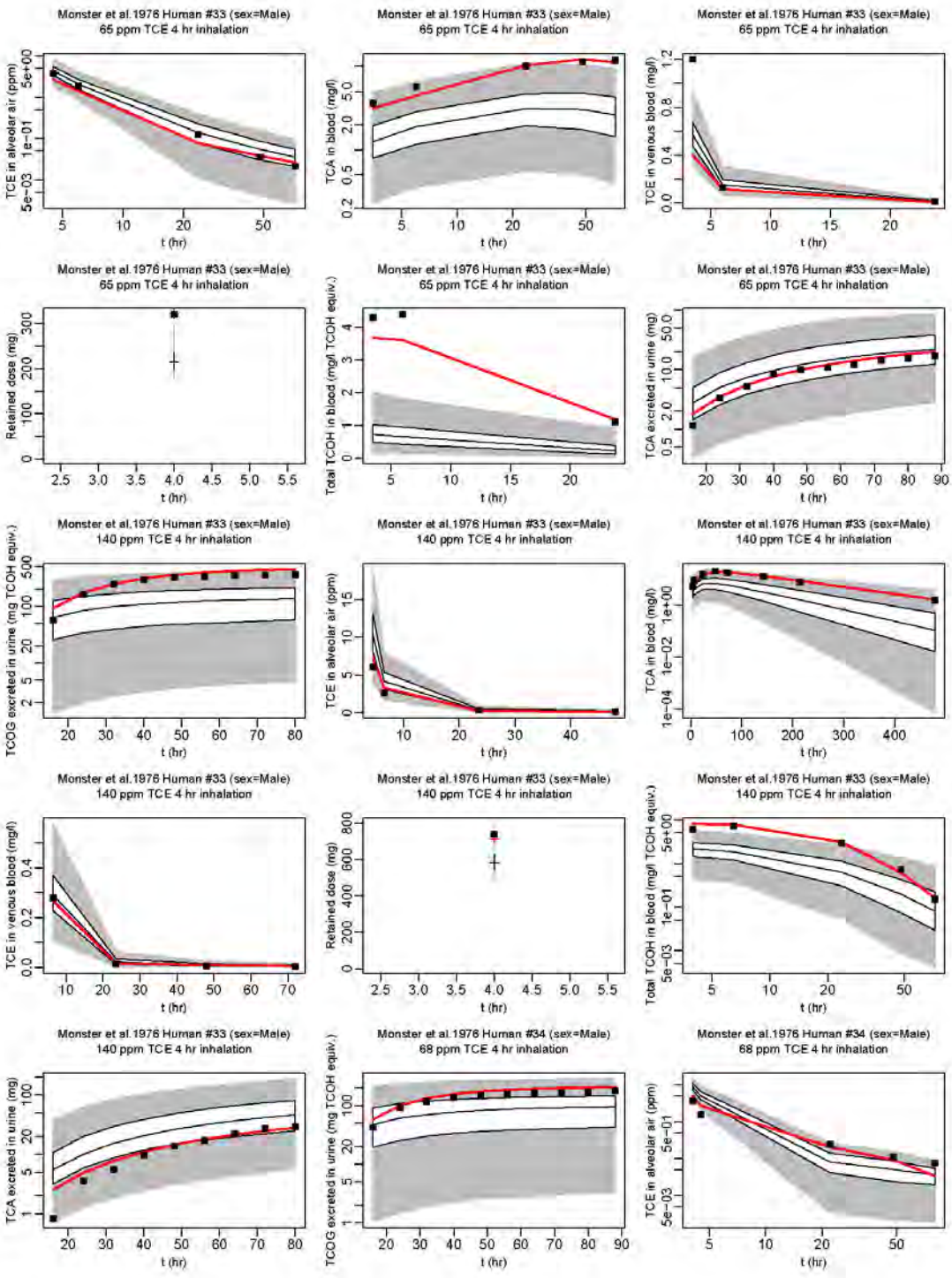


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

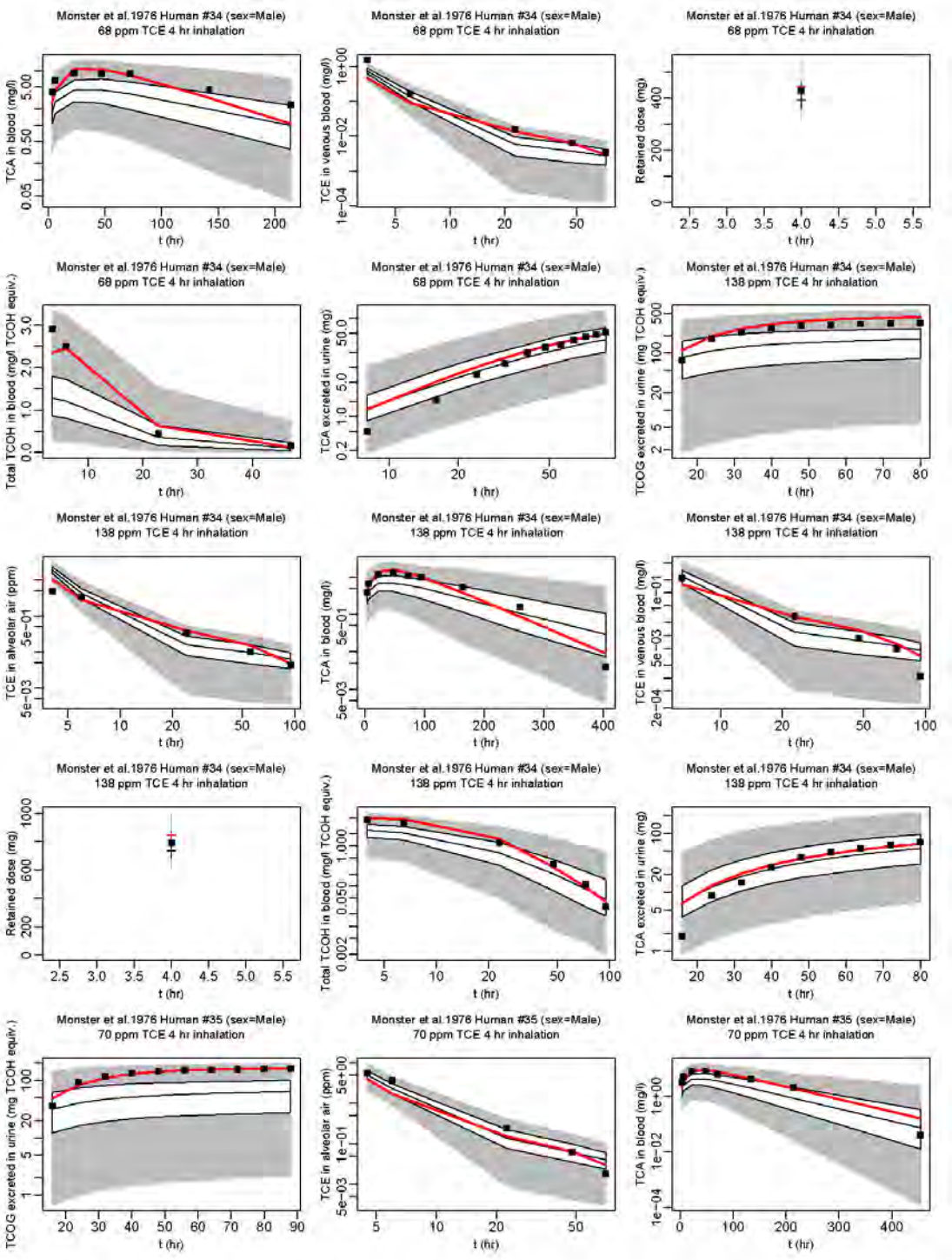


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

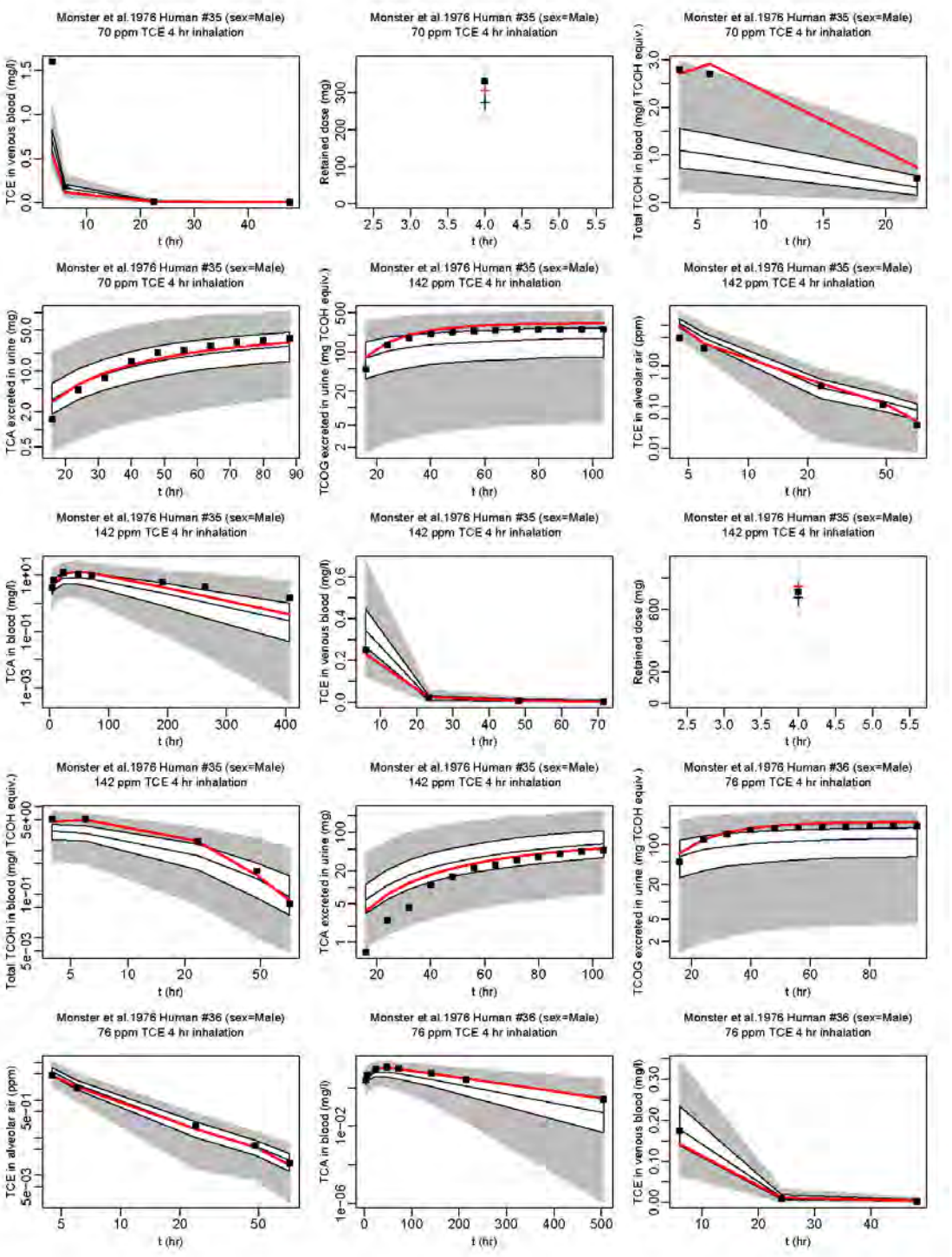
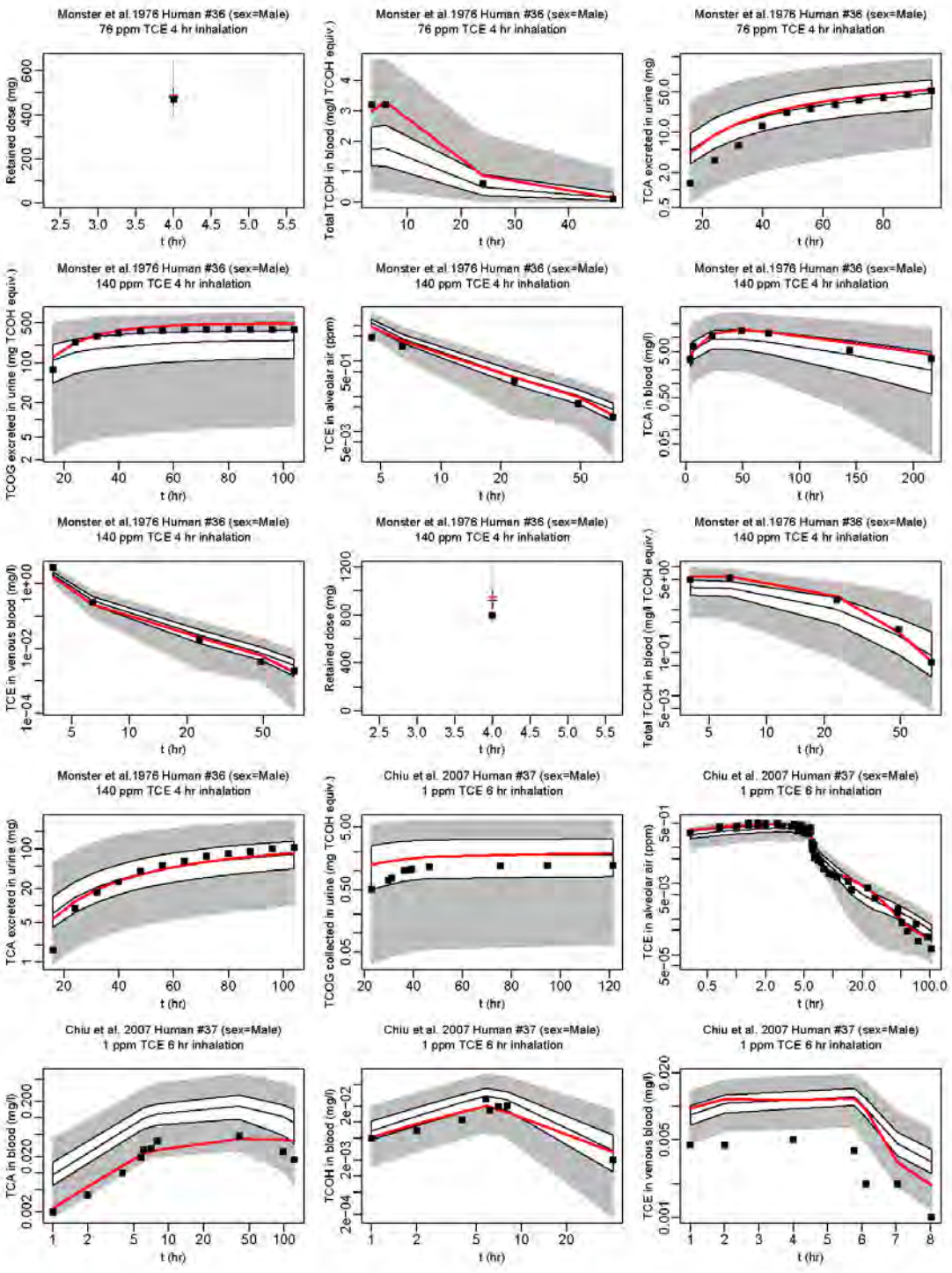


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).



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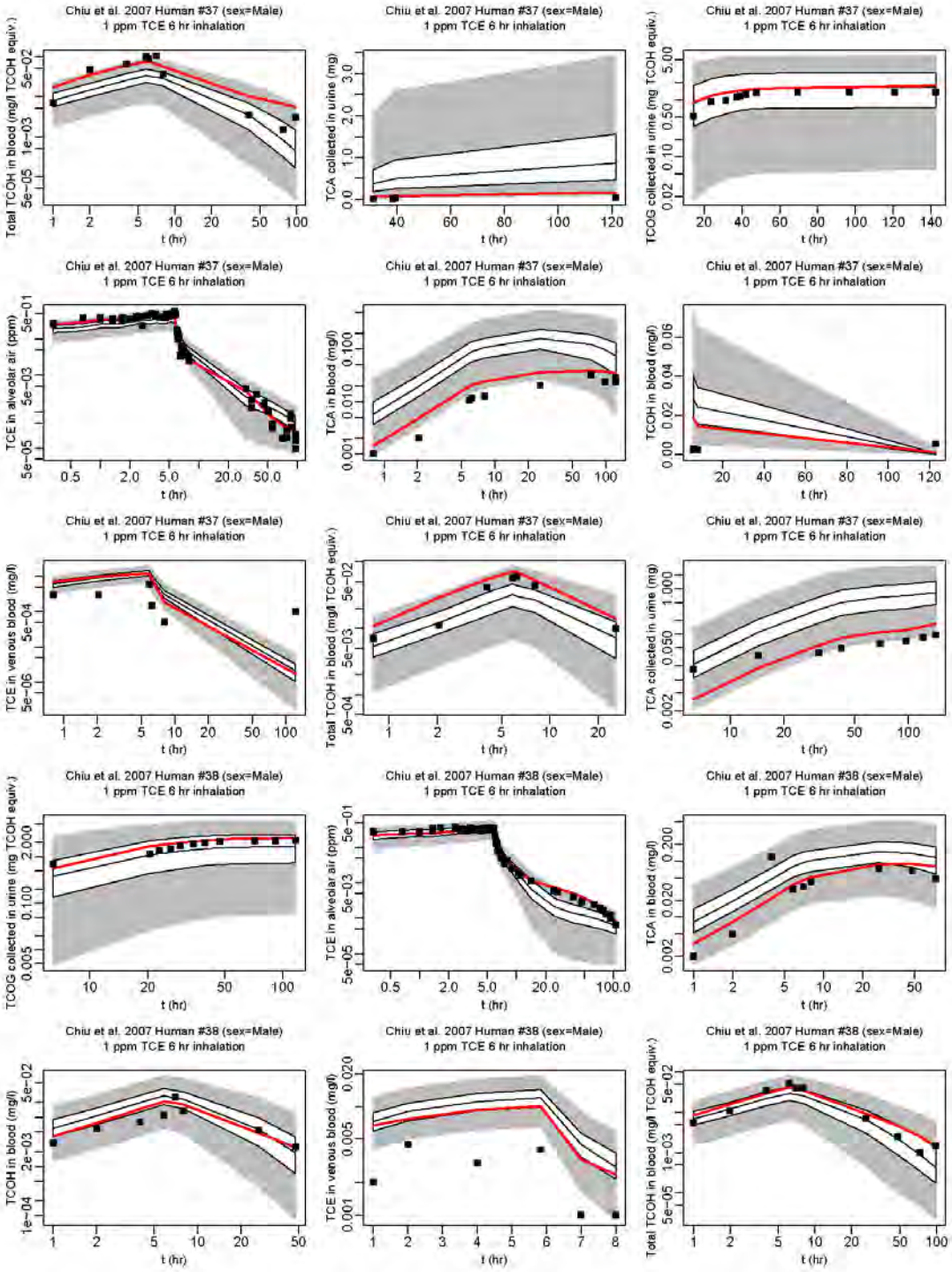

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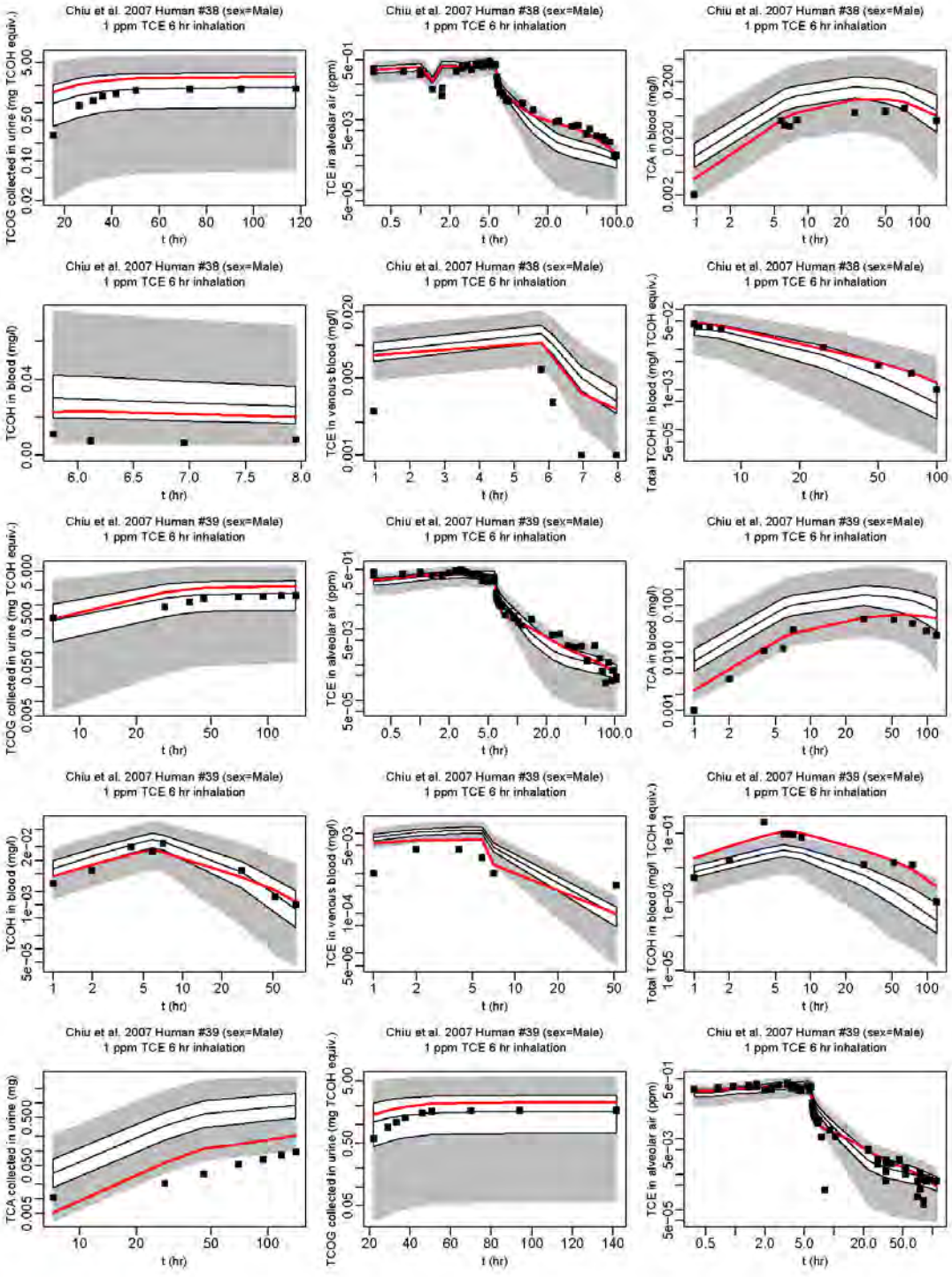
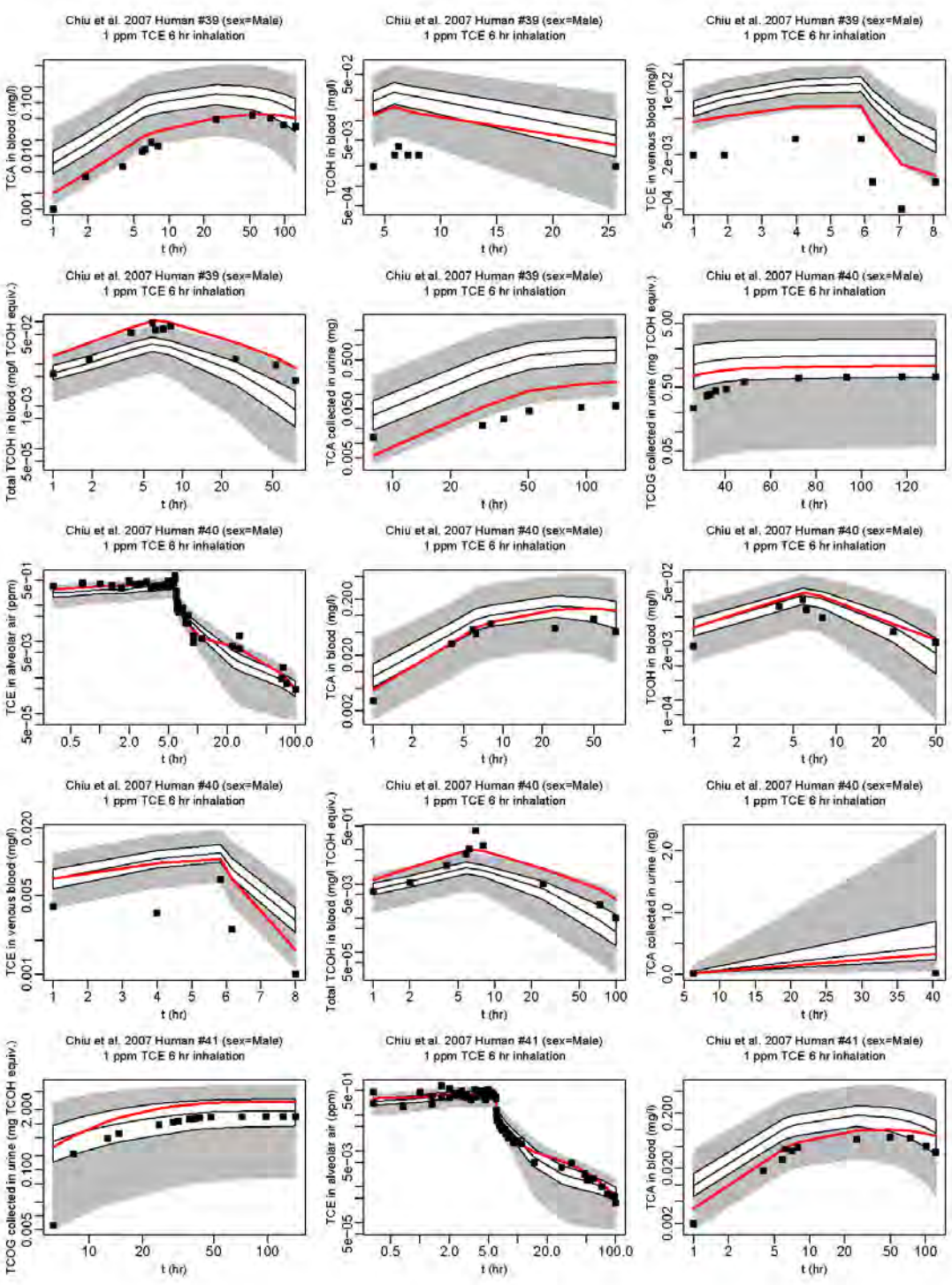


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).



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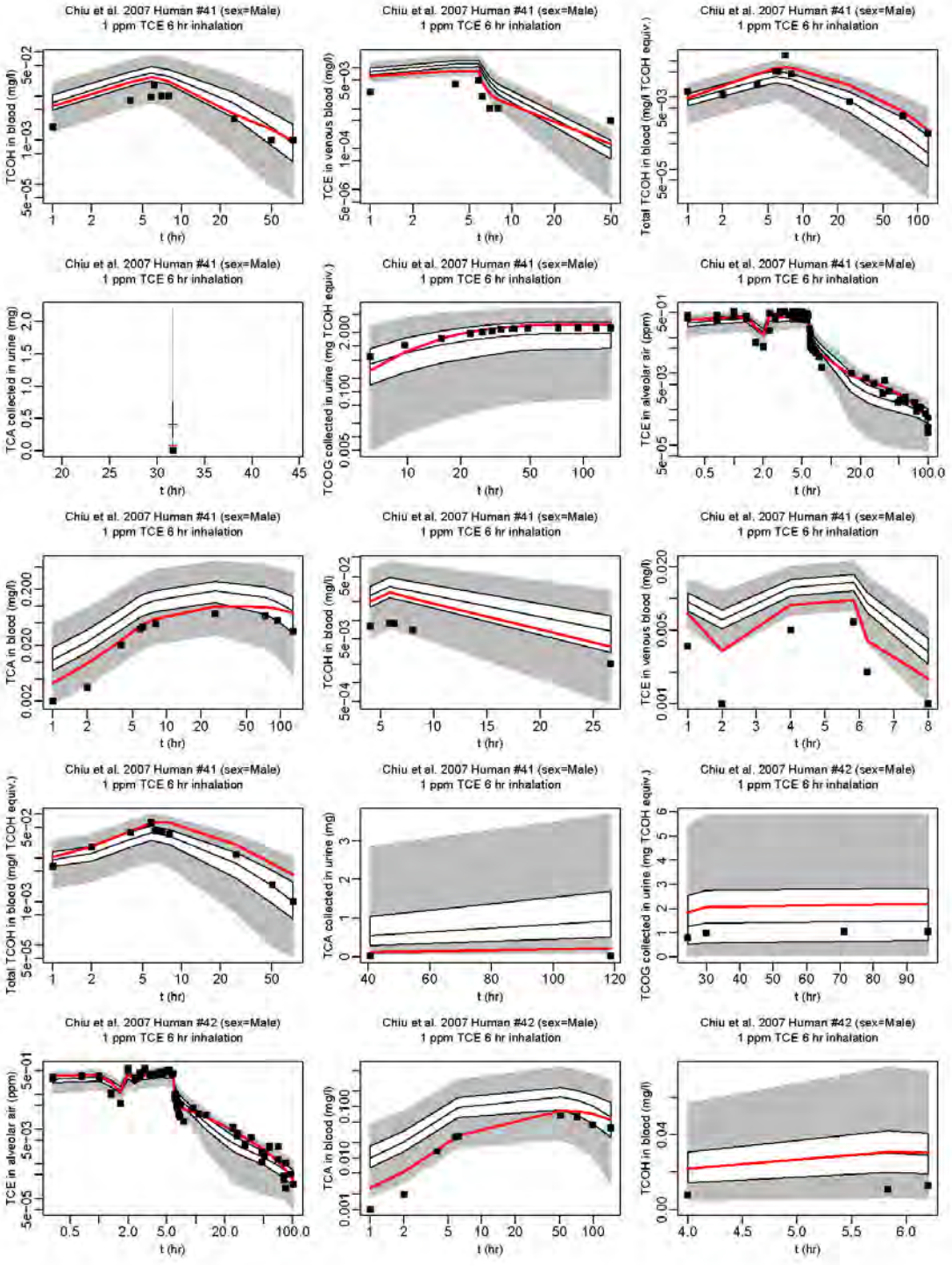


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

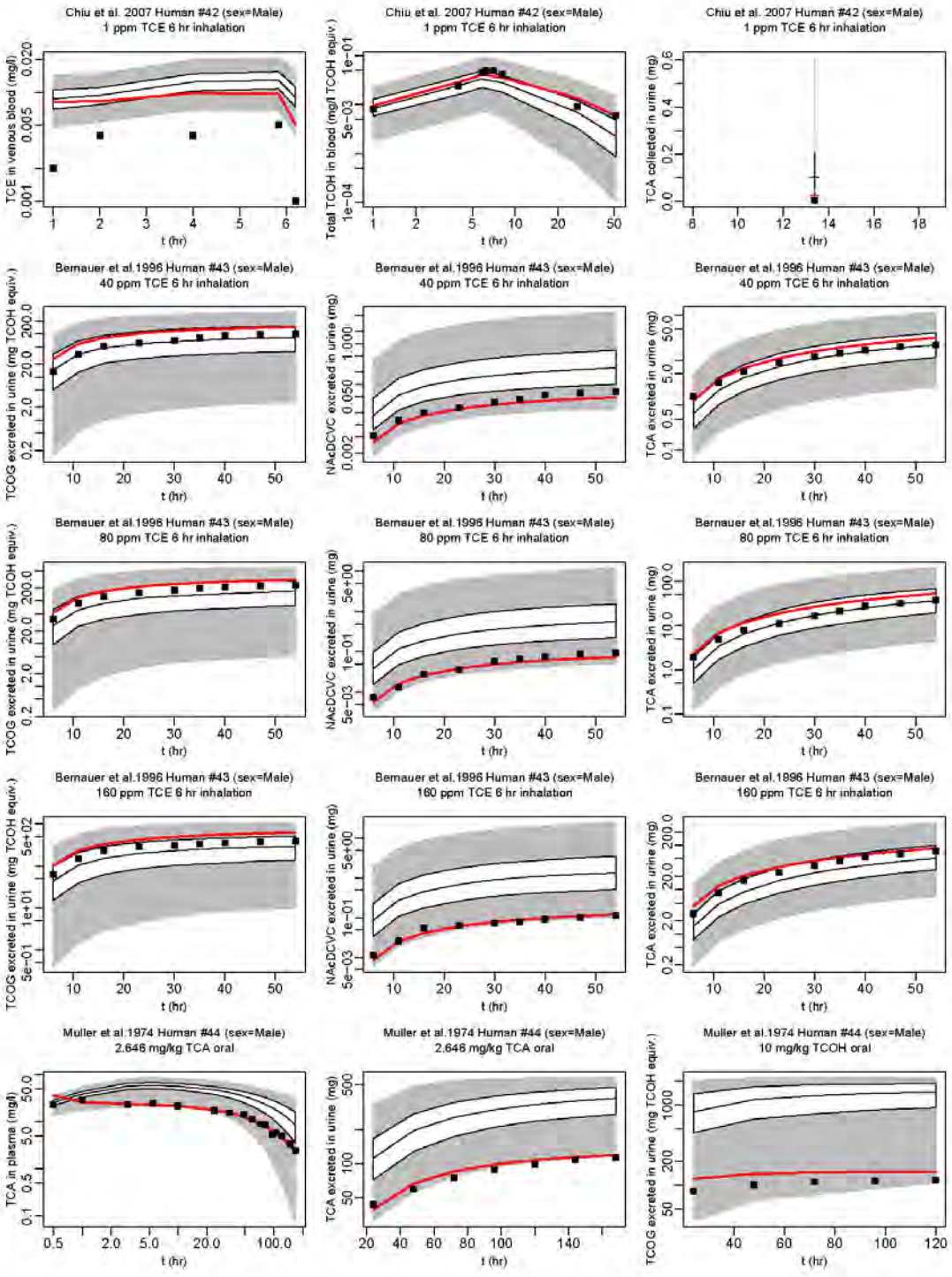


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

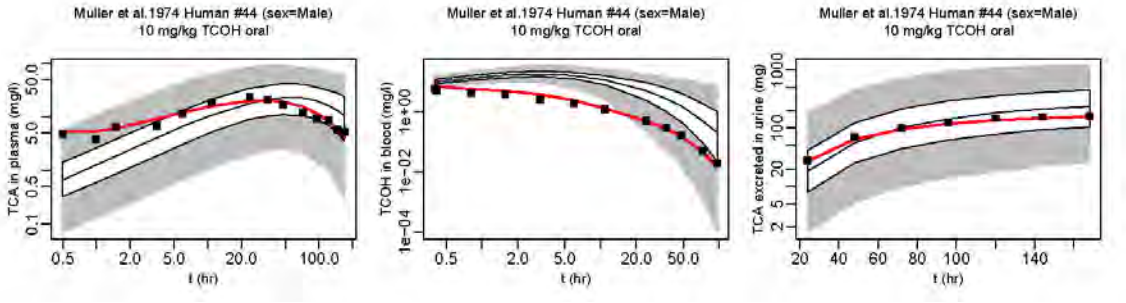


Figure A-34. Comparison of human calibration data (boxes) and PBPK model predictions (red line: using the posterior mean of the subject-specific parameters; + with error bars: single data points; or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

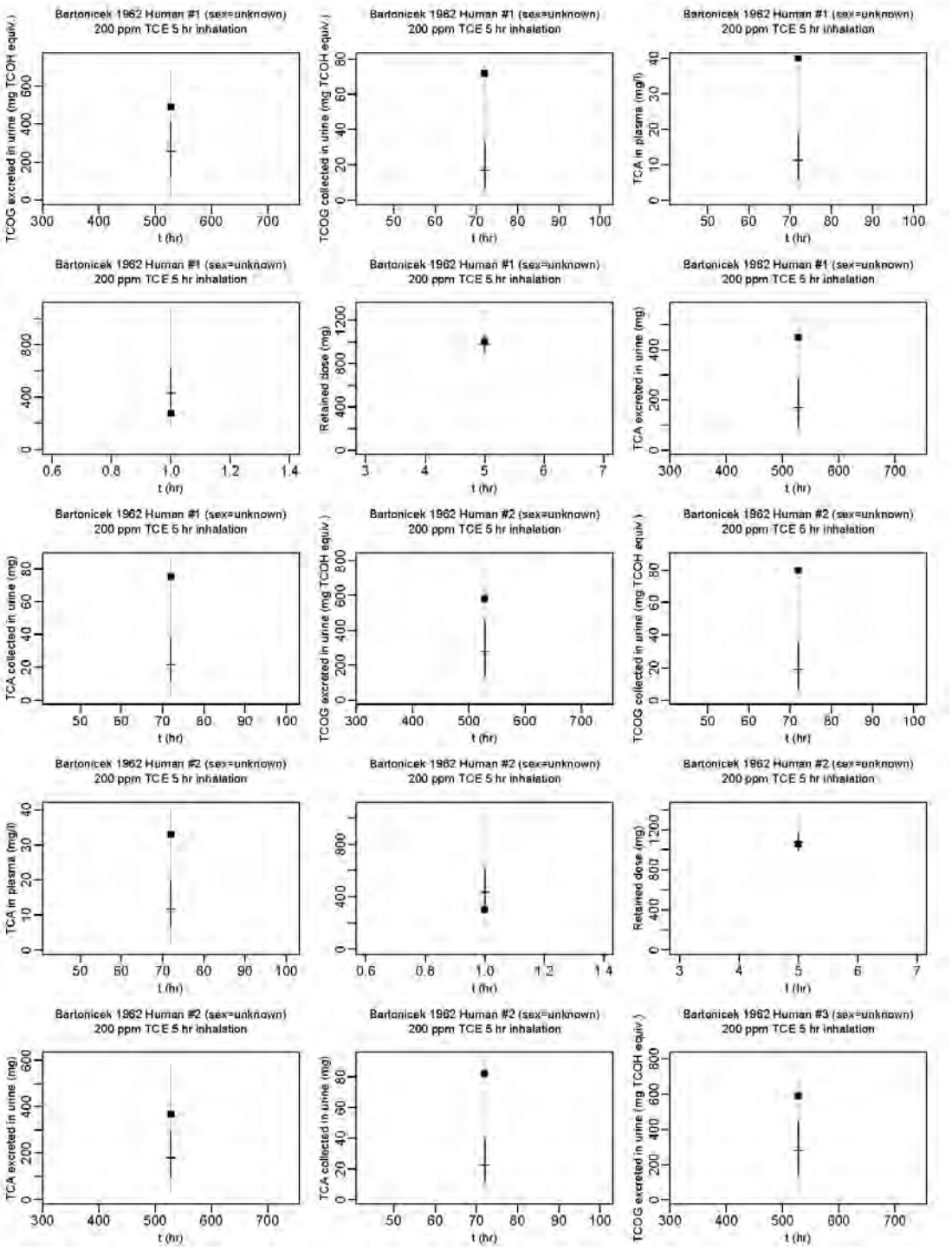


Figure A-35. Comparison of human evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions).

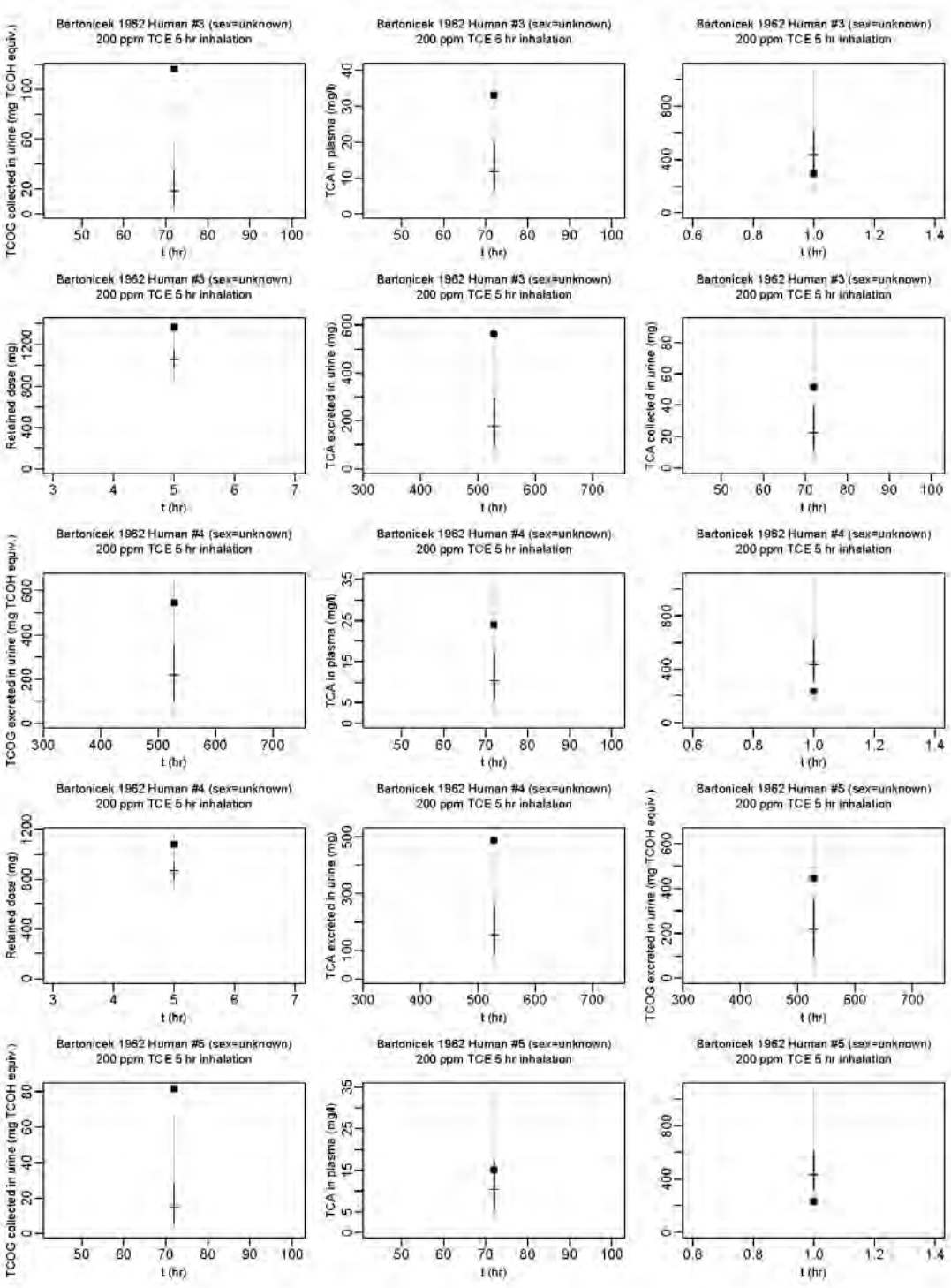


Figure A-35. Comparison of human evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

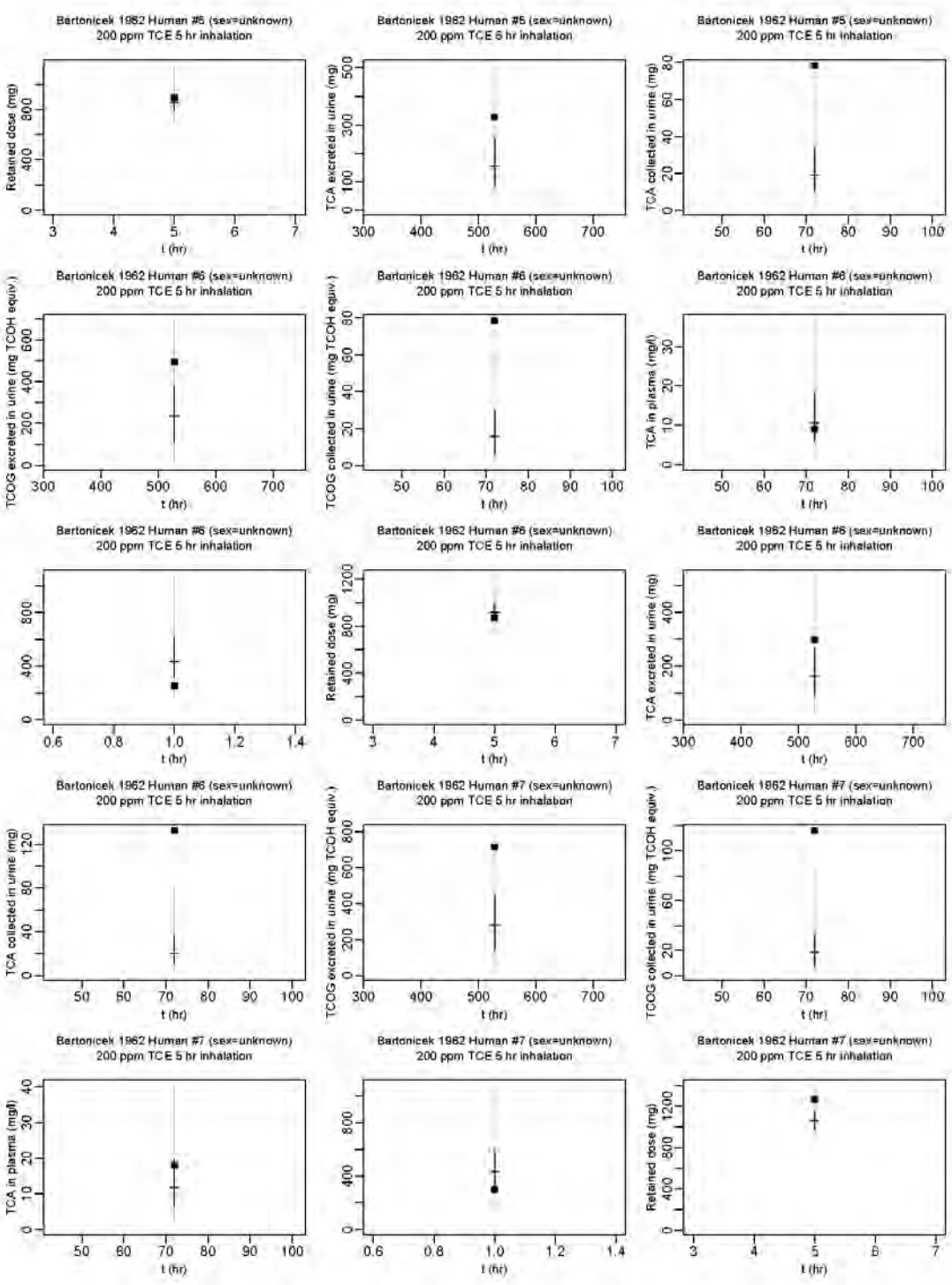


Figure A-35 Comparison of human evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

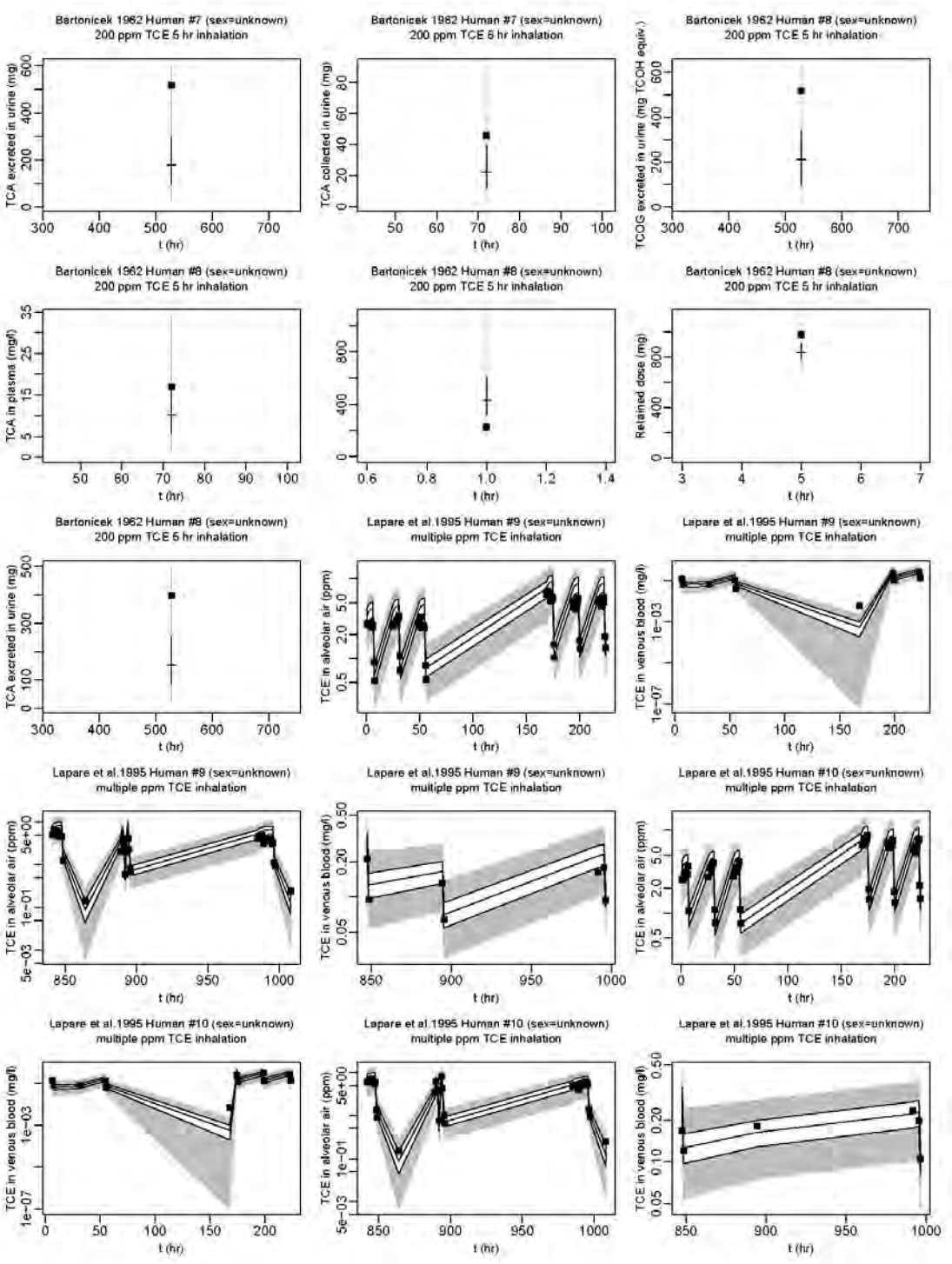


Figure A-35. Comparison of human evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

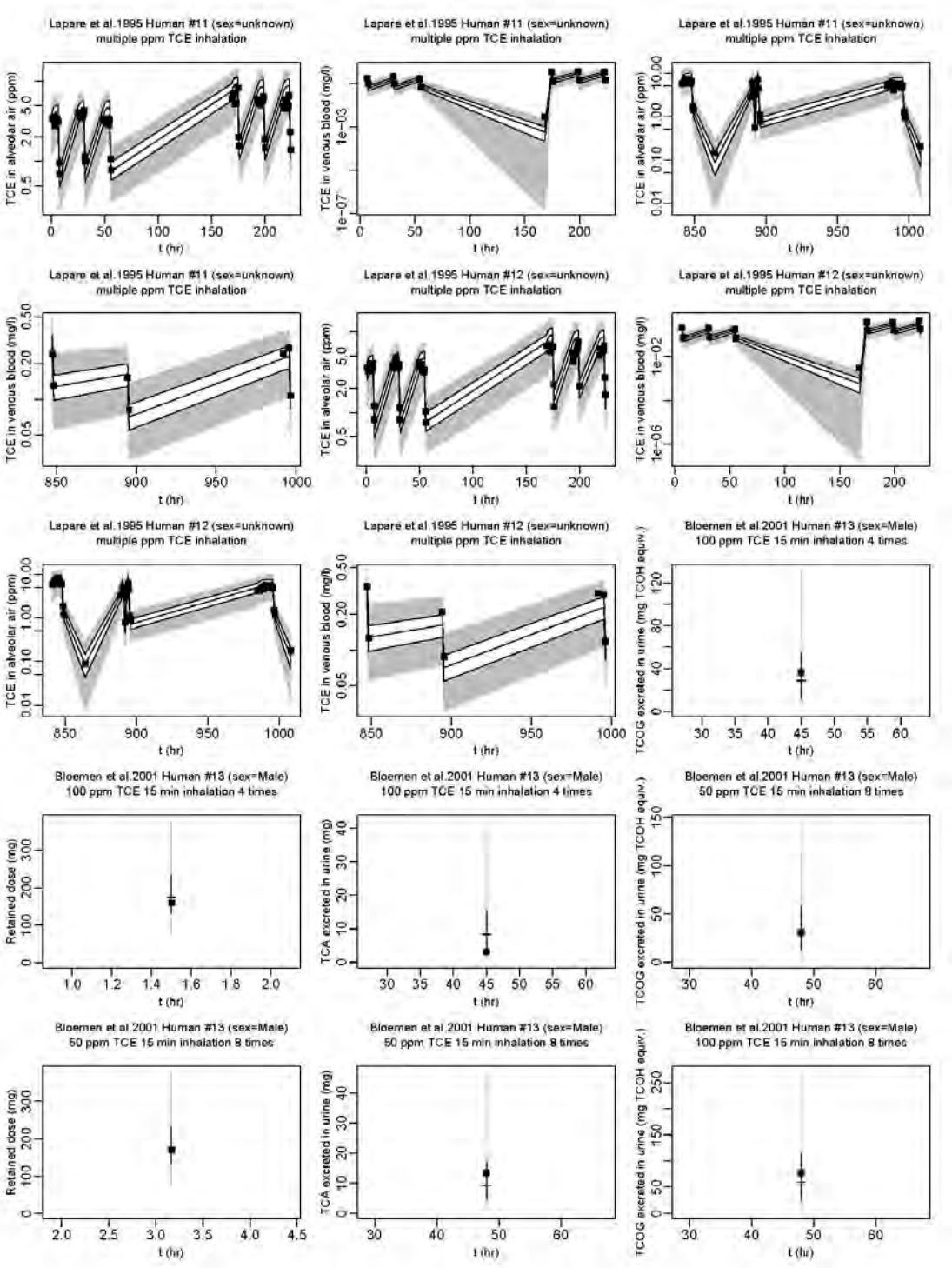


Figure A-35. Comparison of human evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

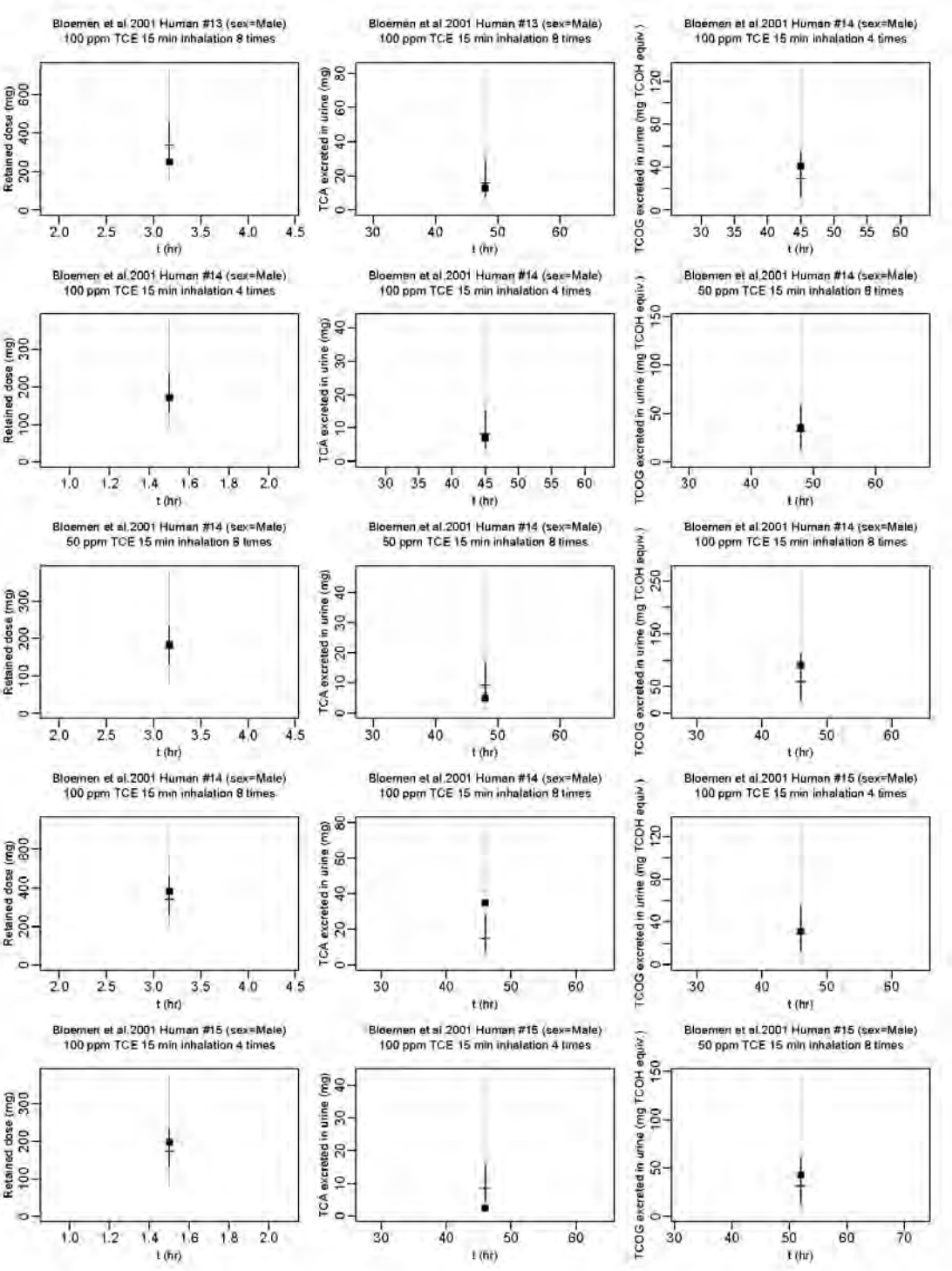


Figure A-35. Comparison of human evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

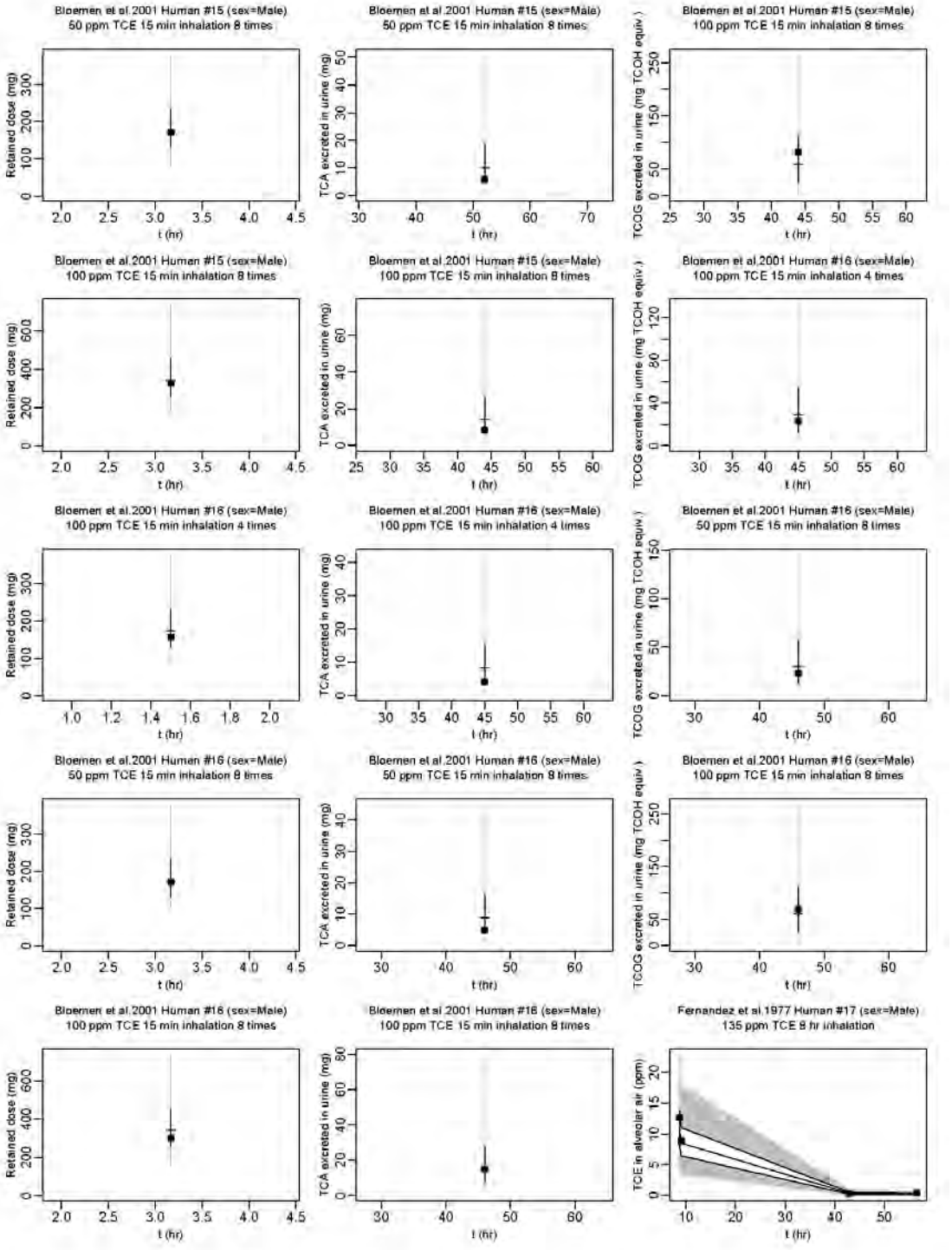


Figure A-35. Comparison of human evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

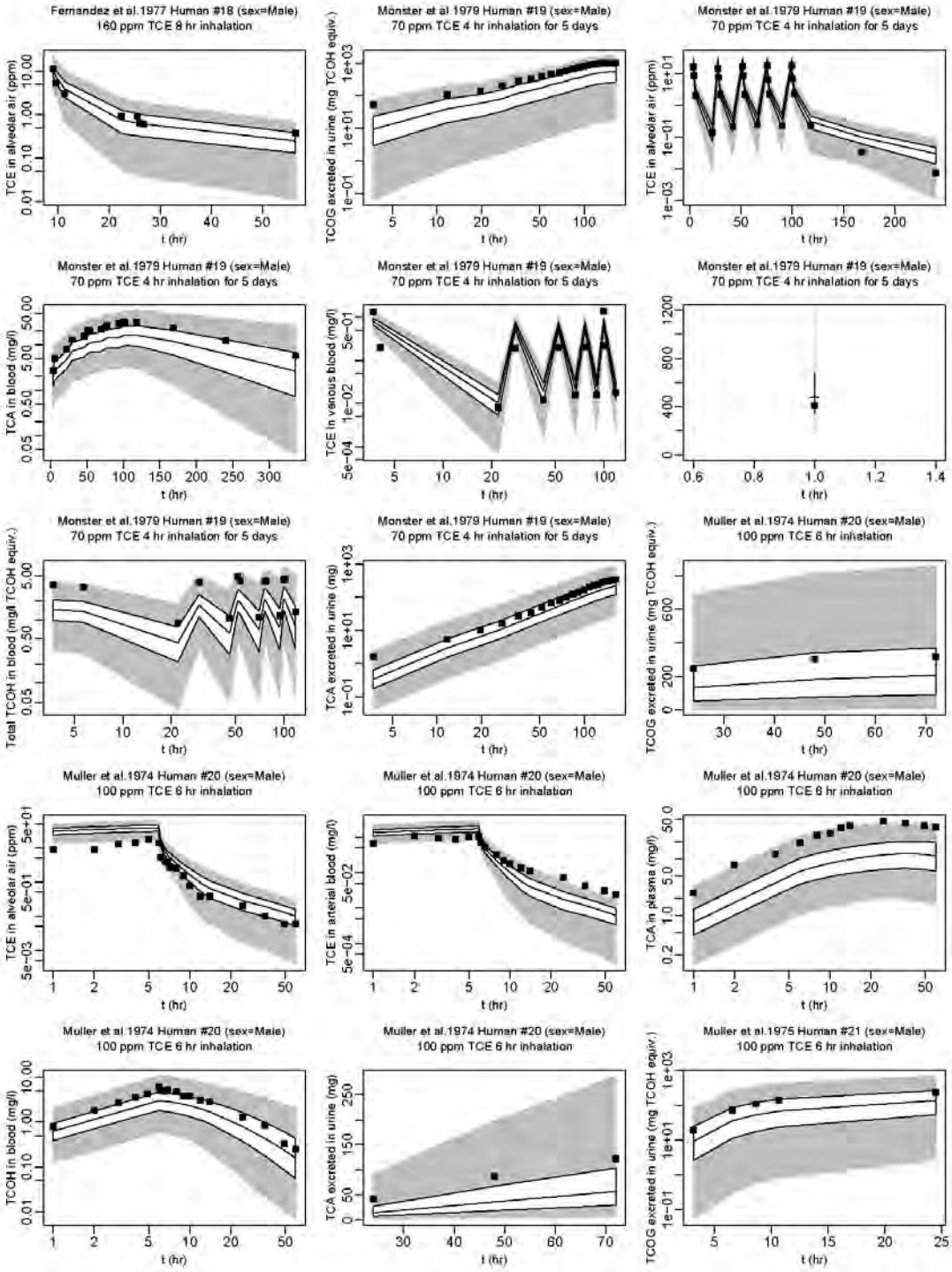


Figure A-35. Comparison of human evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

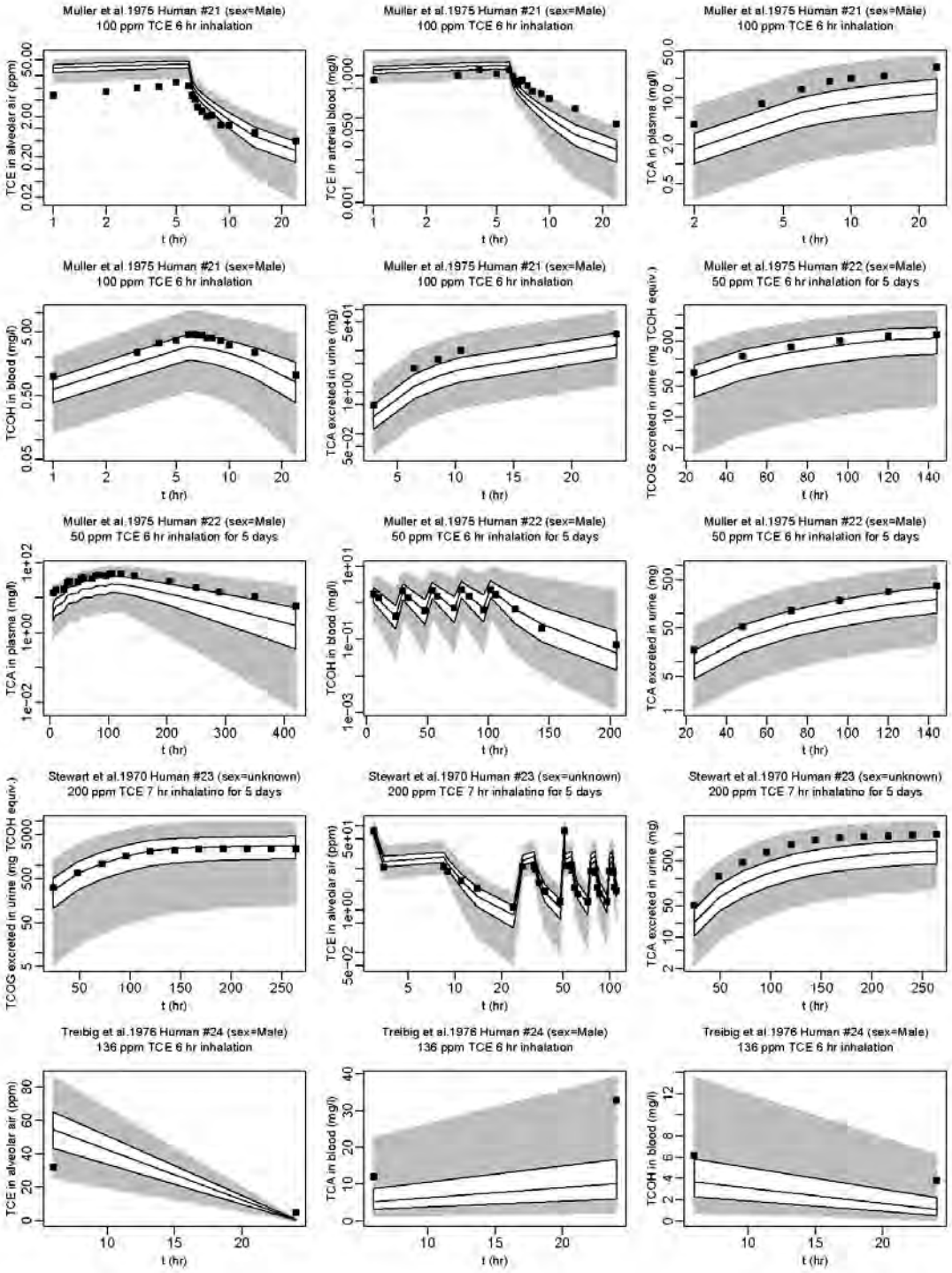


Figure A-35. Comparison of human evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

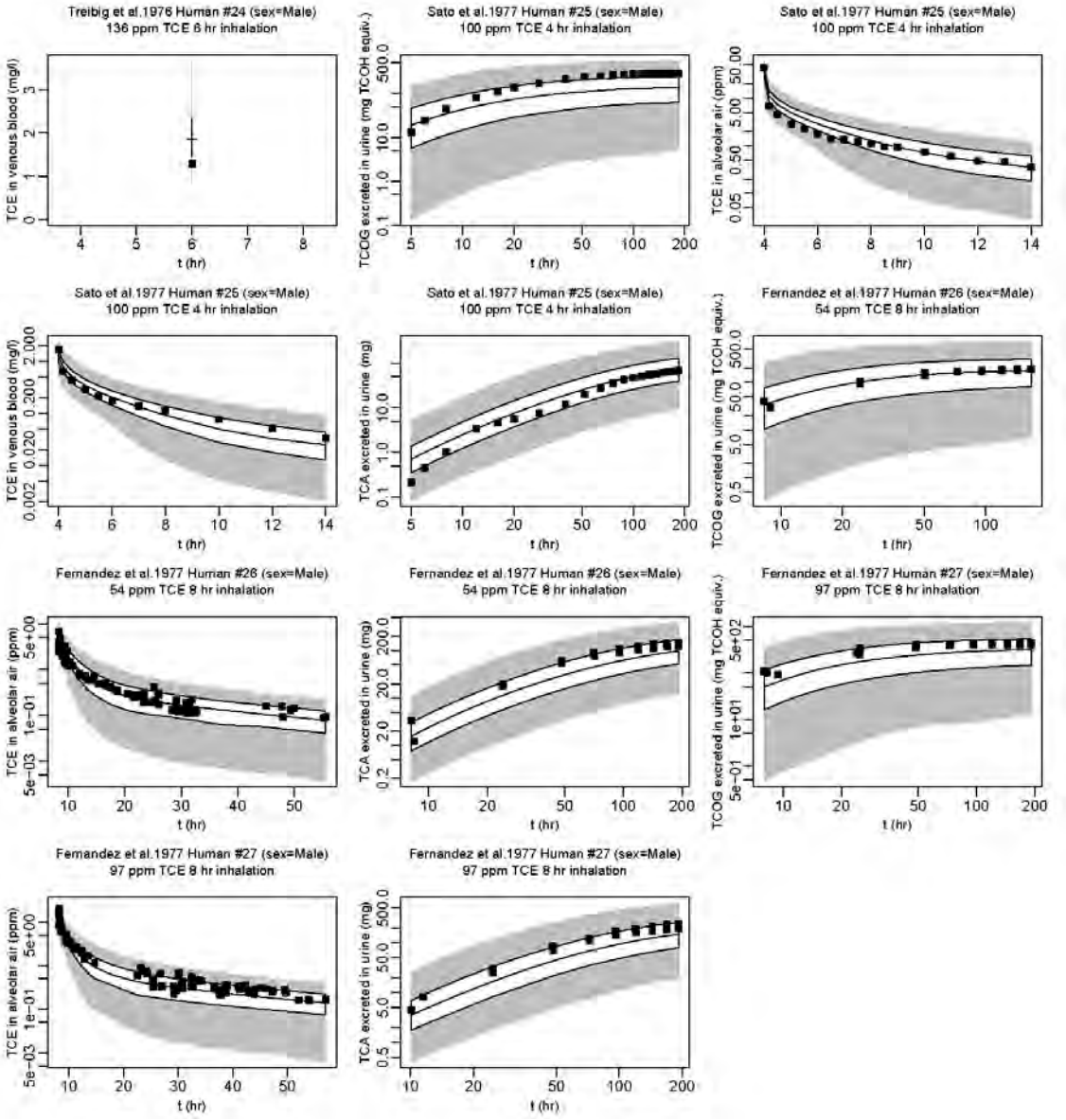


Figure A-35. Comparison of human evaluation data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions) (continued).

A.6. EVALUATION OF RECENTLY PUBLISHED TOXICOKINETIC DATA

Several in vivo toxicokinetic studies were published or became available during internal EPA review and Interagency Consultation, and were not evaluated as part of the originally planned analyses. Preliminary analyses of these data are summarized here. The general approach is the same as that used for the evaluation data in the primary analysis—population predictions from the PBPK model are compared visually with the toxicokinetic data.

A.6.1. TCE Metabolite Toxicokinetics in Mice: Kim et al. (2009)

Kim et al. (2009) measured TCA, DCA, DCVG, and DCVC in blood of male B6C3F$_1$ mice following a single gavage dose of 2,140 mg/kg. Of these data, only TCA and DCVG blood concentrations are predicted by the updated PBPK model, so only those data are compared with PBPK model predictions (prior values for the distribution volume and elimination rate constant of DCVG were used, as there were no calibration data informing those parameters). The TCA data were within the interquartile region of the PBPK model population predictions, as shown in Figure A-36. The DCVG data were at the lower end of the PBPK model population predictions, but within the 95% range.

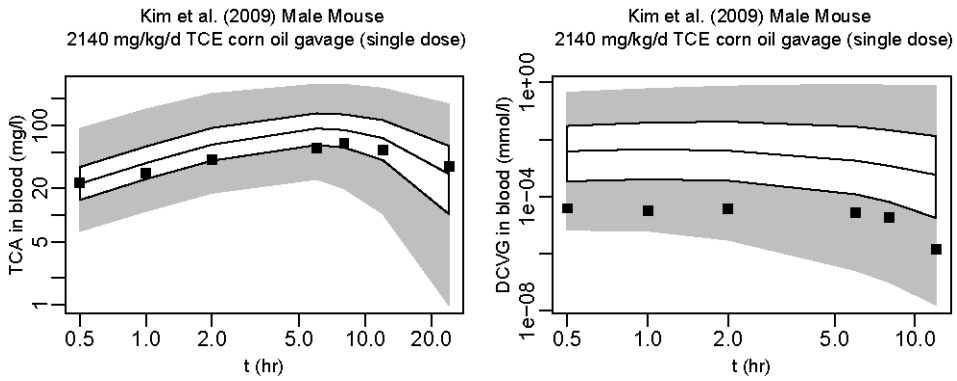


Figure A-36. Comparison of Kim et al. (2009) mouse data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions).

An assessment was made as to whether these data are informative as to the flux of GSH conjugation in mice. First, the best fitting parameter sample (least squares on TCA and DCVG in blood, weighted by inverse of the observed variance, Figures A-37 and A-38) from the posterior distribution was selected out of 50,000 samples generated by Monte Carlo (see Figures A-13 and A-14 for the comparison with predictions with data). This parameter sample was then used to calculate the fraction of intake that is predicted by the PBPK model to undergo GSH metabolism for continuous oral and continuous inhalation exposure, and this point estimate was compared to the full posterior distribution (see Figures A-15 and A-16). The predictions for this "best fitting" parameter set was similar (within threefold) of the median of the full posterior distribution (see Figures A-39 and A-40). While a formal assessment of the impact of these new data (i.e., including its uncertainty and variability) would require a rerunning of the Bayesian analysis, it appears that the median estimates for the mouse GSH conjugation dose-metric used in the dose-response assessment (see Chapter 5) are reasonably consistent with the Kim et al. (2009) data.

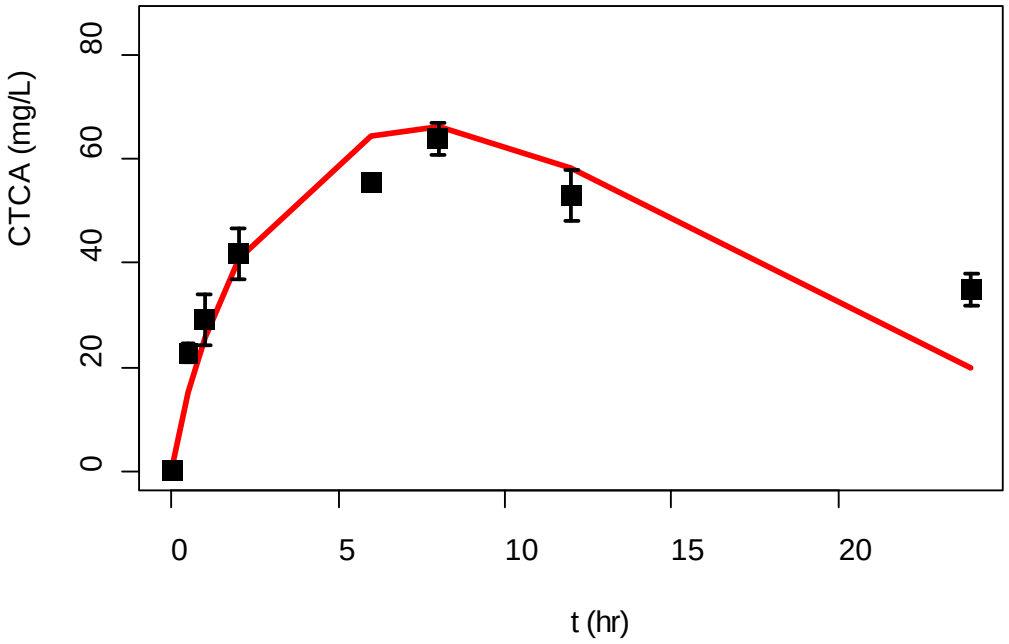


Figure A-37. Comparison of best-fitting (out of 50,000 posterior samples) PBPK model prediction and Kim et al. (2009) TCA blood concentration data for mice gavaged with 2,140 mg/kg TCE.

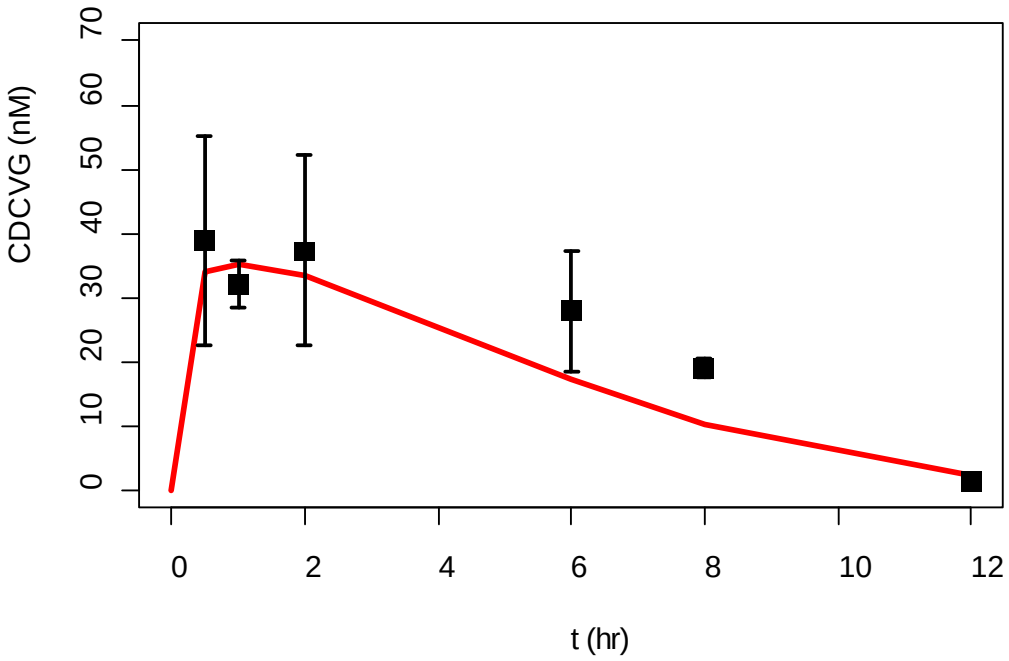
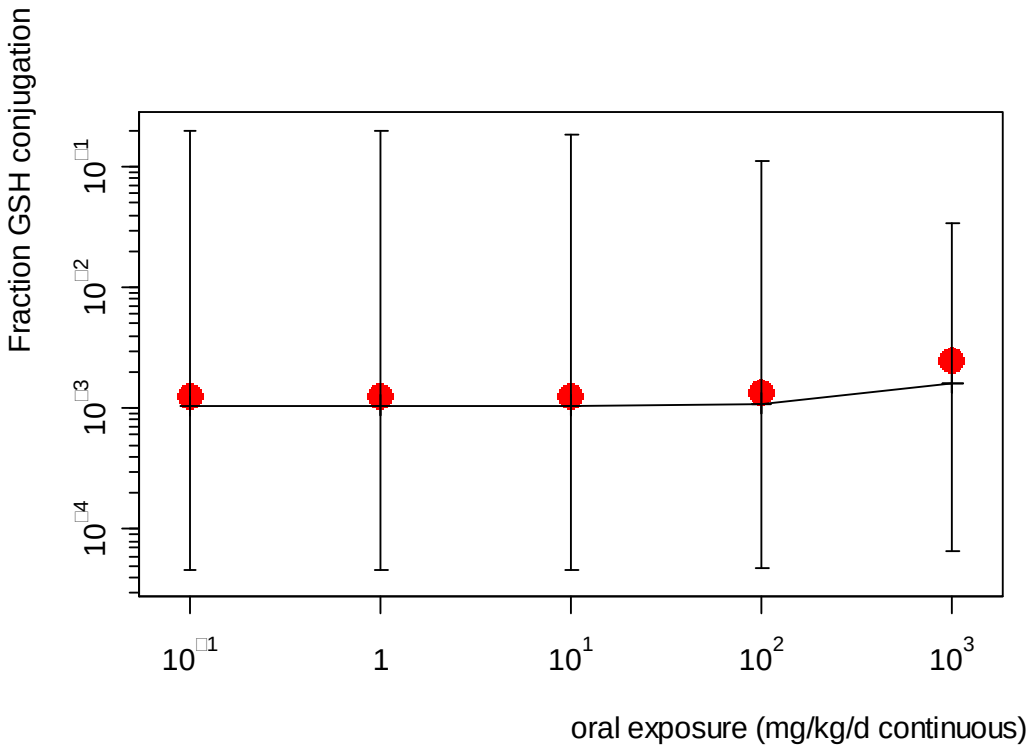
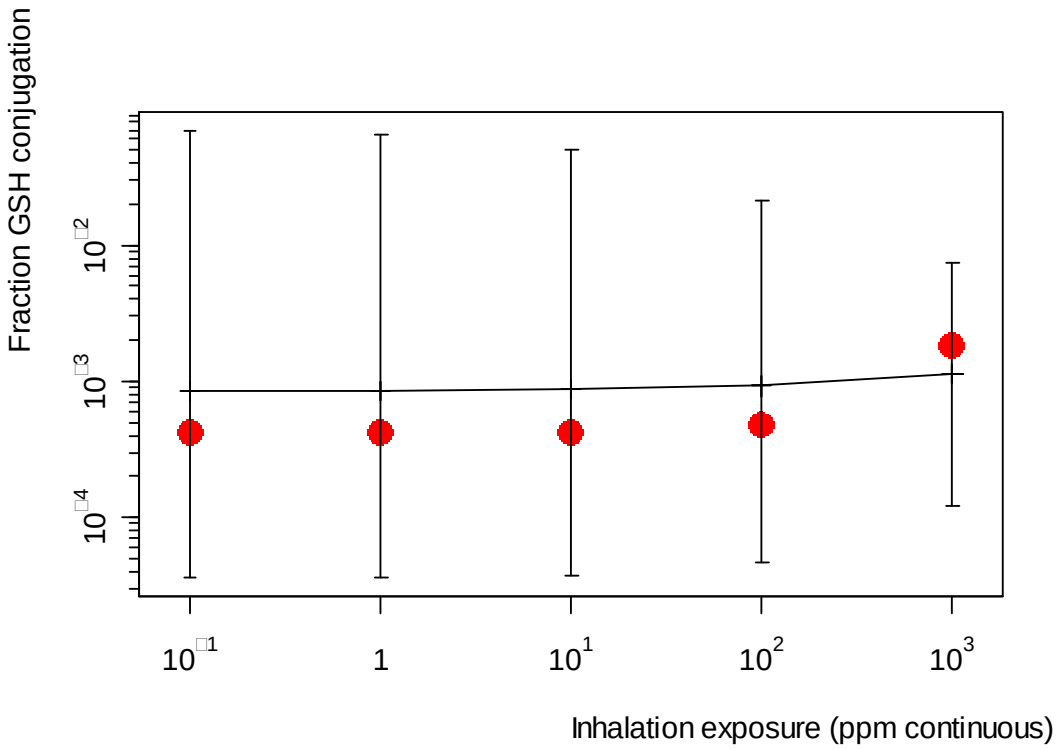


Figure A-38. Comparison of best-fitting (out of 50,000 posterior samples) PBPK model prediction and Kim et al. (2009) DCVG blood concentration data for mice gavaged with 2,140 mg/kg TCE.



Lines and error bars represent the median and 95th percentile CI for the posterior predictions, respectively (also reported in Section 3.5.7.3.1). Filled circles represent the predictions from the sample (out of 50,000 total posterior samples) which provides the best fit to the Kim et al. (2009) TCA and DCVG blood concentration data for mice gavaged with 2,140 mg/kg TCE.

Figure A-39. PBPK model predictions for the fraction of intake undergoing GSH conjugation in mice continuously exposed orally to TCE.



Lines and error bars represent the median and 95th percentile CI for the posterior predictions, respectively (also reported in Section 3.5.7.3.1). Filled circles represent the predictions from the sample (out of 50,000 total posterior samples) which provides the best fit to the Kim et al. (2009) TCA and DCVG blood concentration data for mice gavaged with 2,140 mg/kg TCE.

Figure A-40. PBPK model predictions for the fraction of intake undergoing GSH conjugation in mice continuously exposed via inhalation to TCE.

An additional note of interest from the Kim et al. (2009) data is the interstudy variability in TCA kinetics. In particular, the TCA blood concentrations reported by Kim et al. (2009) are twofold lower than those reported by Abbas and Fisher (1997) in the same sex and strain of mouse, with a very similar corn oil gavage dose of 2,000 mg/kg [as compared to 2,140 mg/kg used in Kim et al. (2009)].

A.6.2. TCE Toxicokinetics in Rats: Liu et al. (2009)

Liu et al. (2009) measured TCE in blood of male rats after treatment with TCE by i.v. injection (0.1, 1.0, or 2.5 mg/kg) or aqueous gavage (0.0001, 0.001, 0.01, 0.1, 1, 2.5, 5, or 10 mg/kg). Almost all of the data from gavage exposures were within the interquartile region of the PBPK model population predictions, with all of it within the 95% CI, as shown in Figure A-41. For i.v. exposures, the data at 1 and 2.5 mg/kg were well simulated, but the time-course data at 0.1 mg/kg were substantially different in shape from that predicted by the PBPK model, with a

lower initial concentration and longer half-life. The slower elimination rat at 0.1 mg/kg was noted by the study authors through use of noncompartamental analysis. There is no clear explanation for this discrepancy, particularly since the gavage data at this and even lower doses were well predicted by the PBPK model.

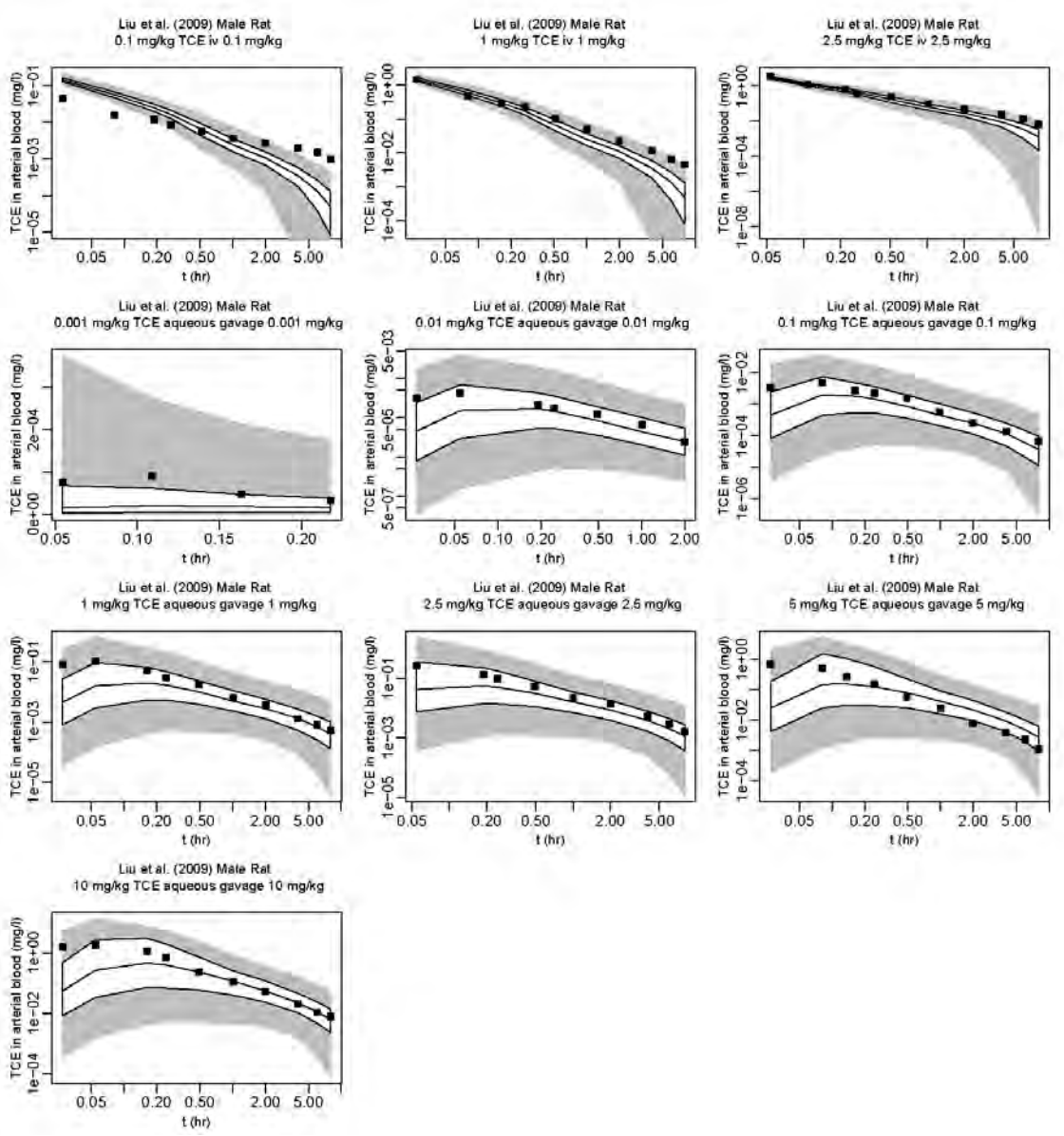


Figure A-41. Comparison of Liu et al. (2009) rat data (boxes) and PBPK model predictions (+ with error bars: single data points or shaded regions: 2.5, 25, 50, 75, and 97.5% population-based predictions).

A.6.3. TCA Toxicokinetics in Mice and Rats: Mahle et al. (1999) and Green (2003a, 2003b)

Three technical reports (Green, 2003b, a; Mahle et al., 1999) described by Sweeney et al. (2009) contained data on TCA toxicokinetics in mice and rats exposed to TCA in drinking water. These technical reports were provided to EPA by the Sweeney et al. (2009) authors.

A.6.3.1. Analysis Using Evans et al. (2009) and Chiu et al. (2009) PBPK Model

TCA blood and liver concentrations were reported by Mahle et al. (1999) for male B6C3F$_1$ mice and male F344 rats exposed to 0.1 g/L to 2 g/L TCA in drinking water for 3 or 14 days (12–270 mg/kg-day in mice and 7–150 mg/kg-day in rats). For mice, these data were all within the 95% CI of PBPK model population predictions, with about half of these data within the interquartile region. For rats, all of these data, except those for the 3-day exposure at 0.1 g/L, were within the 95% CI of the PBPK model predictions. In addition, the median rat predictions were consistently higher than the data, although this could be explained by interstudy (strain, lot, etc.) variability.

TCA blood concentrations were reported by Green (2003a) for male and female B6C3F$_1$ mice exposed to 0.5–2.5 g/L TCA in drinking water for 5 days (130–600 mg/kg-day in males and 160–750 mg/kg-day in females). Notably, these animals consumed around twice as much water per day as compared to the mice reported by Mahle et al. (1999), and therefore, received comparatively higher doses of TCA for the same TCE concentration in drinking water.

In male mice, the data at the lower two doses (130 and 250 mg/kg-day) were within the interquartile region of the PBPK model predictions. The data for male mice at the highest dose (600 mg/kg-day) were below the interquartile region, but within the 95% CI of the PBPK model predictions. In females, the data at the lower two doses (160 and 360 mg/kg-day) were mostly below the interquartile region, but within the 95% CI of the PBPK model predictions, while about half of the data at the highest dose were just below the 95% CI.

TCA blood, plasma, and liver concentrations were reported by Green (2003b) for male PPARα-null mice, male 129/sv mice (the background strain of the PPARα-null mice), and male and female B6C3F$_1$ mice, exposed to 1.0 or 2.5 g/L TCA in drinking water for 5 days (male B6C3F$_1$ only) to 14 days.[2] In male PPARα-null mice, plasma and blood concentrations were within the interquartile region of the PBPK model predictions, while liver concentrations were below the interquartile region but within the 95% CI. In male 129/sv mice, the plasma concentrations were within the interquartile region of the PBPK model predictions, while blood and liver concentrations were below the interquartile region but within the 95% CI. In male B6C3F$_1$ mice, all data were within the 95% CIs of the PBPK model predictions, with about half within the interquartile region, and the rest above (plasma concentrations at the lower dose) or below (liver concentrations at all but the lowest dose at 5 days). In female B6C3F$_1$ mice, plasma concentrations were below the interquartile region but within the 95% confidence region, while liver and blood concentrations were at or below the lower 95% confidence bound.

[2]Sweeney et al. (2009) reported that blood concentrations in Green (2003b) were incorrect due to an arithmetic error owing to a change in chemical analytic methodology, and should have been multiplied by 2. This correction was included in the present analysis.

Overall, the predictions of the TCA submodel of the updated TCE PBPK model appear consistent with these data on the toxicokinetics of TCA after drinking water exposure in male rats and male mice. In female mice, the reported concentrations tends to be at the low end of or lower than those predicted by the PBPK model. Importantly, the data used for calibrating the mouse PBPK model parameters were predominantly in males, with only Fisher et al. (1991) and Fisher and Allen (1993) reporting TCA plasma levels in female mice after TCE exposure. In addition, median PBPK model predictions at higher doses (>300 mg/kg-day), even in males, tended to be higher than the concentrations reported. While TCA kinetics after TCE exposure includes predicted internal production at these higher levels, previously published data on TCA kinetics alone only included doses up to 100 mg/kg, and only in males. Therefore, these results suggest that the median predictions of the TCA submodel of the updated TCE PBPK model are somewhat less accurate for female mice and for higher doses of TCA (>300 mg/kg-day) in mice, though the 95% CIs still cover the majority of the reported data. Finally, the ratio of blood to liver concentrations of ~1.4 reported in the mouse experiments in Mahle et al. (1999) were significantly different from the ratios of ~2.3 reported by Green (2003b), a difference for which there is no clear explanation given the similar experimental designs and common use the $B6C3F_1$ mouse strain. Because median PBPK model predictions for the blood to liver concentration ratio for these studies are ~1.3, they are more consistent with the Mahle et al. (1999) data than with the Green (2003b) data.

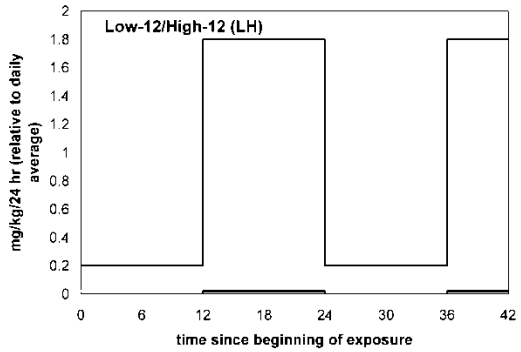
A.6.3.2. Summary of Results From Chiu of Bayesian Updating of Evans et al. (2009) and Chiu et al. (2009) Model Using TCA Drinking Water Data

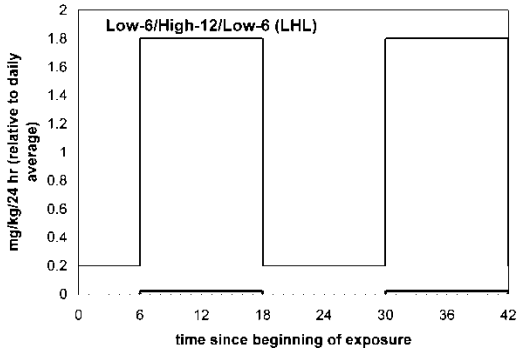
Sweeney et al. (2009) also suggested that the available data, in conjunction with deterministic modeling using the TCA portion of the Hack et al. (2006) TCE PBPK model, supported a hypothesis that the bioavailability of TCA in drinking water in mice is substantially <100%. Classically, oral bioavailability is assessed by comparing blood concentration profiles from oral and i.v. dosing experiments, because blood concentration data from oral dosing alone cannot distinguish fractional uptake from metabolism. Schultz et al. (1999) made this comparison in rats at a single dose of 82 mg/kg, and reported an empirical bioavailability of 116%, consistent with complete absorption. A priori, there would not seem to be a strong reason to suspect that oral absorption in mice would be significantly different from that in rats. As discussed above in the evaluation of Hack et al. (2006) model, available data strongly support clearance of TCA in addition to urinary excretion, based on the finding of <100% recovery in urine after i.v. dosing. In addition, as the current TCE PBPK model assumes 100% absorption for orally-administered TCA, and the PBPK model predictions are consistent with these data, it is likely that the limited bioavailability determined by Sweeney et al. (2009) was confounded by this additional clearance pathway unaccounted for by Hack et al. (2006). Therefore, Chiu

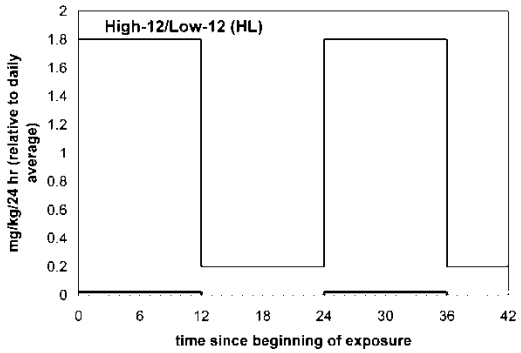
conducted a Bayesian reanalysis of the TCE mouse PBPK model, the results of which are summarized here.

In brief, the TCA submodel from Evans et al. (2009) and Chiu et al. (2009) is augmented by the addition of a fractional absorption parameter for drinking water exposures and parameters reestimated by adding the newly available TCA drinking water kinetic studies in mice. Being nocturnal animals, rodents do not have a steady pattern of drinking water consumption throughout the day. It has been suggested that a 90/10%-split between dark-cycle (night time)/light-cycle (day time) drinking water consumption is a reasonable approximation (Yuan, 1995), and that pattern is assumed here. Most analyses assume something similar (e.g., Sweeney et al., 2009, assumed 100% consumption during the dark cycle).

However, TCA kinetics from drinking water exposures also depends on the relationship between the times of the light/dark cycle and the times of specimen collection (i.e., at what time during the cycle did exposure begin [when is "t = 0"])? These data are not specified in any of the available technical reports cited by Sweeney et al. (2009). Therefore, in the present analysis, three different assumptions that represent a range of possibilities were made, and the results of each were carried through the analysis. These patterns are shown in Figure A-42 and designated low-12/high-12 (LH), low-6/high-12/low-6 (LHL), and high-12/low-12 (HL). In the first, it is assumed that the start of exposure coincided exactly with the start of the light cycle; in the second, it is assumed that the start of exposure was exactly in the middle of the light cycle; and in the last case, it is assumed that the start of exposure was exactly at the end of the light cycle. A priori, one of the first two patterns (LH and LHL) would appear to be most likely, but the last pattern (HL) was included for completeness. Sweeney et al. (2009) assumed drinking water intake was most similar to the LH pattern.







The upper left panel (LH) assumes that t = 0 is at the beginning of the "light" part of the "light/dark" cycle (light is dashed grey line at the bottom, dark is thick black line at the bottom). The upper right panel (LHL) assumes that t = 0 is in the middle of the "light" part of the cycle. The lower left panel (HL) assumes that t = 0 is at the end of the "light" part of the cycle.

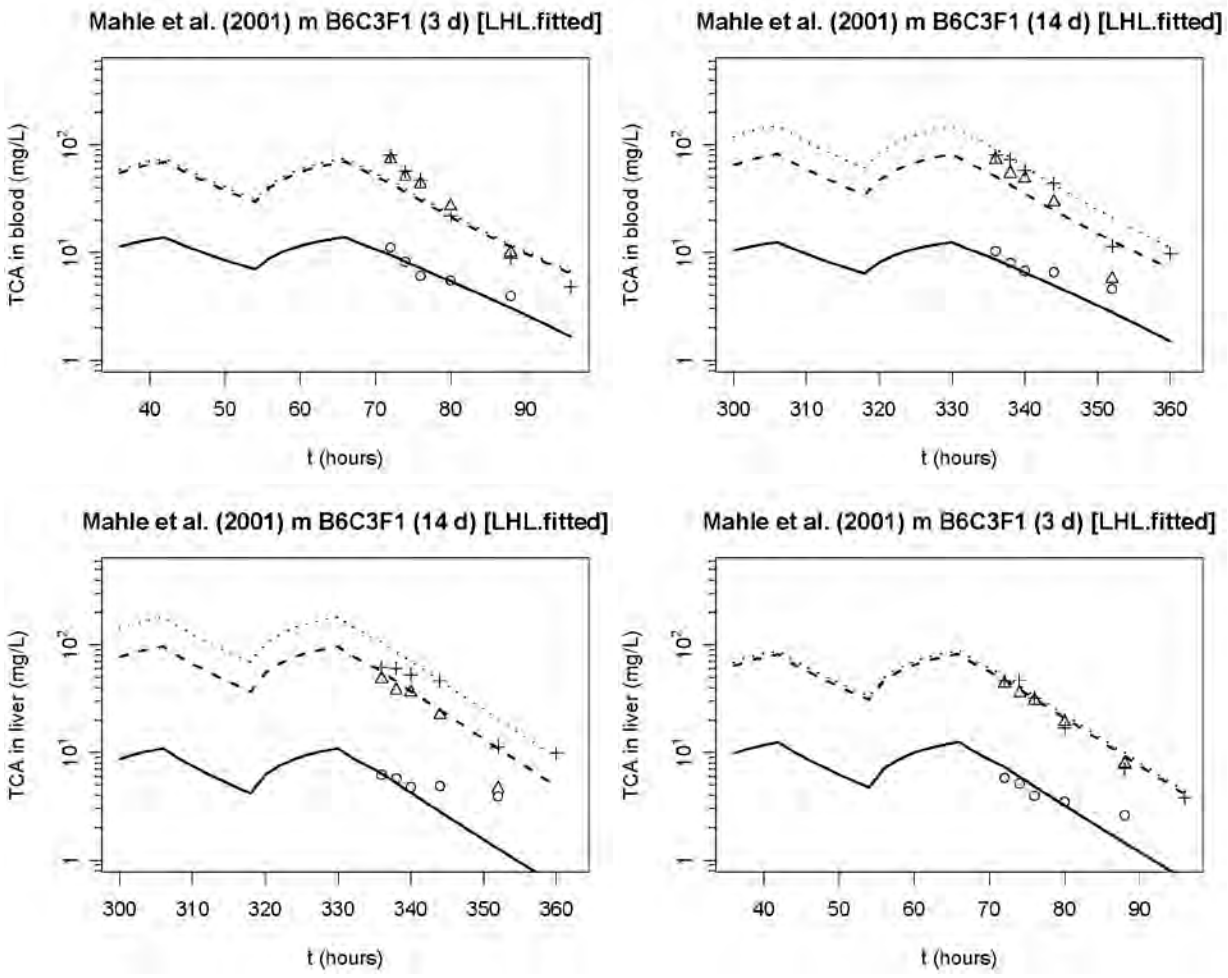
Figure A-42. Assumed drinking water patterns as a function of time since beginning of exposure.

As was done by Evans et al. (2009) and Chiu et al. (2009), the PBPK parameter estimation is performed in a hierarchical Bayesian population statistical framework, with calculations performed using MCMC, using posteriors from the earlier analysis as priors for the reanalysis. A total of six different model runs were made using the "harmonized" PBPK model, as shown in Table A-18, using different assumptions for fractional absorption and for drinking water intake patterns. Comparisons between different modeling assumptions (i.e., fixing or estimating fractional absorption; assumed drinking water patterns) were made using the deviance information criterion (DIC) (Spiegelhalter et al., 2002). The DIC is a Bayesian analogue to the AIC and is used in a similar manner, with smaller values indicating better model fits. As with the AIC, "small" differences in DIC (e.g., <5, as suggested by the WinBUGS "DIC page" [http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/dicpage.shtml]) are not likely to be important, but much lower values suggest substantially better fitting models. Results of these comparison are also shown in Table A-18. Adding the fractional absorption parameter decreases the DIC by about 100 units, which strongly supports inclusion of the parameter. In addition, in both cases of fixed and fitted fractional absorption, the lowest DIC was for the LHL drinking water intake pattern, with the second lowest DIC for the LH pattern, with a difference of 33 units in DIC. Given that these model runs are highly favored relative to the others, the rest of this summary reports the results for the "LHL.fitted" run (see Chiu, 2011, for additional details).

Table A-18. Summary characteristics of model runs

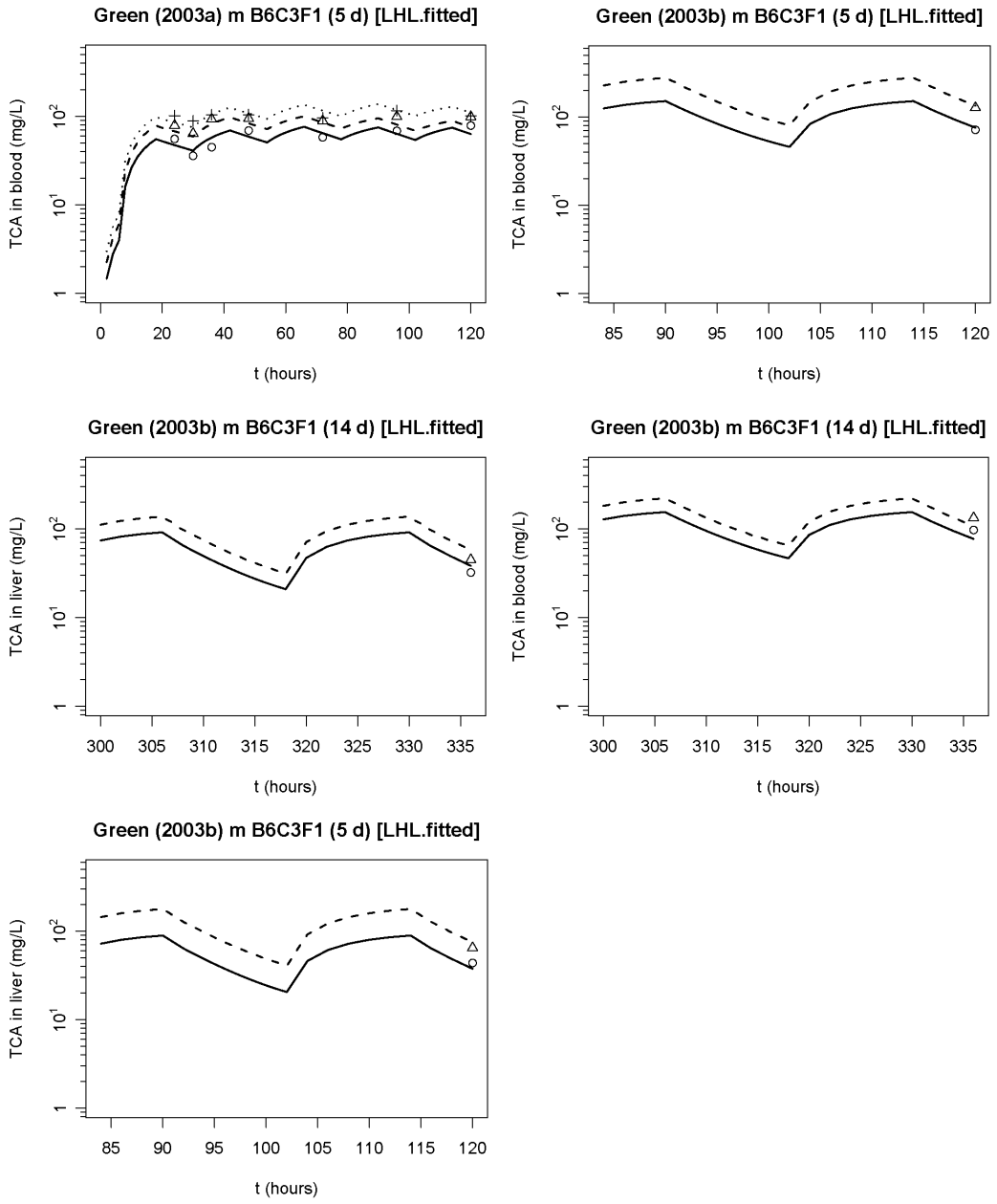
Run designation	Drinking water pattern	Fractional absorption		Convergence	DIC
		Fixed	Fitted		
LH.fixed	Low-12/high-12	√		$R \leq 1.04$	895
LHL.fixed	Low-6/high-12/low-6	√		$R \leq 1.09$	877
HL.fixed	High-12/low-12	√		$R \leq 1.05$	897
LH.fitted	Low-12/high-12		√	$R \leq 1.05$	764
LHL.fitted	Low-6/high-12/low-6		√	$R \leq 1.11$	731
HL.fitted	High-12/low-12		√	$R \leq 1.12$	781

Posterior model fits for the LHL.fitted runs are shown in Figures A-43 and A-44, using a representative sample from the converged MCMC chain. A dose-dependent fractional absorption can account for the less-than-proportional increase in TCA blood concentrations between the middle and high dose groups observed in Mahle et al. (1999) (see Figure A-43) and among all of the dose groups observed in Green (2003a, 2003b) (see Figure A-44).



Three- and 14-day exposures to 0.08 (data: open circles, predictions: solid line), 0.8 (data: open triangle, predictions: dashed line), and 2 g/L TCA in drinking water (data: crosses, predictions: dotted line). Predictions use a representative parameter sample from the converged MCMC chain for the LHL drinking water intake pattern.

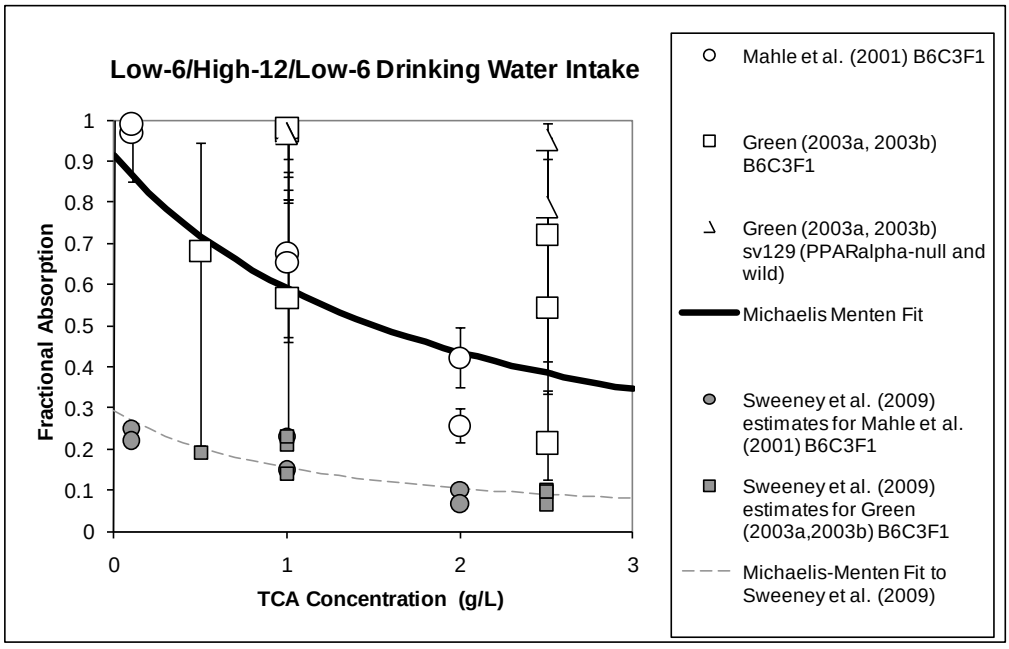
Figure A-43. PBPK model predictions for TCA in blood and liver of male B6C3F$_1$ mice from Mahle et al. (1999).



Green (2003a): 5-day drinking water exposures to 0.5 (data: open circle; predictions: solid line), 1 (data: open triangle; predictions: dashed line), and 2.5 g/L TCA (data: crosses; predictions: dotted lines). Green (2003b): 5- and 14-day drinking water exposures to 1 (data: open circle; predictions: solid line) and 2.5 g/L TCA (data: open triangle; predictions: dashed line). Predictions use a representative parameter sample from the converged MCMC chain for the LHL drinking water intake pattern.

Figure A-44. PBPK model predictions for TCA in blood and liver of male B6C3F$_1$ mice from Green (2003a, 2003b).

As was done by Sweeney et al. (2009), fractional absorption is separately estimated for each drinking water dose group, and the results are fit to a parametric model, shown in Figure A-45. Several features of the data and analysis are worth noting. First, there is a general trend for decrease in fractional absorption with increasing concentration, evident even within studies. Second, there appears to be substantial interstudy and intrastudy variability in the apparent fractional absorption. This is particularly evident across strains in Green (2003b)—the PPARα-null and 129/sv mice appear to have substantially higher fractional absorption than the $B6C3F_1$ mice, even though in all strains, there appeared to be a decreasing trend with increasing TCA concentration. Third, the fractional absorption estimates increase as the "start of exposure" is assumed to be later and later in the "light" cycle. Fourth, the estimated fractional absorption at low concentrations is fairly high, at >80%. Finally, the estimates for fractional absorption from the current analysis are 3–4 times greater than those reported by Sweeney et al. (2009). Because hepatic clearance was not included in the previous Hack et al. (2006) version of the TCE model used by Sweeney et al. (2009), and this could partially explain why they found a very low fractional absorption to be necessary to provide a fit to the observed data from drinking water exposures.



Fits are to a Michaelis-Menten function for "effective" concentration $C_{eff} = C_{max} \times C/(C_{½} + C)$, so that the fractional absorption $Fabs = C_{eff}/C = C_{max}/(C_{½} + C)$. Sweeney et al. (2009) estimates of Fabs, along with a Michaelis-Menten fit, are included for comparison. The ratio $C_{max}/C_{½}$ gives the fractional uptake at low concentrations.

Figure A-45. Distribution of fractional absorption fit to each TCA drinking water kinetic study group in mice, using LHL drinking water intake patterns.

In sum, comparing model results with complete- and less-than-complete-fractional absorption, it is evident (e.g., through the much lower DIC) that including a concentration-dependent fractional absorption substantially improves model fits. Thus, these data are consistent with reduced bioavailability from drinking water, particularly at higher TCA drinking water concentrations. However, the estimates of fractional absorption are three- to fourfold higher than those estimated by Sweeney et al. (2009). In addition, there appeared to be substantial inter- and intrastudy variability, with the fractional absorption for some mouse strains estimated to be nearly complete even at the higher TCA drinking water concentrations. Thus, on the whole, adding a fractional absorption parameter substantially improves the PBPK model predictions, though the degree of absorption is greater than that reported by Sweeney et al. (2009) and appears to be variable between studies and mouse strains. Data are lacking as to a mechanistic basis for reduced absorption of TCA at higher doses. Biliary excretion is a possibility, though data from rats suggest that the degree of biliary excretion of TCA is rather modest (Stenner et al., 1997). It is also possible that the nonlinearity in TCA kinetics reflects a difference in clearance processes, such as saturation of renal reabsorption, which would lead to increased urinary clearance and reduced internal dose. This could be tested experimentally by simultaneously measuring blood and urinary kinetics of TCA at different doses. However, this would not explain differences between drinking water and gavage dosing.

The degree of interexperimental variability raises the question of whether the apparent fractional absorption may be due, in part, to experimental factors, such as analytical errors due to incomplete/inadequate procedures to prevent TCA degradation or experimental losses in estimating drinking water consumption rates. With respect to TCA degradation, Mahle et al. (1999) appeared to be specifically aware of the issue and froze biological samples prior to analysis in order to address it. However, lacking any external validation, the extent to which this was completely successful is unclear. On the other hand, Green (2003a, 2003b) did not appear to have any particular procedure designed to address TCA degradation. Thus, the extent and impact of TCA degradation is not clear, though it may be a plausible explanation for the degree of variability observed across data sets. With respect to drinking water consumption, experimental variance is notable with respect to reported drinking water consumption rates, with Green (2003a) > Green (2003b) > Mahle et al. (1999) > other TCA drinking water studies. One may hypothesize that the *actual* drinking water consumption rates are roughly equal, with differences in *reported* values reflecting experimental losses. However, in this case, reported drinking water consumption would inversely correlate with fractional absorption, and no such correlation is evident. In addition, this does not explain the consistent dose-related trends within a study or data set, even if the slope of the trend varies between experiments.

Overall, then, it may be more accurate to characterize the fractional absorption as an empirical parameter reflecting unaccounted-for biological processes as well as experimental variation.

A.7. UPDATED PBPK MODEL CODE

The following pages contain the updated PBPK model code for the MCSim software (version 5.0.0). Additional details on baseline parameter derivations are included as inline documentation. Example simulation files containing prior distributions and experimental calibration data are available electronically:

- Mouse ("Supplementary data for TCE assessment: Mouse population example," 2011)
- Rat ("Supplementary data for TCE assessment: Rat population example," 2011)
- Human ("Supplementary data for TCE assessment: Human population example," 2011)

```
# TCE.risk.1.2.3.3.pop.model -- Updated TCE Risk Assessment Model
#
#### HISTORY OF HACK ET AL. (2006) MODEL
# Model code to correspond to the block diagram version of the model
# Edited by Deborah Keys to incorporate Lapare et al. 1995 data
# Last edited: August 6, 2004
# Translated into MCSim from acslXtreme CSL file by Eric Hack, started 31Aug2004
# Removed nonessential differential equations (i.e., AUCCBld) for MCMC runs.
# Changed QRap and QSlw calculations and added QTot to scale fractional flows
# back to 1 after sampling.
# Finished translating and verifying results on 15Sep2004.
# Changed QSlw calculation and removed QTot 21Sep2004.
# Removed diffusion-limited fat uptake 24Sep2004.
#### HISTORY OF U.S. EPA (2009) MODEL (CHIU ET AL., 2009)
# Extensively revised by U.S. EPA June 2007-June 2008
#         - Fixed hepatic plasma flow for TCA-submodel to include
#           portal vein (i.e., QGutLivPlas -- originally was just
#           QLivPlas, which was only hepatic artery).
#         - Clearer coding and in-line documentation
#         - Single model for 3 species
#         - Revised physiological parameters, with discussion of
#                 uncertainty and variability,
#         - In vitro data used for default metabolism parameters,
#                 with discussion of uncertainty and variability
#         - added TCE blood compartment
#         - added TCE kidney compartment, with GSH metabolism
#         - added DCVG compartment
#         - added additional outputs available from in vivo data
#         - removed DCA compartment
#         - added IA and PV dosing (for rats)
#         - Version 1.1 -- fixed urinary parameter scaling
#                         -- fixed VBod in kUrnTCOG (should be VBodTCOH)
#         - Version 1.1.1 -- changed some truncation limits (in commments only)
#         - Version 1.2 --
#                 -- removed TB compartment as currently coded
#                 -- added respiratory oxidative metabolism:
#                         3 states: AInhResp, AResp, AExhResp
#                 -- removed clearance from respiratory metabolism
#         - Version 1.2.1 -- changed oral dosing to be similar to IV
#         - Version 1.2.2 -- fixed default lung metabolism (additional
#                          scaling by lung/liver weight ratio)
#         - Version 1.2.3 -- fixed FracKidDCVC scaling
#         - Version 1.2.3.1 -- added output CDCVG_ND (no new dynamics)
#                 for non-detects of DCVG in blood
#         - Version 1.2.3.2 -- Exact version of non-detects likelihood
#         - Version 1.2.3.3 -- Error variances changed to "Ve_xxx"
#         NOTE -- lines with comment "(vrisk)" are used only for
#                 calculating dose metrics, and are commented out
#                 when doing MCMC runs.
#*****************************************************************************
#***                  State Variable Specifications                        ***
#*****************************************************************************

States = {
##-- TCE uptake
          AStom,              # Amount of TCE in stomach
          ADuod,              # oral gavage absorption -- mice and rats only
          AExc,               #(vrisk) excreted in feces from gavage (currently 0)
          AO,                 #(vrisk) total absorbed
          InhDose,  # Amount inhaled
##-- TCE in the body
          ARap,               # Amount in rapidly perfused tissues
          ASlw,               # Amount in slowly perfused tissues
          AFat,               # Amount in fat
          AGut,               # Amount in gut
          ALiv,               # Amount in liver
          AKid,               # Amount in Kidney -- previously in Rap tissue
          ABld,               # Amount in Blood -- previously in Rap tissue
          AInhResp, # Amount in respiratory lumen during inhalation
          AResp,              # Amount in respiratory tissue
          AExhResp, # Amount in respiratory lumen during exhalation
##-- TCA in the body
          AOTCA,              #(vrisk)
          AStomTCA, # Amount of TCA in stomach
          APlasTCA, # Amount of TCA in plasma #comment out for
          ABodTCA,  # Amount of TCA in lumped body compartment
          ALivTCA,  # Amount of TCA in liver
##-- TCA metabolized
          AUrnTCA,  # Cumulative Amount of TCA excreted in urine
          AUrnTCA_sat,        # Amount of TCA excreted that during times that had
                              # saturated measurements (for lower bounds)
          AUrnTCA_collect,# Cumulative Amount of TCA excreted in urine during
                              # collection times (for intermittent collection)
##-- TCOH in body
          AOTCOH,             #(vrisk)
          AStomTCOH,          # Amount of TCOH in stomach
          ABodTCOH,           # Amount of TCOH in lumped body compartment
          ALivTCOH,           # Amount of TCOH in liver
##-- TCOG in body
          ABodTCOG,           # Amount of TCOG in lumped body compartment
          ALivTCOG,           # Amount of TCOG in liver
          ABileTCOG,          # Amount of TCOG in bile (incl. gut)
          ARecircTCOG,        #(vrisk)
##-- TCOG excreted
          AUrnTCOG, # Amount of TCOG excreted in urine
          AUrnTCOG_sat,       # Amount of TCOG excreted that during times that had
                              # saturated measurements (for lower bounds)
          AUrnTCOG_collect,# Cumulative Amount of TCA excreted in urine during
                              # collection times (for intermittent collection)
##-- DCVG in body
          ADCVGIn,  #(vrisk)
          ADCVGmol, # Amount of DCVG in body in mmoles
          AMetDCVG, #(vrisk)
##-- DCVC in body
          ADCVCIn,  #(vrisk)
         ADCVC,               # Amount of DCVC in body
          ABioactDCVC,        #(vrisk)
##-- NAcDCVC excreted
          AUrnNDCVC,          # Amount of NAcDCVC excreted
##-- Other states for TCE
          ACh,                # Amount in closed chamber -- mice and rats only
          AExh,               # Amount exhaled
          AExhExp,  # Amount exhaled during expos  [to calc. retention]
##-- Metabolism
```

```
          AMetLiv1, #(vrisk) Amount metabolized by P450 in liver
          AMetLiv2, #(vrisk) Amount metabolized by GSH conjugation in liver
          AMetLng,  #(vrisk) Amount metabolized in the lung
          AMetKid,  #(vrisk)
          AMetTCOHTCA,        #(vrisk) Amount of TCOH metabolized to TCA
          AMetTCOHGluc,       #(vrisk) Amount of TCOH glucuronidated
          AMetTCOHOther,      #(vrisk)
          AMetTCA,  #(vrisk) Amount of TCA metabolized
##-- Other Dose metrics
          AUCCBld,  #(vrisk)
          AUCCLiv,  #(vrisk)
          AUCCKid,  #(vrisk)
          AUCCRap,  #(vrisk)
          AUCCTCOH, #(vrisk)
          AUCCBodTCOH,        #(vrisk)
          AUCTotCTCOH,        #(vrisk)
          AUCPlasTCAFree,     #(vrisk)
          AUCPlasTCA,         #(vrisk)
          AUCLivTCA,          #(vrisk)
          AUCCDCVG  #(vrisk)
};

#*****************************************************************************
#***                  Input Variable Specifications                        ***
#*****************************************************************************

Inputs = {
##-- TCE dosing
          Conc,               # Inhalation exposure conc. (ppm)
          IVDose,             # IV dose (mg/kg)
          PDose,              # Oral gavage dose (mg/kg)
          Drink,              # Drinking water dose (mg/kg-day)
          IADose,                       # Inter-arterial
          PVDose,                       # Portal Vein
##-- TCA dosing
          IVDoseTCA,          # IV dose (mg/kg) of TCA
          PODoseTCA,          # Oral dose (mg/kg) of TCA
##-- TCOH dosing
          IVDoseTCOH,         # IV dose (mg/kg) of TCOH
          PODoseTCOH,         # Oral dose (mg/kg) of TCOH
##-- Potentially time-varying parameters
          QPmeas,             # Measured value of Alveolar ventilation QP
          TCAUrnSat,          # Flag for saturated TCA urine
          TCOGUrnSat,         # Flag for saturated TCOG urine
          UrnMissing          # Flag for missing urine collection times
};

#*****************************************************************************
#***                  Output Variable Specifications                       ***
#*****************************************************************************
Outputs = {
#*****************************************************************************
#*** Outputs for mass balance check
MassBalTCE,
TotDose,
TotTissue,
MassBalTCOH,
TotTCOHIn,
TotTCOHDose,
TotTissueTCOH,
TotMetabTCOH,
MassBalTCA,
TotTCAIn,
TotTissueTCA,
MassBalTCOG,
TotTCOGIn,
TotTissueTCOG,
MassBalDCVG,
MassBalDCVC,
AUrnNDCVCequiv,

#*****************************************************************************
#*** Outputs that are potential dose metrics
          TotMetab, #(vrisk) Total metabolism
          TotMetabBW34, #(vrisk) Total metabolism/BW^3/4
          ATotMetLiv, #(vrisk) Total metabolism in liver
          AMetLiv1Liv, #(vrisk) Total oxidation in liver/liver volume
          AMetLivOther, #(vrisk) Total "other" oxidation in liver
          AMetLivOtherLiv, #(vrisk) Total "other" oxidation in liver/liver vol
          AMetLngResp, #(vrisk) oxiation in lung/respiratory tissue volume
          AMetGSH, #(vrisk) total GSH conjugation
          AMetGSHBW34, #(vrisk) total GSH conjugation/BW^3/4
          ABioactDCVCKid,     #(vrisk) Amount of DCVC bioactivated/kidney volume
# NEW
          TotDoseBW34, #(vrisk) mg intake / BW^3/4
          AMetLiv1BW34, #(vrisk) mg hepatic oxidative metabolism / BW^3/4
          TotOxMetabBW34, #(vrisk) mg oxidative metabolism / BW^3/4
          TotTCAInBW, #(vrisk) TCA production / BW
          AMetLngBW34, #(vrisk) oxiation in lung/BW^3/4
          ABioactDCVCBW34, #(vrisk) Amount of DCVC bioactivated/BW^3/4
          AMetLivOtherBW34, #(vrisk) Total "other" oxidation in liver/BW^3/4
#*****************************************************************************
#*** Outputs for comparison to in vivo data
# TCE
RetDose,  # human - = (InhDose - AExhExp)
CAlv,     # needed for CAlvPPM
CAlvPPM,  # human
CInhPPM,  # mouse, rat
CInh,     # needed for CMixExh
CMixExh,  # rat - Mixed exhaled breath (mg/l)
CArt,     # rat, human - Arterial blood concentration
CVen,     # mouse, rat, human
CBldMix,  # rat - Concentration in mixed arterial+venous blood
                  # (used for cardiac puncture)
CFat,     # mouse, rat - Concentration in fat
CGut,     # rat
CRap,     # needed for unlumped tissues
CSlw,     # needed for unlumped tissues
CHrt,     # rat - Concentration in heart tissue [use CRap]
CKid,     # mouse, rat -  Concentration in kidney
CLiv,     # mouse, rat - Concentration in liver
CLung,    # mouse, rat - Concentration in lung [use CRap]
CMus,     # rat - Concentration in muscle [use CSlw]
CSpl,     # rat - Concentration in spleen [use CRap]
```

```
CBrn,     # rat - Concentration in brain [use CRap]
zAExh,    # mouse
zAExhpost,          # rat - Amount exhaled post-exposure (mg)

# TCOH
CTCOH,    # mouse, rat, human - TCOH concentration in blood
CKidTCOH, # mouse - TCOH concentration in kidney
CLivTCOH, # mouse - TCOH concentration in liver
CLungTCOH,          # mouse - TCOH concentration in lung

# TCA
CPlasTCA, # mouse, rat, human - TCA concentration in plasma
CBldTCA,  # mouse, rat, human - TCA concentration in blood
CBodTCA,  # needed for CKidTCA and CLungTCA
CKidTCA,  # mouse - TCA concentration in kidney
CLivTCA,  # mouse, rat - TCA concentration in liver
CLungTCA, # mouse - TCA concentration in lung
zAUrnTCA, # mouse, rat, human - Cumulative Urinary TCA
zAUrnTCA_collect,   # human - TCA measurements for intermittent collection
zAUrnTCA_sat,       # human - Saturated TCA measurements

# TCOG
zABileTCOG,         # rat - Amount of TCOG in bile (mg)
CTCOG,    # needed for CTCOGTCOH
CTCOGTCOH,          # mouse - TCOG concentration in blood (in TCOH-equiv)
CKidTCOGTCOH,       # mouse - TCOG concentration in kidney (in TCOH-equiv)
CLivTCOGTCOH,       # mouse - TCOG concentration in liver (in TCOH-equiv)
CLungTCOGTCOH,      # mouse - TCOG concentration in lung (in TCOH-equiv)
AUrnTCOGTCOH,       # mouse, rat, human - Cumulative Urinary TCOG (in TCOH-equiv)
AUrnTCOGTCOH_collect,         # human - TCOG (in TCOH-equiv) measurements for
                              # intermittent collection
AUrnTCOGTCOH_sat,   # human - Saturated TCOG (in TCOH-equiv) measurements

# Other
CDCVGmol,           # concentration of DCVG (mmol/l)
CDCVGmol0,          # Dummy variable without likelihood (for plotting)#(v1.2.3.1)
CDCVG_ND, # Non-detect of DCVG (<0.05 pmol/ml= 5e-5 mmol/l )#(v1.2.3.1)
                    # Output -ln(likelihood)#(v1.2.3.1)
zAUrnNDCVC,         # rat, human - Cumulative urinary NAcDCVC
AUrnTCTotMole,      # rat, human - Cumulative urinary TCOH+TCA in mmoles
TotCTCOH, # mouse, human - TCOH+TCOG Concentration (in TCOH-equiv)
TotCTCOHcomp,       # ONLY FOR COMPARISON WITH HACK
ATCOG,              # ONLY FOR COMPARISON WITH HACK
QPsamp,   # human - sampled value of alveolar ventilation rate

## PARAMETERS #(vrisk)

  QCnow, # (vrisk) #Cardiac output (L/hr)
  QP, # (vrisk) #Alveolar ventilation (L/hr)
  QFatCtmp, # (vrisk) #Scaled fat blood flow
  QGutCtmp, # (vrisk) #Scaled gut blood flow
  QLivCtmp, # (vrisk) #Scaled liver blood flow
  QSlwCtmp, # (vrisk) #Scaled slowly perfused blood flow
  QRapCtmp, # (vrisk) #Scaled rapidly perfused blood flow
  QKidCtmp, # (vrisk) #Scaled kidney blood flow
  DResp, # (vrisk) #Respiratory lumen:tissue diffusive clearance rate
  VFatCtmp, # (vrisk) #Fat fractional compartment volume
  VGutCtmp, # (vrisk) #Gut fractional compartment volume
  VLivCtmp, # (vrisk) #Liver fractional compartment volume
  VRapCtmp, # (vrisk) #Rapidly perfused fractional compartment volume
  VRespLumCtmp, # (vrisk) # Fractional volume of respiratory lumen
  VRespEffCtmp, # (vrisk) #Effective fractional volume of respiratory tissue
  VKidCtmp, # (vrisk) #Kidney fractional compartment volume
  VBldCtmp, # (vrisk) #Blood fractional compartment volume
  VSlwCtmp, # (vrisk) #Slowly perfused fractional compartment volume
  VPlasCtmp, # (vrisk) #Plasma fractional compartment volume
  VBodCtmp, # (vrisk) #TCA Body fractional compartment volume [not incl.
blood+liver]
  VBodTCOHCtmp, # (vrisk) #TCOH/G Body fractional compartment volume [not incl.
liver]
  PB, # (vrisk) #TCE Blood/air partition coefficient
  PFat, # (vrisk) #TCE Fat/Blood partition coefficient
  PGut, # (vrisk) #TCE Gut/Blood partition coefficient
  PLiv, # (vrisk) #TCE Liver/Blood partition coefficient
  PRap, # (vrisk) #TCE Rapidly perfused/Blood partition coefficient
  PResp, # (vrisk) #TCE Respiratory tissue:air partition coefficient
  PKid, # (vrisk) #TCE Kidney/Blood partition coefficient
  PSlw, # (vrisk) #TCE Slowly perfused/Blood partition coefficient
  TCAPlas, # (vrisk) #TCA blood/plasma concentration ratio
  PBodTCA, # (vrisk) #Free TCA Body/blood plasma partition coefficient
  PLivTCA, # (vrisk) #Free TCA Liver/blood plasma partition coefficient
  kDissoc, # (vrisk) #Protein/TCA dissociation constant (umole/L)
  BMax, # (vrisk) #Maximum binding concentration (umole/L)
  PBodTCOH, # (vrisk) #TCOH body/blood partition coefficient
  PLivTCOH, # (vrisk) #TCOH liver/body partition coefficient
  PBodTCOG, # (vrisk) #TCOG body/blood partition coefficient
  PLivTCOG, # (vrisk) #TCOG liver/body partition coefficient
  VDCVG, # (vrisk) #DCVG effective volume of distribution
  kAS, # (vrisk) #TCE Stomach absorption coefficient (/hr)
  kTSD, # (vrisk) #TCE Stomach-duodenum transfer coefficient (/hr)
  kAD, # (vrisk) #TCE Duodenum absorption coefficient (/hr)
  kTD, # (vrisk) #TCE Duodenum-feces transfer coefficient (/hr)
  kASTCA, # (vrisk) #TCA Stomach absorption coefficient (/hr)
  kASTCOH, # (vrisk) #TCOH Stomach absorption coefficient (/hr)
  VMAX, # (vrisk) #VMAX for hepatic TCE oxidation (mg/hr)
  KM, # (vrisk) #KM for hepatic TCE oxidation (mg/L)
  FracOther, # (vrisk) #Fraction of hepatic TCE oxidation not to TCA+TCOH
  FracTCA, # (vrisk) #Fraction of hepatic TCE oxidation to TCA
  VMAXDCVG, # (vrisk) #VMAX for hepatic TCE GSH conjugation (mg/hr)
  KMDCVG, # (vrisk) #KM for hepatic TCE GSH conjugation (mg/L)
  VMAXKidDCVG, # (vrisk) #VMAX for renal TCE GSH conjugation (mg/hr)
  KMKidDCVG, # (vrisk) #KM for renal TCE GSH conjugation (mg/L)
  FracKidDCVC, # (vrisk) #Fraction of renal TCE GSH conj. "directly" to DCVC
               # (vrisk) #(i.e., via first pass)
  VMAXClara, # (vrisk) #VMAX for Tracheo-bronchial TCE oxidation (mg/hr)
  KMClara, # (vrisk) #KM for Tracheo-bronchial TCE oxidation (mg/L)
  FracLungSys, # (vrisk) #Fraction of respiratory metabolism to systemic circ.
  VMAXTCOH, # (vrisk) #VMAX for hepatic TCOH->TCA (mg/hr)
  KMTCOH, # (vrisk) #KM for hepatic TCOH->TCA (mg/L)
  VMAXGluc, # (vrisk) #VMAX for hepatic TCOH->TCOG (mg/hr)
  KMGluc, # (vrisk) #KM for hepatic TCOH->TCOG (mg/L)
  kMetTCOH, # (vrisk) #Rate constant for hepatic TCOH->other (/hr)
  kUrnTCA, # (vrisk) #Rate constant for TCA plasma->urine (/hr)
  kMetTCA, # (vrisk) #Rate constant for hepatic TCA->other (/hr)
```

```
  kBile, # (vrisk) #Rate constant for TCOG liver->bile (/hr)
  kEHR, # (vrisk) #Lumped rate constant for TCOG bile->TCOH liver (/hr)
  kUrnTCOG, # (vrisk) #Rate constant for TCOG->urine (/hr)
  kDCVG, # (vrisk) #Rate constant for hepatic DCVG->DCVC (/hr)
  kNAT, # (vrisk) #Lumped rate constant for DCVC->Urinary NAcDCVC (/hr)
  kKidBioact, # (vrisk) #Rate constant for DCVC bioactivation (/hr)

## Misc
  RUrnTCA, #(vrisk)
  RUrnTCOGTCOH, #(vrisk)
  RUrnNDCVC, #(vrisk)
  RAO,
  CVenMole,
  CPlasTCAMole,
  CPlasTCAFreeMole
};

#*****************************************************************************
#***                  Global Constants                                     ***
#*****************************************************************************

# Molecular Weights
        MWTCE = 131.39;        # TCE
        MWDCA = 129.0;        # DCA
       MWDCVC = 216.1;        # DCVC
        MWTCA = 163.5;        # TCA
      MWChlor = 147.5;        # Chloral
       MWTCOH = 149.5;        # TCOH
   MWTCOHGluc = 325.53;       # TCOH-Gluc
     MWNADCVC = 258.8;        # N Acetyl DCVC

# Stoichiometry
 StochChlorTCE = MWChlor / MWTCE;
   StochTCATCE = MWTCA / MWTCE;
  StochTCATCOH = MWTCA / MWTCOH;
  StochTCOHTCE = MWTCOH / MWTCE;
 StochGlucTCOH = MWTCOHGluc / MWTCOH;
 StochTCOHGluc = MWTCOH / MWTCOHGluc;
  StochTCEGluc = MWTCE / MWTCOHGluc;
  StochDCVCTCE = MWDCVC / MWTCE;
        StochN = MWNADCVC / MWDCVC;
   StochDCATCE = MWDCA / MWTCE;

#*****************************************************************************
#***                  Global Model Parameters                              ***
#*****************************************************************************
# These are the actual model parameters used in "dynamics."
# Values that are assigned in the "initialize" section,
# are all set to 1 to avoid confusion.

#*****************************************************************************
# Flows
QC        = 1;      # Cardiac output (L/hr)
QPsamp    = 1;      # Alveolar ventilation (L/hr)
VPR       = 1;      # Alveolar ventilation-perfusion ratio
QFatCtmp  = 1;      # Scaled fat blood flow
QGutCtmp  = 1;      # Scaled gut blood flow
QLivCtmp  = 1;      # Scaled liver blood flow
QSlwCtmp  = 1;      # Scaled slowly perfused blood flow
DResptmp  = 1;      # Respiratory lumen:tissue diffusive clearance rate (L/hr)
[scaled to QP]
QKidCtmp  = 1;      # Scaled kidney blood flow
FracPlas  = 1;      # Fraction of blood that is plasma (1-hematocrit)
#*****************************************************************************
# Volumes
VFat      = 1;      # Fat compartment volume (L)
VGut      = 1;      # Gut compartment volume (L)
VLiv      = 1;      # Liver compartment volume (L)
VRap      = 1;      # Rapidly perfused compartment volume (L)
VRespLum  = 1;      # Volume of respiratory lumen (L air)
VRespEfftmp         = 1;      #(vrisk) volume for respiratory tissue (L)
VRespEff  = 1;      # Effective volume for respiratory tissue (L air) = V(tissue) *
Resp:Air partition coefficient
VKid      = 1;      # Kidney compartment volume (L)
VBld      = 1;      # Blood compartment volume (L)
VSlw      = 1;      # Slowly perfused compartment volume (L)
VPlas     = 1;      # Plasma compartment volume [fraction of blood] (L)
VBod      = 1;      # TCA Body compartment volume [not incl. blood+liver] (L)
VBodTCOH  = 1;      # TCOH/G Body compartment volume [not incl. liver] (L)
#*****************************************************************************
# Distribution/partitioning
PB        = 1;      # TCE Blood/air partition coefficient
PFat      = 1;      # TCE Fat/Blood partition coefficient
PGut      = 1;      # TCE Gut/Blood partition coefficient
PLiv      = 1;      # TCE Liver/Blood partition coefficient
PRap      = 1;      # TCE Rapidly perfused/Blood partition coefficient
PResp     = 1;      # TCE Respiratory tissue:air partition coefficient
PKid      = 1;      # TCE Kidney/Blood partition coefficient
PSlw      = 1;      # TCE Slowly perfused/Blood partition coefficient
TCAPlas   = 1;      # TCA blood/plasma concentration ratio
PBodTCA   = 1;      # Free TCA Body/blood plasma partition coefficient
PLivTCA   = 1;      # Free TCA Liver/blood plasma partition coefficient
kDissoc   = 1;      # Protein/TCA dissociation constant (umole/L)
BMax      = 1;      # Protein concentration (UNITS?)
PBodTCOH  = 1;      # TCOH body/blood partition coefficient
PLivTCOH  = 1;      # TCOH liver/body partition coefficient
PBodTCOG  = 1;      # TCOG body/blood partition coefficient
PLivTCOG  = 1;      # TCOG liver/body partition coefficient
VDCVG     = 1;      # DCVG effective volume of distribution
#*****************************************************************************
# Oral absorption
kTSD      = 1.4;    # TCE Stomach-duodenum transfer coefficient (/hr)
kAS       = 1.4;    # TCE Stomach absorption coefficient (/hr)
kTD       = 0.1;    # TCE Duodenum-feces transfer coefficient (/hr)
kAD       = 0.75;   # TCE Duodenum absorption coefficient (/hr)
kASTCA    = 0.75;   # TCA Stomach absorption coefficient (/hr)
kASTCOH   = 0.75;   # TCOH Stomach absorption coefficient (/hr)
#*****************************************************************************
# TCE Metabolism
VMAX      = 1;      # VMAX for hepatic TCE oxidation (mg/hr)
KM        = 1;      # KM for hepatic TCE oxidation (mg/L)
FracOther = 1;      # Fraction of hepatic TCE oxidation not to TCA+TCOH
FracTCA   = 1;      # Fraction of hepatic TCE oxidation to TCA
VMAXDCVG  = 1;      # VMAX for hepatic TCE GSH conjugation (mg/hr)
```

```
KMDCVG    = 1;      # KM for hepatic TCE GSH conjugation (mg/L)
VMAXKidDCVG         = 1;      # VMAX for renal TCE GSH conjugation (mg/hr)
KMKidDCVG = 1;      # KM for renal TCE GSH conjugation (mg/L)
VMAXClara = 1;      # VMAX for Tracheo-bronchial TCE oxidation (mg/hr)
KMClara   = 1;      # KM for Tracheo-bronchial TCE oxidation (mg/L)
                    # but in units of air concentration
FracLungSys         = 1;      # Fraction of respiratory oxidative metabolism that
enters systemic circulation

#*******************************************************************************
# TCOH metabolism
VMAXTCOH  = 1;      # VMAX for hepatic TCOH->TCA (mg/hr)
KMTCOH    = 1;      # KM for hepatic TCOH->TCA (mg/L)
VMAXGluc  = 1;      # VMAX for hepatic TCOH->TCOG (mg/hr)
KMGluc    = 1;      # KM for hepatic TCOH->TCOG (mg/L)
kMetTCOH  = 1;      # Rate constant for hepatic TCOH->other (/hr)
#*******************************************************************************
# TCA metabolism/clearance
kUrnTCA   = 1;      # Rate constant for TCA plasma->urine (/hr)
kMetTCA   = 1;      # Rate constant for hepatic TCA->other (/hr)
#*******************************************************************************
# TCOG metabolism/clearance
kBile     = 1;      # Rate constant for TCOG liver->bile (/hr)
kEHR      = 1;      # Lumped rate constant for TCOG bile->TCOH liver (/hr)
kUrnTCOG  = 1;      # Rate constant for TCOG->urine (/hr)
#*******************************************************************************
# DCVG metabolism
kDCVG     = 1;      # Rate constant for hepatic DCVG->DCVC (/hr)
FracKidDCVC         = 1;      # Fraction of renal TCE GSH conj. "directly" to DCVC
(i.e., via first pass)
#*******************************************************************************
# DCVC metabolism/clearance
kNAT      = 1;      # Lumped rate constant for DCVC->Urinary NAcDCVC (/hr)
kKidBioact          = 1;      # Rate constant for DCVC bioactivation (/hr)
#*******************************************************************************
# Closed chamber and other exposure parameters
Rodents   = 1;      # Number of rodents in closed chamber data
VCh       = 1;      # Chamber volume for closed chamber data
kLoss     = 1;      # Rate constant for closed chamber air loss
CC        = 0.0;    # Initial chamber concentration (ppm)
TChng     = 0.003;  # IV infusion duration (hour)
#*******************************************************************************
## Flag for species, sex -- these are global parameters
BW        = 0.0;  # Species-specific defaults during initialization
BW75      = 0.0;    #(vrisk) Variable for BW^3/4
Male      = 1.0;    # 1 = male, 0 = female
Species   = 1.0;    # 1 = human, 2 = rat, 3 = mouse

#*******************************************************************************
#***                  Potentially measured covariates (constants)           ***
#*******************************************************************************
BWmeas    = 0.0;    # Body weight
VFatCmeas = 0.0; # Fractional volume fat
PBmeas    = 0.0;    # Measured blood-air partition coefficient
Hematocritmeas = 0.0; # Measured hematocrit -- used for FracPlas = 1 - HCt
CDCVGmolLD = 5e-5; # Detection limit of CDCVGmol#(v1.2.3.1)

#*******************************************************************************
#***                  Global Sampling Parameters                             ***
#*******************************************************************************
# These parameters are potentially sampled/calibrated in the MCMC or MC
# analyses.  The default values here are used if no sampled value is given.
# M_ indicates population mean parameters used only in MC sampling
# V_ indicates a population variance parameter used in MC and MCMC sampling

# Flow Rates
lnQCC     = 0.0;    # Scaled by BW^0.75 and species-specific central estimates
lnVPRC    = 0.0;    # Scaled to species-specific central estimates

# Fractional Blood Flows to Tissues (fraction of cardiac output)
QFatC     = 1.0;    # Scaled to species-specific central estimates
QGutC     = 1.0;    # Scaled to species-specific central estimates
QLivC     = 1.0;    # Scaled to species-specific central estimates
QSlwC     = 1.0;    # Scaled to species-specific central estimates
QKidC     = 1.0;    # Scaled to species-specific central estimates
FracPlasC = 1.0;    # Scaled to species-specific central estimates
lnDRespC  = 0.0;    # Scaled to alveolar ventilation rate in dynamics

# Fractional Tissue Volumes (fraction of BW)
VFatC     = 1.0;    # Scaled to species-specific central estimates
VGutC     = 1.0;    # Scaled to species-specific central estimates
VLivC     = 1.0;    # Scaled to species-specific central estimates
VRapC     = 1.0;    # Scaled to species-specific central estimates
VRespLumC = 1.0;    # Scaled to species-specific central estimates
VRespEffC = 1.0;    # Scaled to species-specific central estimates

VKidC     = 1.0;    # Scaled to species-specific central estimates
VBldC     = 1.0;    # Scaled to species-specific central estimate

# Partition Coefficients for TCE
lnPBC     = 0.0;    # Scaled to species-specific central estimates
lnPFatC   = 0.0;    # Scaled to species-specific central estimates
lnPGutC   = 0.0;    # Scaled to species-specific central estimates
lnPLivC   = 0.0;    # Scaled to species-specific central estimates
lnPRapC   = 0.0;    # Scaled to species-specific central estimates
lnPRespC  = 0.0;    # Scaled to species-specific central estimates
lnPKidC   = 0.0;    # Scaled to species-specific central estimates
lnPSlwC   = 0.0;    # Scaled to species-specific central estimates

# Partition Coefficients for TCA
lnPRBCPlasTCAC      = 0.0;    # Scaled to species-specific central estimates
lnPBodTCAC          = 0.0;    # Scaled to species-specific central estimates
lnPLivTCAC          = 0.0;    # Scaled to species-specific central estimates

# Plasma Binding for TCA
lnkDissocC          = 0.0;    # Scaled to species-specific central estimates
lnBMaxkDC = 0.0;    # Scaled to species-specific central estimates

# Partition Coefficients for TCOH and TCOG
lnPBodTCOHC         = 0.0;    # Scaled to species-specific central estimates
lnPLivTCOHC         = 0.0;    # Scaled to species-specific central estimates
lnPBodTCOGC         = 0.0;    # Scaled to species-specific central estimates
lnPLivTCOGC         = 0.0;    # Scaled to species-specific central estimates
lnPeffDCVG          = 0.0;    # Scaled to species-specific central estimates
```

```
# Oral Absorption rates
lnkTSD    = 0.336;
lnkAS     = 0.336;
lnkTD     = -2.303;
lnkAD     = -0.288;
lnkASTCA  = -0.288;
lnkASTCOH = -0.288;

# TCE Metabolism
lnVMAXC   = 0.0;    # Scaled by liver weight and species-specific central estimates
lnKMC     = 0.0;    # Scaled to species-specific central estimates
lnClC     = 0.0;    # Scaled to species-specific central estimates
lnFracOtherC        = 0.0;    # Ratio of DCA to non-DCA
lnFracTCAC          = 0.0;    # Ratio of TCA to TCOH
lnVMAXDCVGC         = 0.0;    # Scaled by liver weight and species-specific central
estimates
lnClDCVGC = 0.0;    # Scaled to species-specific central estimates
lnKMDCVGC = 0.0;    # Scaled to species-specific central estimates
lnVMAXKidDCVGC      = 0.0;    # Scaled by kidney weight and species-specific central
estimates
lnClKidDCVGC        = 0.0;    # Scaled to species-specific central estimates
lnKMKidDCVGC        = 0.0;    # Scaled to species-specific central estimates
lnVMAXLungLivC      = 0.0;    # Ratio of lung VMAX to liver VMAX,
                              # Scaled to species-specific central estimates
lnKMClara = 0.0;    # now in units of air concentration

# Clearance in lung
lnFracLungSysC      = 0.0;    # ratio of systemic to local clearance of lung
oxidation

# TCOH Metabolism
lnVMAXTCOHC         = 0.0;    # Scaled by BW^0.75
lnClTCOHC = 0.0;    # Scaled by BW^0.75
lnKMTCOH  = 0.0;    #
lnVMAXGlucC         = 0.0;    # Scaled by BW^0.75
lnClGlucC = 0.0;    # Scaled by BW^0.75
lnKMGluc  = 0.0;    #
lnkMetTCOHC         = 0.0;    # Scaled by BW^-0.25

# TCA Metabolism/clearance
lnkUrnTCAC          = 0.0;    # Scaled by (plasma volume)^-1 and species-specific
central estimates
lnkMetTCAC          = 0.0;    # Scaled by BW^-0.25

# TCOG excretion and reabsorption
lnkBileC  = 0.0;    # Scaled by BW^-0.25
lnkEHRC   = 0.0;    # Scaled by BW^-0.25
lnkUrnTCOGC         = 0.0;    # Scaled by (blood volume)^-1 and species-specific
central estimates

# DCVG metabolism
lnFracKidDCVCC      = 0.0;    # Ratio of "directly" to DCVC to systemic DCVG
lnkDCVGC  = 0.0;    # Scaled by BW^-0.25

# DCVC metabolism
lnkNATC   = 0.0;    # Scaled by BW^-0.25
lnkKidBioactC       = 0.0;    # Scaled by BW^-0.25

# Closed chamber parameters
NRodents  = 1;      #
VChC      = 1;      #
lnkLossC  = 0;      #

#***************************************************************************
# Population means
#
# These are given truncated normal or uniform distributions, depending on
#         what prior information is available.  Note that these distributions
#         reflect uncertainty in the population mean, not inter-individual
#         variability.  Normal distributions are truncated at 2, 3, or 4 SD.
#                   For fractional volumes and flows, 2xSD
#                   For plasma fraction, 3xSD
#                   For cardiac output and ventilation-perfusion ratio, 4xSD
#                   For all others, 3xSD
#         For uniform distributions, range of 1e2 to 1e8 fold, centered on
#                   central estimate.
#
M_lnQCC   = 1.0;
M_lnVPRC  = 1.0;
M_QFatC   = 1.0;
M_QGutC   = 1.0;
M_QLivC   = 1.0;
M_QSlwC   = 1.0;
M_QKidC   = 1.0;
M_FracPlasC         = 1.0;
M_lnDRespC = 1.0;
M_VFatC   = 1.0;
M_VGutC   = 1.0;
M_VLivC   = 1.0;
M_VRapC   = 1.0;
M_VRespLumC = 1.0;
M_VRespEffC = 1.0;
M_VKidC   = 1.0;
M_VBldC   = 1.0;
M_lnPBC   = 1.0;
M_lnPFatC = 1.0;
M_lnPGutC = 1.0;
M_lnPLivC = 1.0;
M_lnPRapC = 1.0;
M_lnPRespC          = 1.0;
M_lnPKidC = 1.0;
M_lnPSlwC = 1.0;
M_lnPRBCPlasTCAC    = 1.0;
M_lnPBodTCAC        = 1.0;
M_lnPLivTCAC        = 1.0;
M_lnkDissocC        = 1.0;
M_lnBMaxkDC         = 1.0;
M_lnPBodTCOHC       = 1.0;
M_lnPLivTCOHC       = 1.0;
M_lnPBodTCOGC       = 1.0;
M_lnPLivTCOGC       = 1.0;
M_lnPeffDCVG        = 1.0;
M_lnkTSD  = 1.0;
```

```
M_lnkAS   = 1.0;
M_lnkTD   = 1.0;
M_lnkAD   = 1.0;
M_lnkASTCA          = 1.0;
M_lnkASTCOH         = 1.0;
M_lnVMAXC = 1.0;
M_lnKMC   = 1.0;
M_lnClC   = 1.0;
M_lnFracOtherC      = 1.0;
M_lnFracTCAC        = 1.0;
M_lnVMAXDCVGC       = 1.0;
M_lnClDCVGC         = 1.0;
M_lnKMDCVGC         = 1.0;
M_lnVMAXKidDCVGC    = 1.0;
M_lnClKidDCVGC      = 1.0;
M_lnKMKidDCVGC      = 1.0;
M_lnVMAXLungLivC    = 1.0;
M_lnKMClara         = 1.0;
M_lnFracLungSysC    = 1.0;
M_lnVMAXTCOHC       = 1.0;
M_lnClTCOHC         = 1.0;
M_lnKMTCOH          = 1.0;
M_lnVMAXGlucC       = 1.0;
M_lnClGlucC         = 1.0;
M_lnKMGluc          = 1.0;
M_lnkMetTCOHC       = 1.0;
M_lnkUrnTCAC        = 1.0;
M_lnkMetTCAC        = 1.0;
M_lnkBileC          = 1.0;
M_lnkEHRC = 1.0;
M_lnkUrnTCOGC       = 1.0;
M_lnFracKidDCVCC    = 1.0;
M_lnkDCVGC          = 1.0;
M_lnkNATC = 1.0;
M_lnkKidBioactC     = 1.0;


#*****************************************************************************
# Population Variances
#
# These are given InvGamma(alpha,beta) distributions.  The parameterization
#         for alpha and beta is given by:
#                   alpha = (n-1)/2
#                   beta = s^2*(n-1)/2
#         where n = number of data points, and s^2 is the sample variance
#         Sum(x_i^2)/n - <x>^2.
# Generally, for parameters for which there is no direct data, assume a
#         value of n = 5 (alpha = 2).  For a sample variance s^2, this gives
#         an expected value for the standard deviation <sigma> = 0.9*s,
#         a median [2.5%,97.5%] of 1.1*s [0.6*s,2.9*s].
#
V_lnQCC   = 1.0;
V_lnVPRC  = 1.0;
V_QFatC   = 1.0;
V_QGutC   = 1.0;
V_QLivC   = 1.0;
V_QSlwC   = 1.0;
V_QKidC   = 1.0;
V_FracPlasC         = 1.0;
V_lnDRespC = 1.0;
V_VFatC   = 1.0;
V_VGutC   = 1.0;
V_VLivC   = 1.0;
V_VRapC   = 1.0;
V_VRespLumC = 1.0;
V_VRespEffC = 1.0;
V_VKidC   = 1.0;
V_VBldC   = 1.0;
V_lnPBC   = 1.0;
V_lnPFatC = 1.0;
V_lnPGutC = 1.0;
V_lnPLivC = 1.0;
V_lnPRapC = 1.0;
V_lnPRespC          = 1.0;
V_lnPKidC = 1.0;
V_lnPSlwC = 1.0;
V_lnPRBCPlasTCAC    = 1.0;
V_lnPBodTCAC        = 1.0;
V_lnPLivTCAC        = 1.0;
V_lnkDissocC        = 1.0;
V_lnBMaxkDC         = 1.0;
V_lnPBodTCOHC       = 1.0;
V_lnPLivTCOHC       = 1.0;
V_lnPBodTCOGC       = 1.0;
V_lnPLivTCOGC       = 1.0;
V_lnPeffDCVG        = 1.0;
V_lnkTSD  = 1.0;
V_lnkAS   = 1.0;
V_lnkTD   = 1.0;
V_lnkAD   = 1.0;
V_lnkASTCA          = 1.0;
V_lnkASTCOH         = 1.0;
V_lnVMAXC = 1.0;
V_lnKMC   = 1.0;
V_lnClC   = 1.0;
V_lnFracOtherC      = 1.0;
V_lnFracTCAC        = 1.0;
V_lnVMAXDCVGC       = 1.0;
V_lnClDCVGC         = 1.0;
V_lnKMDCVGC         = 1.0;
V_lnVMAXKidDCVGC    = 1.0;
V_lnClKidDCVGC      = 1.0;
V_lnKMKidDCVGC      = 1.0;
V_lnVMAXLungLivC    = 1.0;
V_lnKMClara         = 1.0;
V_lnFracLungSysC    = 1.0;
V_lnVMAXTCOHC       = 1.0;
V_lnClTCOHC         = 1.0;
V_lnKMTCOH          = 1.0;
V_lnVMAXGlucC       = 1.0;
V_lnClGlucC         = 1.0;
V_lnKMGluc          = 1.0;
V_lnkMetTCOHC       = 1.0;
V_lnkUrnTCAC        = 1.0;
```

```
V_lnkMetTCAC        = 1.0;
V_lnkBileC          = 1.0;
V_lnkEHRC = 1.0;
V_lnkUrnTCOGC       = 1.0;
V_lnFracKidDCVCC    = 1.0;
V_lnkDCVGC          = 1.0;
V_lnkNATC = 1.0;
V_lnkKidBioactC     = 1.0;

#*****************************************************************************
# Measurement error variances for output

Ve_RetDose          = 1;
Ve_CAlv   = 1;
Ve_CAlvPPM          = 1;
Ve_CInhPPM          = 1;
Ve_CInh   = 1;
Ve_CMixExh          = 1;
Ve_CArt   = 1;
Ve_CVen   = 1;
Ve_CBldMix          = 1;

Ve_CFat   = 1;
Ve_CGut   = 1;
Ve_CRap   = 1;
Ve_CSlw   = 1;
Ve_CHrt   = 1;
Ve_CKid   = 1;
Ve_CLiv   = 1;
Ve_CLung  = 1;
Ve_CMus   = 1;
Ve_CSpl   = 1;
Ve_CBrn   = 1;
Ve_zAExh  = 1;
Ve_zAExhpost        = 1;


Ve_CTCOH  = 1;
Ve_CKidTCOH         = 1;
Ve_CLivTCOH         = 1;
Ve_CLungTCOH        = 1;


Ve_CPlasTCA         = 1;
Ve_CBldTCA          = 1;
Ve_CBodTCA          = 1;
Ve_CKidTCA          = 1;
Ve_CLivTCA          = 1;
Ve_CLungTCA         = 1;
Ve_zAUrnTCA         = 1;
Ve_zAUrnTCA_collect = 1;
Ve_zAUrnTCA_sat     = 1;


Ve_zABileTCOG       = 1;
Ve_CTCOG  = 1;
Ve_CTCOGTCOH        = 1;

Ve_CKidTCOGTCOH     = 1;
Ve_CLivTCOGTCOH     = 1;
Ve_CLungTCOGTCOH    = 1;
Ve_AUrnTCOGTCOH     = 1;
Ve_AUrnTCOGTCOH_collect       = 1;

Ve_AUrnTCOGTCOH_sat = 1;


Ve_CDCVGmol         = 1;
Ve_zAUrnNDCVC       = 1;
Ve_AUrnTCTotMole    = 1;
Ve_TotCTCOH         = 1;
Ve_QPsamp = 1;

#*****************************************************************************
#***                  Defaults for input parameters                        ***
#*****************************************************************************
##-- TCE dosing
          Conc = 0.0;         # Inhalation exposure conc. (ppm)
          IVDose = 0.0;       # IV dose (mg/kg)
          PDose = 0.0;        # Oral gavage dose (mg/kg)
          Drink = 0.0;        # Drinking water dose (mg/kg-day)
          IADose = 0.0;       # Intraarterial dose (mg/kg)
          PVDose = 0.0;       # Portal vein dose (mg/kg)
##-- TCA dosing
          IVDoseTCA = 0.0;# IV dose (mg/kg) of TCA
          PODoseTCA = 0.0;# Oral dose (mg/kg) of TCA
##-- TCOH dosing
          IVDoseTCOH = 0.0;# IV dose (mg/kg) of TCOH
          PODoseTCOH = 0.0;# Oral dose (mg/kg) of TCOH
##-- Potentially time-varying parameters
          QPmeas = 0.0;       # Measured value of Alveolar ventilation QP
          TCAUrnSat = 0.0;# Flag for saturated TCA urine
          TCOGUrnSat = 0.0;# Flag for saturated TCOG urine
          UrnMissing = 0.0;# Flag for missing urine collection times

Initialize {

#*****************************************************************************
#***                  Parameter Initialization and Scaling                 ***
#*****************************************************************************
# Model Parameters (used in dynamics):
#         QC                  Cardiac output (L/hr)
#         VPR                 Ventilation-perfusion ratio
#         QPsamp              Alveolar ventilation (L/hr)
#         QFatCtmp  Scaled fat blood flow
#         QGutCtmp  Scaled gut blood flow
#         QLivCtmp  Scaled liver blood flow
#         QSlwCtmp  Scaled slowly perfused blood flow
#         DResptmp  Respiratory lumen:tissue diffusive clearance rate
#         QKidCtmp  Scaled kidney blood flow
#         FracPlas  Fraction of blood that is plasma (1-hematocrit)
#         VFat                Fat compartment volume (L)
#         VGut                Gut compartment volume (L)
#         VLiv                Liver compartment volume (L)
#         VRap                Rapidly perfused compartment volume (L)
```

```
#         VRespLum  Volume of respiratory lumen (L air)
#         VRespEff  Effective volume of respiratory tissue (L air)
#         VKid              Kidney compartment volume (L)
#         VBld              Blood compartment volume (L)
#         VSlw              Slowly perfused compartment volume (L)
#         VPlas             Plasma compartment volume [fraction of blood] (L)
#         VBod              TCA Body compartment volume [not incl. blood+liver]
(L)
#         VBodTCOH  TCOH/G Body compartment volume [not incl. liver] (L)
#         PB                TCE Blood/air partition coefficient
#         PFat              TCE Fat/Blood partition coefficient
#         PGut              TCE Gut/Blood partition coefficient
#         PLiv              TCE Liver/Blood partition coefficient
#         PRap              TCE Rapidly perfused/Blood partition coefficient
#         PResp             TCE Respiratory tissue:air partition coefficient
#         PKid              TCE Kidney/Blood partition coefficient
#         PSlw              TCE Slowly perfused/Blood partition coefficient
#         TCAPlas           TCA blood/plasma concentration ratio
#         PBodTCA           Free TCA Body/blood plasma partition coefficient
#         PLivTCA           Free TCA Liver/blood plasma partition coefficient
#         kDissoc           Protein/TCA dissociation constant (umole/L)
#         BMax              Maximum binding concentration (umole/L)
#         PBodTCOH  TCOH body/blood partition coefficient
#         PLivTCOH  TCOH liver/body partition coefficient
#         PBodTCOG  TCOG body/blood partition coefficient
#         PLivTCOG  TCOG liver/body partition coefficient
#         kAS               TCE Stomach absorption coefficient (/hr)
#         kTSD              TCE Stomach-duodenum transfer coefficient (/hr)
#         kAD               TCE Duodenum absorption coefficient (/hr)
#         kTD               TCE Duodenum-feces transfer coefficient (/hr)
#         kASTCA            TCA Stomach absorption coefficient (/hr)
#         kASTCOH           TCOH Stomach absorption coefficient (/hr)
#         VMAX              VMAX for hepatic TCE oxidation (mg/hr)
#         KM                KM for hepatic TCE oxidation (mg/L)
#         FracOther Fraction of hepatic TCE oxidation not to TCA+TCOH
#         FracTCA           Fraction of hepatic TCE oxidation to TCA
#         VMAXDCVG  VMAX for hepatic TCE GSH conjugation (mg/hr)
#         KMDCVG            KM for hepatic TCE GSH conjugation (mg/L)
#         VMAXKidDCVG       VMAX for renal TCE GSH conjugation (mg/hr)
#         KMKidDCVG KM for renal TCE GSH conjugation (mg/L)
#         VMAXClara VMAX for Tracheo-bronchial TCE oxidation (mg/hr)
#         KMClara           KM for Tracheo-bronchial TCE oxidation (mg/L)
#         FracLungSys       Fraction of respiratory metabolism to systemic circ.
#         VMAXTCOH  VMAX for hepatic TCOH->TCA (mg/hr)
#         KMTCOH            KM for hepatic TCOH->TCA (mg/L)
#         VMAXGluc  VMAX for hepatic TCOH->TCOG (mg/hr)
#         KMGluc            KM for hepatic TCOH->TCOG (mg/L)
#         kMetTCOH  Rate constant for hepatic TCOH->other (/hr)
#         kUrnTCA           Rate constant for TCA plasma->urine (/hr)
#         kMetTCA           Rate constant for hepatic TCA->other (/hr)
#         kBile             Rate constant for TCOG liver->bile (/hr)
#         kEHR              Lumped rate constant for TCOG bile->TCOH liver (/hr)
#         kUrnTCOG  Rate constant for TCOG->urine (/hr)
#         kDCVG             Rate constant for hepatic DCVG->DCVC (/hr)
#         FracKidDCVC       Fraction of renal TCE GSH conj. "directly" to DCVC
#                           (i.e., via first pass)
#         VDCVG             DCVG effective volume of distribution
#         kNAT              Lumped rate constant for DCVC->Urinary NAcDCVC (/hr)
#         kKidBioact        Rate constant for DCVC bioactivation (/hr)
#         Rodents           Number of rodents in closed chamber data
#         VCh               Chamber volume for closed chamber data
#         kLoss             Rate constant for closed chamber air loss
# Parameters used (not assigned here)
#         BW                Body weight in kg
#         Species           1 = human (default), 2 = rat, 3 = mouse
#         Male              0 = female, 1 (default) = male
#         CC                Closed chamber initial concentration
# Sampling/scaling parameters (assigned or sampled)
#         lnQCC
#         lnVPRC
#         lnDRespC
#         QFatC
#         QGutC
#         QLivC
#         QSlwC
#         QKidC
#         FracPlasC
#         VFatC
#         VGutC
#         VLivC
#         VRapC
#         VRespLumC
#         VRespEffC
#         VKidC
#         VBldC
#         lnPBC
#         lnPFatC
#         lnPGutC
#         lnPLivC
#         lnPRapC
#         lnPSlwC
#         lnPRespC
#         lnPKidC
#         lnPRBCPlasTCAC
#         lnPBodTCAC
#         lnPLivTCAC
#         lnkDissocC
#         lnBMaxkDC
#         lnPBodTCOHC
#         lnPLivTCOHC
#         lnPBodTCOGC
#         lnPLivTCOGC
#         lnPeffDCVG
#         lnkTSD
#         lnkAS
#         lnkTD
#         lnkAD
#         lnkASTCA
#         lnkASTCOH
#         lnVMAXC
#         lnKMC
#         lnClC
#         lnFracOtherC
#         lnFracTCAC
```

```
#         lnVMAXDCVGC
#         lnClDCVGC
#         lnKMDCVGC
#         lnVMAXKidDCVGC
#         lnClKidDCVGC
#         lnKMKidDCVGC
#         lnVMAXLungLivC
#         lnKMClara
#         lnFracLungSysC
#         lnVMAXTCOHC
#         lnClTCOHC
#         lnKMTCOH
#         lnVMAXGlucC
#         lnClGlucC
#         lnKMGluc
#         lnkMetTCOHC
#         lnkUrnTCAC
#         lnkMetTCAC
#         lnkBileC
#         lnkEHRC
#         lnkUrnTCOGC
#         lnFracKidDCVCC
#         lnkDCVGC
#         lnkNATC
#         lnkKidBioactC
#         NRodents
#         VChC
#         lnkLossC
# Input parameters
#         none
# Notes:
#*****************************************************************************
          # use measured value of > 0, otherwise use 0.03 for mouse,
          #         0.3 for rat, 60 for female human, 70 for male human
          BW = (BWmeas > 0.0 ? BWmeas : (Species == 3 ? 0.03 : (Species == 2 ? 0.3 :
(Male == 0 ? 60.0 : 70.0) )));

          BW75 = pow(BW, 0.75);
          BW25 = pow(BW, 0.25);

# Cardiac Output and alveolar ventilation (L/hr)
          QC = exp(lnQCC) * BW75 *      # Mouse, Rat, Human (default)
                    (Species == 3 ? 11.6 : (Species == 2 ? 13.3 : 16.0 ));
          # Mouse: CO=13.98 +/- 2.85 ml/min, BW=30 g (Brown et al. 1997, Tab. 22)
          #         Uncertainty CV is 0.20
          # Rat: CO=110.4 ml/min +/- 15.6, BW=396 g (Brown et al. 1997, Tab. 22,
          #         p 441).  Uncertainty CV is 0.14.
          # Human: Average of Male CO=6.5 l/min, BW=73 kg
          #         and female CO= 5.9 l/min, BW=60 kg (ICRP #89, sitting at rest)
          #         From Price et al. 2003, estimates of human perfusion rate were
          #         4.7~6.5 for females and 5.5~7.1 l/min for males (note
          #         portal blood was double-counted, and subtracted off here)
          #         Thus for uncertainty use CV of 0.2, truncated at 4xCV
          #         Variability from Price et al. (2003) had CV of 0.14~0.20,
          #         so use 0.2 as central estimate
          VPR = exp(lnVPRC)*
                    (Species == 3 ? 2.5 : (Species == 2 ? 1.9 : 0.96 ));
          # Mouse: QP/BW=116.5 ml/min/100 g (Brown et al. 1997, Tab. 31), VPR=2.5
          #         Assume uncertainty CV of 0.2 similar to QC, truncated at 4xCV
          #         Consistent with range of QP in Tab. 31
          # Rat: QP/BW=52.9 ml/min/100 g (Brown et al. 1997, Tab. 31), VPR=1.9
          #         Assume uncertainty CV of 0.3 similar to QC, truncated at 4xCV
          #         Used larger CV because Tab. 31 shows a very large range of QP
          # Human: Average of Male VE=9 l/min, resp. rate=12 /min,
          #         dead space=0.15 l (QP=7.2 l/min), and Female
          #         VE=6.5 l/min, resp. rate=14 /min, dead space=0.12 l
          #         (QP=4.8 l/min), VPR = 0.96
          #         Assume uncertainty CV of 0.2 similar to QC, truncated at 4xCV
          #         Consistent with range of QP in Tab. 31
          QPsamp = QC*VPR;

#         Respiratory diffusion flow rate
#         Will be scaled by QP in dynamics
#         Use log-uniform distribution from 1e-5 to 10
          DResptmp = exp(lnDRespC);

# Fractional Flows scaled to the appropriate species
# Fat = Adipose only
# Gut = GI tract + pancreas + spleen (all drain to portal vein)
# Liv = Liver, hepatic artery
# Slw = Muscle + Skin
# Kid = Kidney
# Rap = Rapidly perfused (rest of organs, plus bone marrow, lymph, etc.),
#         derived by difference in dynamics
#
# Mouse and rat data from Brown et al. (1997).  Human data from
#         ICRP-89 (2002), and is sex-specific.

          QFatCtmp = QFatC*
          (Species == 3 ? 0.07 : (Species == 2 ? 0.07 : (Male == 0 ? 0.085 : 0.05)
));
          QGutCtmp = QGutC*
          (Species == 3 ? 0.141 : (Species == 2 ? 0.153 : (Male == 0 ? 0.21 : 0.19)
));
          QLivCtmp = QLivC*
          (Species == 3 ? 0.02 : (Species == 2 ? 0.021 : 0.065 ));
          QSlwCtmp = QSlwC*
          (Species == 3 ? 0.217 : (Species == 2 ? 0.336 : (Male == 0 ? 0.17 : 0.22)
));
          QKidCtmp = QKidC*
                    (Species == 3 ? 0.091 : (Species == 2 ? 0.141 : (Male == 0 ?
0.17 : 0.19) ));

# Plasma Flows to Tissues (L/hr)
## Mice and rats from Hejtmancik et al. 2002,
##        control F344 rats and B6C3F1 mice at 19 weeks of age
## However, there appear to be significant strain differences in rodents, so
##        assume uncertainty CV=0.2 and variability CV=0.2.
## Human central estimate from ICRP.  Well measured in humans, from Price et al.,
##        human SD in hematocrit was 0.029 in females, 0.027 in males,
##        corresponding to FracPlas CV of 0.047 in females and
##        0.048 in males.  Use rounded CV = 0.05 for both uncertainty and
variability
## Use measured 1-hematocrit if available
```

```
## Truncate distributions at 3xCV to encompass clinical "normal range"
         FracPlas = (Hematocritmeas > 0.0 ? (1-Hematocritmeas) : (FracPlasC *
         (Species == 3 ? 0.52 : (Species == 2 ? 0.53 : (Male == 0 ? 0.615 :
0.567)))));

# Tissue Volumes (L)
# Fat = Adipose only
# Gut = GI tract (not contents) + pancreas + spleen (all drain to portal vein)
# Liv = Liver
# Rap = Brain + Heart + (Lungs-TB) + Bone marrow + "Rest of the body"
# VResp = Tracheobroncial region (trachea+broncial basal+
#                   broncial secretory+bronchiolar)
# Kid = Kidney
# Bld = Blood
# Slw = Muscle + Skin, derived by difference
# residual (assumed unperfused) = (Bone-Marrow)+GI contents+other
#
# Mouse and rat data from Brown et al. (1997).  Human data from
#         ICRP-89 (2002), and is sex-specific.

       VFat = BW * (VFatCmeas > 0.0 ? VFatCmeas : (VFatC * (Species == 3 ? 0.07 :
(Species == 2 ? 0.07 : (Male == 0 ? 0.317 : 0.199)  ))));
       VGut = VGutC * BW *
         (Species == 3 ? 0.049 : (Species == 2 ? 0.032 : (Male == 0 ? 0.022 :
0.020) ));
       VLiv = VLivC * BW *
         (Species == 3 ? 0.055 : (Species == 2 ? 0.034 : (Male == 0 ? 0.023 :
0.025) ));
       VRap = VRapC * BW *
         (Species == 3 ? 0.100 : (Species == 2 ? 0.088 : (Male == 0 ? 0.093 :
0.088) ));
         VRespLum = VRespLumC * BW *
         (Species == 3 ? (0.00014/0.03) : (Species == 2 ? (0.0014/0.3) : (0.167/70)
)); # Lumenal volumes from Styrene model (Sarangapani et al. 2002)
         VRespEfftmp = VRespEffC * BW *
         (Species == 3 ? 0.0007 : (Species == 2 ? 0.0005 : 0.00018 ));
         # Respiratory tract volume is TB region
         # will be multiplied by partition coef. below
         VKid = VKidC * BW *
         (Species == 3 ? 0.017 : (Species == 2 ? 0.007 : (Male == 0 ? 0.0046 :
0.0043) ));
       VBld = VBldC * BW *
         (Species == 3 ? 0.049 : (Species == 2 ? 0.074 : (Male == 0 ? 0.068 :
0.077) ));
       VSlw = (Species == 3 ? 0.8897 : (Species == 2 ? 0.8995 : (Male == 0 ?
0.85778 : 0.856))) * BW
                  - VFat - VGut - VLiv - VRap - VRespEfftmp - VKid - VBld;
# Slowly perfused:
# Baseline mouse: 0.8897-0.049-0.017-0.0007-0.1-0.055-0.049-0.07= 0.549
# Baseline rat: 0.8995 -0.074-0.007-0.0005-0.088-0.034-0.032-0.07= 0.594
# Baseline human F: 0.85778-0.068-0.0046-0.00018-0.093-0.023-0.022-0.317= 0.33
# Baseline human M: 0.856-0.077-0.0043-0.00018-0.088-0.025-0.02-0.199= 0.4425

      VPlas = FracPlas * VBld;
         VBod = VFat + VGut + VRap + VRespEfftmp + VKid + VSlw; # For TCA
         VBodTCOH = VBod + VBld;       # for TCOH and TCOG -- body without liver

# Partition coefficients
      PB = (PBmeas > 0.0 ? PBmeas : (exp(lnPBC) * (Species == 3 ? 15. : (Species ==
2 ? 22. : 9.5 )))); # Blood-air
         # Mice: pooling Abbas and Fisher 1997, Fisher et al. 1991
         #         each a single measurement, with overall CV = 0.07.
         #         Given small number of measurements, and variability
         #         in rat, use CV of 0.25 for uncertainty and variability.
         # Rats: pooling Sato et al. 1977, Gargas et al. 1989,
         #         Barton et al. 1995, Simmons et al. 2002, Koizumi 1989,
         #         Fisher et al. 1989.  Fisher et al. measurement substantially
         #         smaller than others (15 vs. 21~26).  Recent article
         #         by Rodriguez et al. 2007 shows significant change with
         #         age (13.1 at PND10, 17.5 at adult, 21.8 at aged), also seems
         #         to favor lower values than previously reported.  Therefore
         #         use CV = 0.25 for uncertainty and variability.
         # Humans: pooling Sato and Nakajima 1979, Sato et al. 1977,
         #         Gargas et al. 1989, Fiserova-Bergerova et al. 1984,
         #         Fisher et al. 1998, Koizumi 1989
         #         Overall variability CV = 0.185.  Consistent with
         #         within study inter-individual variability CV = 0.07~0.22.
         #         Study-to-study, sex-specific means range 8.1~11, so
         #         uncertainty CV = 0.2.
      PFat = exp(lnPFatC) *           # Fat/blood
                  (Species == 3 ? 36. : (Species == 2 ? 27. : 67. ));
         # Mice: Abbas and Fisher 1997.  Single measurement.  Use
         #         rat uncertainty of CV = 0.3.
         # Rats: Pooling Barton et al. 1995, Sato et al. 1977,
         #         Fisher et al. 1989.  Recent article by Rodriguez et al.
         #         (2007) shows higher value of 36., so assume uncertainty
         #         CV of 0.3.
         # Humans: Pooling Fiserova-Bergerova et al. 1984, Fisher et al. 1998,
         #         Sato et al. 1977.  Variability in Fat:Air has CV = 0.07.
         #         For uncertainty, dominated by PB uncertainty CV = 0.2
         #         For variability, add CVs in quadrature for
         #         sqrt(0.07^2+0.185^2)=0.20
      PGut = exp(lnPGutC) *           # Gut/blood
                  (Species == 3 ? 1.9 : (Species == 2 ? 1.4 : 2.6 ));
         # Mice: Geometric mean of liver, kidney
         # Rats: Geometric mean of liver, kidney
         # Humans: Geometric mean of liver, kidney
         #         Uncertainty of CV = 0.4 due to tissue extrapolation
      PLiv = exp(lnPLivC) *           # Liver/blood
                  (Species == 3 ? 1.7 : (Species == 2 ? 1.5 : 4.1 ));
         # Mice: Fisher et al. 1991, single datum, so assumed uncert CV = 0.4
         # Rats: Pooling Barton et al. 1995, Sato et al. 1977,
         #         Fisher et al. 1989, with little variation (range 1.3~1.7).
         #         Recent article by Rodriguez et al.reports 1.34.  Use
         #         uncertainty CV = 0.15.
         # Humans: Pooling Fiserova-Bergerova et al. 1984, Fisher et al. 1998
         #         almost 2-fold difference in Liver:Air values, so uncertainty
         #         CV = 0.4
      PRap = exp(lnPRapC) *           # Rapidly perfused/blood
                  (Species == 3 ? 1.9 : (Species == 2 ? 1.3 : 2.6 ));
         # Mice: Similar to liver, kidney.  Uncertainty CV = 0.4 due to
         #         tissue extrapolation
         # Rats: Use brain values Sato et al. 1977.  Recent article by
         #         Rodriguez et al. (2007) reports 0.99 for brain.  Uncertainty
```

```
          #         CV of 0.4 due to tissue extrapolation.
          # Humans: Use brain from Fiserova-Bergerova et al. 1984
          #         Uncertainty of CV = 0.4 due to tissue extrapolation
        PResp = exp(lnPRespC) *                    # Resp/blood =
                  (Species == 3 ? 2.6 : (Species == 2 ? 1.0 : 1.3 ));
          # Mice: Abbas and Fisher 1997, single datum, so assumed uncert CV = 0.4
          # Rats: Sato et al. 1977, single datum, so assumed uncert CV = 0.4
          # Humans: Pooling Fiserova-Bergerova et al. 1984, Fisher et al. 1998
          #         > 2-fold difference in lung:air values, so uncertainty
          #         CV = 0.4
        VRespEff = VRespEfftmp * PResp * PB; # Effective air volume
        PKid = exp(lnPKidC) *            # Slowly perfused/blood
                  (Species == 3 ? 2.1 : (Species == 2 ? 1.3 : 1.6 ));
          # Mice: Abbas and Fisher 1997, single datum, so assumed uncert CV = 0.4
          # Rats: Pooling Barton et al. 1995, Sato et al. 1977.  Recent article
          #         by Rodriguez et al. (2007) reports 1.01, so use uncertainty
          #         CV of 0.3.  Pooled variability CV = 0.39.
          # Humans: Pooling Fiserova-Bergerova et al. 1984, Fisher et al. 1998
          #         For uncertainty, dominated by PB uncertainty CV = 0.2
          #         Variability in kidney:air CV = 0.23, so add to PB variability
          #         in quadrature       sqrt(0.23^2+0.185^2)=0.30
        PSlw = exp(lnPSlwC) *            # Slowly perfused/blood
                  (Species == 3 ? 2.4 : (Species == 2 ? 0.58 : 2.1 ));
          # Mice: Muscle - Abbas and Fisher 1997, single datum, so assumed
          #         uncert CV = 0.4
          # Rats: Pooling Barton et al. 1995, Sato et al. 1977,
          #         Fisher et al. 1989.  Recent article by Rodriguez et al. (2007)
          #         reported 0.72, so use uncertainty CV of 0.25.  Variability
          #         in Muscle:air and muscle:blood ~ CV = 0.3
          # Humans: Pooling Fiserova-Bergerova et al. 1984, Fisher et al. 1998
          #         Range of values 1.4~2.4, so uncertainty CV = 0.3
          #         Variability in muscle:air CV = 0.3, so add to PB variability
          #         in quadrature sqrt(0.3^2+0.185^2)=0.35

# TCA partitioning
    TCAPlas = FracPlas + (1 - FracPlas) * 0.5 * exp(lnPRBCPlasTCAC);
          #         Blood/Plasma concentration ratio.  Note dependence
          #         on fraction of blood that is plasma.  Here
          #         exp(lnPRBCPlasTCA) = partition coefficient
          #                   C(blood minus plasma)/C(plasma)
          #         Default of 0.5, corresponding to Blood/Plasma
          #                   concentration ratio of 0.76 in
          #                   rats (Schultz et al 1999)
          #         For rats, Normal uncertainty with GSD = 1.4
          #         For mice and humans, diffuse prior uncertainty of
          #         100-fold up/down
    PBodTCA = TCAPlas * exp(lnPBodTCAC) *
                  (Species == 3 ? 0.88 : (Species == 2 ? 0.88 : 0.52 ));
          # Note -- these were done at 10~20 microg/ml (Abbas and Fisher 1997),
          #         which is 1.635-3.27 mmol/ml (1.635-3.27 x 10^6 microM).
          #         At this high concentration, plasma binding should be
          #         saturated -- e.g., plasma albumin concentration was
          #         measured to be P=190-239 microM in mouse, rat, and human
          #         plasma by Lumpkin et al. 2003, or > 6800 molecules of
          #         TCA per molecule of albumin.  So the measured partition
          #         coefficients should reflect free blood-tissue partitioning.
          # Used muscle values, multiplied by blood:plasma ratio to get
          #         Body:Plasma partition coefficient
          # Rats = mice from Abbas and Fisher 1997
          # Humans from Fisher et al. 1998
          #         Uncertainty in mice, humans GSD = 1.4
          #         For rats, GSD = 2.0, based on difference between mice
          #         and humans.
        PLivTCA = TCAPlas * exp(lnPLivTCAC) *
                  (Species == 3 ? 1.18 : (Species == 2 ? 1.18 : 0.66 ));
          # Multiplied by blood:plasma ratio to get Liver:Plasma
          # Rats = mice from Abbas and Fisher 1997
          # Humans from Fisher et al. 1998
          #         Uncertainty in mice, humans GSD = 1.4
          #         For rats, GSD = 2.0, based on difference between mice
          #         and humans.

# Binding Parameters for TCA
          # GM of Lumpkin et al. 2003; Schultz et al. 1999;
          #         Templin et al. 1993, 1995; Yu et al. 2000
          # Protein/TCA dissociation constant (umole/L)
          #         note - GSD = 3.29, 1.84, and 1.062 for mouse, rat, human
          kDissoc = exp(lnkDissocC) *
                  (Species == 3 ? 107. : (Species == 2 ? 275. : 182. ));
          # BMax = NSites * Protein concentration.  Sampled parameter is
          #         BMax/kD (determines binding at low concentrations)
          #         note - GSD = 1.64, 1.60, 1.20 for mouse, rat, human
          BMax = kDissoc * exp(lnBMaxkDC) *
                  (Species == 3 ? 0.88 : (Species == 2 ? 1.22 : 4.62 ));

# TCOH partitioning
          # Data from Abbas and Fisher 1997 (mouse) and Fisher et al.
          #         1998 (human).  For rat, used mouse values.
          #         Uncertainty in mice, humans GSD = 1.4
          #         For rats, GSD = 2.0, based on difference between mice
          #         and humans.

        PBodTCOH = exp(lnPBodTCOHC) *
                  (Species == 3 ? 1.11 : (Species == 2 ? 1.11 : 0.91 ));
        PLivTCOH = exp(lnPLivTCOHC) *
                  (Species == 3 ? 1.3 : (Species == 2 ? 1.3 : 0.59 ));

# TCOG partitioning
          # Use TCOH as a proxy, but uncertainty much greater
          # (e.g., use uniform prior, 100-fold up/down)
        PBodTCOG = exp(lnPBodTCOGC) *
                  (Species == 3 ? 1.11 : (Species == 2 ? 1.11 : 0.91 ));
        PLivTCOG = exp(lnPLivTCOGC) *
                  (Species == 3 ? 1.3 : (Species == 2 ? 1.3 : 0.59 ));

# DCVG distribution volume
          # exp(lnPeffDCVG) is the effective partition coefficient for
          # the "body" (non-blood) compartment
          # Diffuse prior distribution: loguniform 1e-3 to 1e3
          VDCVG = VBld +      # blood plus body (with "effective" PC)
          exp(lnPeffDCVG) * (VBod + VLiv);

# Absorption Rate Constants (/hr)
          # All priors are diffuse (log)uniform distributions
```

```
          # transfer from stomach centered on 1.4/hr, range up or down 100-fold,
          #         based on human stomach half-time of 0.5 hr.
        kTSD = exp(lnkTSD);
          # stomach absorption centered on 1.4/hr, range up or down 1000-fold
          kAS = exp(lnkAS);
          # assume no fecal excretion -- 100% absorption
          kTD = 0.0 * exp(lnkTD);
          # intestinal absorption centered on 0.75/hr, range up or down
          #         1000-fold, based on human transit time of small intestine
          #         of 4 hr   (95% throughput in 4 hr)
          kAD = exp(lnkAD);
          kASTCA = exp(lnkASTCA);
          kASTCOH = exp(lnkASTCOH);

# TCE Oxidative Metabolism Constants
# For rodents, in vitro microsomal data define priors (pooled).
# For human, combined in vitro microsomoal+hepatocellular individual data
#         define priors.
# All data from Elfarra et al. 1998; Lipscomb et al. 1997, 1998a,b
# For VMAX, scaling from in vitro data were (Barter et al. 2007):
#         32 mg microsomal protein/g liver
#         99 x 1e6 hepatocytes/g liver
#         Here, human data assumed representative of mouse and rats.
# For KM, two different scaling methods were used for microsomes:
#         Assume microsomal concentration = liver concentration, and
#                 use central estimate of liver:blood PC (see above)
#         Use measured microsome:air partition coefficient (1.78) and
#                 central estimate of blood:air PC (see above)
# For human KM from hepatocytes, used measured human hepatocyte:air
#         partition coefficient (21.62, Lipscomb et al. 1998), and
#         central estimate of blood:air PC.
#         Note that to that the hepatocyte:air PC is similar to that
#         found in liver homogenates (human: 29.4+/-5.1 from Fiserova-
#         Bergerova et al. 1984, and 54 for Fisher et al. 1998; rat:
#         27.2+/-3.4 from Gargas et al. 1989, 62.7 from Koisumi 1989,
#         43.6 from Sato et al. 1977; mouse: 23.2 from Fisher et al. 1991).
# For humans, sampled parameters are VMAX and ClC (VMAX/KM), due to
#         improved convergence.  VMAX is kept as a parameter because it
#         appears less uncertain (i.e., more consistent across microsomal
#         and hepatocyte data).

          # Central estimate of VMAX is 342, 76.2, and 32.3 (micromol/min/
          #         kg liver) for mouse, rat, human.  Converting to /hr by
          #         * (60 min/hr * 0.1314 mg/micromol) gives
          #         2700, 600, and 255 mg/hr/kg liver
          # Observed variability of about 2-fold GSD.  Assume 2-fold GSD for
          #         both uncertainty and variability
        VMAX = VLiv*exp(lnVMAXC)*
                  (Species == 3 ? 2700. : (Species == 2 ? 600. : 255.));

          # For mouse and rat central estimates for KM are 0.068~1.088 and
          #         0.039~0.679 mmol/l in blood, depending on the scaling
          #         method used.  Taking the geometric mean, and converting
          #         to mg/l by 131.4 mg/mmol gives 36. and 21. mg/l in blood.
          # For human, central estimate
          #         for Cl are 0.306~3.95 l/min/kg liver.  Taking the geometric
          #         mean and converting to /hr gives a central estimate of
          #         66. l/hr/kg.
          #         KM is then derived from KM = VMAX/(Cl*Vliv) (central estimate
          #         of
          # Note uncertainty due to scaling is about 4-fold.
          #         Variability is about 3-fold in mice, 1.3-fold in rats, and
          #         2- to 4- fold in humans (depending on scaling).
        KM = (Species == 3 ? 36.*exp(lnKMC) : (Species == 2 ? 21.*exp(lnKMC) :
VMAX/(VLiv*66.*exp(lnClC))));

# Oxidative metabolism splits
          # Fractional split of TCE to DCA
          # exp(lnFracOtherC) = ratio of DCA to non-DCA
          # Diffuse prior distribution: loguniform 1e-4 to 1e2
          FracOther = exp(lnFracOtherC)/(1+exp(lnFracOtherC));
          # Fractional split of TCE to TCA
          # exp(lnFracTCAC) = ratio of TCA to TCOH
          # TCA/TCOH = 0.1 from Lipscomb et al. 1998 using fresh hepatocytes,
          # but TCA/TCOH ~ 1 from Bronley-DeLancey et al 2006
          # GM = 0.32, GSD = 3.2
          FracTCA = 0.32*exp(lnFracTCAC)*(1-FracOther)/(1+0.32*exp(lnFracTCAC));

# TCE GSH Metabolism Constants
# Human in vitro data from Lash et al. 1999, define human priors.
#                             VMAX (nmol/min/     KM (mM)             CLeff (ml/min/
#                                   g tissue)                             g tissue)
#                       ------------------------------------------
#                             [high affinity pathway only]  [total]
# Human liver cytosol:        ~423                0.0055~0.023        21.2~87.0
# Human liver cytosol+        ~211                --                  --
#         microsomes
#                             [total]             [total]             [total]
# Human hepatocytes*          12~30**             0.012~0.039***      0.2~0.5****
# Human kidney cytosol:       81                  0.0164~0.0263       3.08~4.93
#         * estimated visually from Fig 1, Lash et al. 1999
#         ** Fig 1A, data from 50~500 ppm headspace at 60 min
#                 and Fig 1B, data at 100~5000 ppm in headspace for 120 min
#         *** Fig 1B, 30~100 ppm headspace, converted to blood concentration
#                 using blood:air PC of 9.5
#         **** Fig 1A, data at 50 ppm headspace at 120 min and Fig 1B, data at
#                 25 and 50 ppm headspace at 120 min.
# Overall, human liver hepatocytes are probably most like the
#         intact liver (e.g., accounting for the competition between
#         GSH conjugation and oxidation).  So central estimates based
#         on those: CLeff ~ 0.32 ml/min/g tissue, KM ~ 0.022 mM in blood.
#         CLeff converted to 19 l/hr/kg; KM converted to 2.9 mg/l in blood
#         However, uncertainty in CLeff is large (values in cytosol
#         ~100-fold larger).  Moreover, Green et al. 1997 reported
#         DCVG formation in cytosol that was ~30,000-fold smaller
#         than Lash et al. (1998) in cytosol, which would be a VMAX
#         ~300-fold smaller than Lash et al. (1998) in hepatocytes.
#         Uncertainty in KM appears smaller (~4-fold)
#         CLC: GM = 19., GSD = 100; KM: GM = 2.9., GSD = 4.
#         In addition, at a single concentration, the variability
#         in human liver cytosol samples had a GSD=1.3.
# For the human kidney, the kidney cytosol values are used, with the same
#         uncertainty as for the liver.  Note that the DCVG formation rates
#         in rat kidney cortical cells and rat cytosol are quite similar
```

```
#         (see below).
#         CLC: GM = 230., GSD = 100; KM: GM = 2.7., GSD = 4.
# Rat and mouse in vitro data from Lash et al. 1995,1998 define rat and mouse
#         priors.  However, rats and mice are only assayed at 1 and 2 mM
#         providing only a bound on VMAX and very little data on KM.
#                             Rate at 2 mM        Equivalent          CLeff
#                                                 blood conc.         at 2 mM
#                             (nmol/min/          (mM)                (ml/min/
#                             g tissue)                       g tissue)
#                        ---------------------------------------------
# Rat     hepatocytes:        4.4~16              2.0                 0.0022~0.0079
#         liver cytosol:      8.0~12              1.7~2.0             0.0040~0.0072
#         kidney cells:       0.79~1.1  2.2                 0.00036~0.00049
#         kidney cytosol:     0.53~0.75 1.1~2.0             0.00027~0.00068
# Mouse   liver cytosol:      36~40               1.1~2.0             0.018~0.036
#         kidney cytosol:     6.2~9.3             0.91~2.0  0.0031~0.0102
#
# In most cases, rates were increased over the same sex/species at 1 mM,
#         indicating VMAX has not yet been reached.  The values between cells
#         and cytosol are more much consistent that in the human data.
#         These data therefore put a lower bound on VMAX and a lower bound
#         on CLC.  To account for in vitro-in vivo uncertainty, the lower
#         bound of the prior distribution is set 100-fold below the central
#         estimate of the measurements here.  In addition, Green et al.
#         (1997) found values 100-fold smaller than Lash et al. 1995, 1998.
#         Therefore diffuse prior distributions set to 1e-2~1e4.
# Rat liver: Bound on VMAX of 4.4~16, with GM of 8.4.  Converting to
#         mg/hr/kg tissue (* 131.4 ng/nmol * 60 min/hr * 1e3 g/kg / 1e6 mg/ng)
#         gives a central estimate of 66. mg/hr/kg tissue.  Bound on CL of
#         0.0022~0.0079, with GM of 0.0042.  Converting to l/hr/kg tissue
#         (* 60 min/hr) gives 0.25 l/hr/kg tissue.
# Rat kidney: Bound on VMAX of 0.53~1.1, with GM of 0.76.  Converting
#         to mg/hr/kg tissue gives a central estimate of 6.0 mg/hr/kg.
#         Bound on CL of 0.00027~0.00068, with GM of 0.00043.  Converting
#         to l/hr/kg tissue gives 0.026 l/hr/kg tissue.
# Mouse liver: Bound on VMAX of 36~40, with GM of 38.  Converting
#         to mg/hr/kg tissue gives a central estimate of 300. mg/hr/kg.
#         Bound on CL of 0.018~0.036, with GM of 0.025.  Converting
#         to l/hr/kg tissue gives 1.53 l/hr/kg tissue.
# Mouse kidney: Bound on VMAX of 6.2~9.3, with GM of 7.6.  Converting
#         to mg/hr/kg tissue gives a central estimate of 60. mg/hr/kg.
#         Bound on CL of 0.0031~0.0102, with GM of 0.0056.  Converting
#         to l/hr/kg tissue gives 0.34 l/hr/kg tissue.

         VMAXDCVG = VLiv*(Species == 3 ? (300.*exp(lnVMAXDCVGC)) : (Species == 2 ?
(66.*exp(lnVMAXDCVGC)) : (2.9*19.*exp(lnClDCVGC+lnKMDCVGC))));
        KMDCVG = (Species == 3 ? (VMAXDCVG/(VLiv*1.53*exp(lnClDCVGC))) : (Species ==
2 ? (VMAXDCVG/(VLiv*0.25*exp(lnClDCVGC))) : 2.9*exp(lnKMDCVGC)));
         VMAXKidDCVG = VKid*(Species == 3 ? (60.*exp(lnVMAXKidDCVGC)) : (Species ==
2 ? (6.0*exp(lnVMAXKidDCVGC)) : (2.7*230.*exp(lnClKidDCVGC+lnKMKidDCVGC))));
        KMKidDCVG = (Species == 3 ? (VMAXKidDCVG/(VKid*0.34*exp(lnClKidDCVGC))) :
(Species == 2 ? (VMAXKidDCVG/(VKid*0.026*exp(lnClKidDCVGC))) :
2.7*exp(lnKMKidDCVGC)));

# TCE Metabolism Constants for Chloral Kinetics in Lung (mg/hr)
#         Scaled to liver VMAX using data from Green et al. (1997)
#         in microsomal preparations (nmol/min/mg protein) at ~1 mM.
#         For humans, used detection limit of 0.03
#         Additional scaling by lung/liver weight ratio
#         from Brown et al. Table 21 (mouse and rat) or
#         ICRP Pub 89 Table 2.8 (Human female and male)
#         Uncertainty ~ 3-fold truncated at 3 GSD
   VMAXClara = exp(lnVMAXLungLivC) * VMAX *
         (Species == 3 ? (1.03/1.87*0.7/5.5):(Species == 2 ?
(0.08/0.82*0.5/3.4):(0.03/0.33*(Male == 0 ? (0.42/1.4) : (0.5/1.8)))));
   KMClara = exp(lnKMClara);
# Fraction of Respiratory Metabolism that goes to system circulation
# (translocated to the liver)
   FracLungSys = exp(lnFracLungSysC)/(1 + exp(lnFracLungSysC));

# TCOH Metabolism Constants (mg/hr)
         # No in vitro data.  So use diffuse priors of
         #         1e-4 to 1e4 mg/hr/kg^0.75 for VMAX
         #                  (4e-5 to 4000 mg/hr for rat),
         #         1e-4 to 1e4 mg/l for KM,
         #         and 1e-5 to 1e3 l/hr/kg^0.75 for Cl
         #                  (2e-4 to 2.4e4 l/hr for human)
         VMAXTCOH = BW75*
                  (Species == 3 ? (exp(lnVMAXTCOHC)) : (Species == 2 ?
(exp(lnVMAXTCOHC)) : (exp(lnClTCOHC+lnKMTCOH))));
         KMTCOH = exp(lnKMTCOH);
         VMAXGluc = BW75*
                  (Species == 3 ? (exp(lnVMAXGlucC)) : (Species == 2 ?
(exp(lnVMAXGlucC)) : (exp(lnClGlucC+lnKMGluc))));
         KMGluc = exp(lnKMGluc);
         # No in vitro data.  So use diffuse priors of
         #         1e-5 to 1e3 kg^0.25/hr (3.5e-6/hr to 3.5e2/hr for human)
         kMetTCOH = exp(lnkMetTCOHC) / BW25;

# TCA kinetic parameters
         # Central estimate based on GFR clearance per unit body weight
         #         10.0, 8.7, 1.8 ml/min/kg for mouse, rat, human
         #         (= 0.6, 0.522, 0.108 l/hr/kg) from Lin 1995.
         #         = CL_GFR / BW (BW=0.02 for mouse, 0.265 for rat, 70 for human)
         #         kUrn = CL_GFR / VPlas
         #         Diffuse prior with uncertainty of up,down 100-fold
         kUrnTCA = exp(lnkUrnTCAC) * BW / VPlas *
                  (Species == 3 ? 0.6 : (Species == 2 ? 0.522 : 0.108));
         # No in vitro data.  So use diffuse priors of
         #         1e-4 to 1e2 /hr/kg^0.25 (0.3/hr to 35/hr for human)
         kMetTCA = exp(lnkMetTCAC) / BW25;

# TCOG kinetic parameters
         # No in vitro data.  So use diffuse priors of
         #         1e-4 to 1e2 /hr/kg^0.25 (0.3/hr to 35/hr for human)
         kBile = exp(lnkBileC) / BW25;
        kEHR = exp(lnkEHRC) / BW25;
         # Central estimate based on GFR clearance per unit body weight
         #         10.0, 8.7, 1.8 ml/min/kg for mouse, rat, human
         #         (= 0.6, 0.522, 0.108 l/hr/kg) from Lin 1995.
         #         = CL_GFR / BW (BW=0.02 for mouse, 0.265 for rat, 70 for human)
         #         kUrn = CL_GFR / VBld
         #         Diffuse prior with Uncertainty of up,down 1000-fold
         kUrnTCOG = exp(lnkUrnTCOGC) * BW / (VBodTCOH * PBodTCOG) *
```

```
                  (Species == 3 ? 0.6 : (Species == 2 ? 0.522 : 0.108));

# DCVG Kinetics (/hr)
          # Fraction of renal TCE GSH conj. "directly" to DCVC via "first pass"
          # exp(lnFracOtherCC) = ratio of direct/non-direct
          # Diffuse prior distribution: loguniform 1e-3 to 1e3
          # FIXED in v1.2.3
          # In ".in" files, set to 1, so that all kidney GSH conjugation
          # is assumed to directly produce DCVC (model lacks identifiability
          # otherwise).
          FracKidDCVC = exp(lnFracKidDCVCC)/(1 + exp(lnFracKidDCVCC));
          # No in vitro data.  So use diffuse priors of
          #         1e-4 to 1e2 /hr/kg^0.25 (0.3/hr to 35/hr for human)
          kDCVG = exp(lnkDCVGC) / BW25;

# DCVC Kinetics in Kidney (/hr)
          # No in vitro data.  So use diffuse priors of
          #         1e-4 to 1e2 /hr/kg^0.25 (0.3/hr to 35/hr for human)
          kNAT = exp(lnkNATC) / BW25;
          kKidBioact = exp(lnkKidBioactC) / BW25;

# CC data initialization
          Rodents = (CC > 0 ? NRodents : 0.0); # Closed chamber simulation
          VCh = (CC > 0 ? VChC - (Rodents * BW) : 1.0);
                    # Calculate net chamber volume
          kLoss = (CC > 0 ? exp(lnkLossC) : 0.0);

#*****************************************************************************
#***                   State Variable Initialization and Scaling             ***
#*****************************************************************************
# NOTE: All State Variables are automatically set to 0 initially,
# unless re-initialized here

          ACh = (CC * VCh * MWTCE) / 24450.0;    # Initial amount in chamber

};
###################### End of Initialization ########################

Dynamics{

#*****************************************************************************
#***                        Dynamic physiological parameter scaling          ***
#*****************************************************************************
# State Variables with dynamics:
#         none
# Input Variables:
#         QPmeas
# Other State Variables and Global Parameters:
#         QC
#         VPR
#         DResptmp
#         QPsamp
#         QFatCtmp
#         QGutCtmp
#         QLivCtmp
#         QSlwCtmp
#         QKidCtmp
#         FracPlas
# Temporary variables used:
#         none
# Temporary variables assigned:
#         QP
#         DResp
#         QCnow
#         QFat
#         QGut
#         QLiv
#         QSlw
#         QKid
#         QGutLiv
#         QRap
#         QCPlas
#         QBodPlas
#         QGutLivPlas
# Notes:
#*****************************************************************************

# QP uses QPmeas if value is > 0, otherwise uses sampled value
          QP = (QPmeas > 0 ? QPmeas : QPsamp);
          DResp = DResptmp * QP;

# QCnow uses QPmeas/VPR if QPmeas > 0, otherwise uses sampled value
          QCnow = (QPmeas > 0 ? QPmeas/VPR : QC);

# These done here in dynamics in case QCnow changes
# Blood Flows to Tissues (L/hr)
        QFat = (QFatCtmp) * QCnow; #
        QGut = (QGutCtmp) * QCnow; #
        QLiv = (QLivCtmp) * QCnow; #
        QSlw = (QSlwCtmp) * QCnow; #

        QKid = (QKidCtmp) * QCnow; #
     QGutLiv = QGut + QLiv; #
          QRap = QCnow - QFat - QGut - QLiv - QSlw - QKid;
          QRapCtmp = QRap/QCnow; #(vrisk)
          QBod = QCnow - QGutLiv;

# Plasma Flows to Tissues (L/hr)
      QCPlas = FracPlas * QCnow; #
    QBodPlas = FracPlas * QBod; #
    QGutLivPlas = FracPlas * QGutLiv; #

#*****************************************************************************
#***                  Exposure and Absorption calculations                 ***
#*****************************************************************************
# State Variables with dynamics:
#         AStom
#         ADuod
#         AStomTCA
#         AStomTCOH
# Input Variables:
#         IVDose
#         PDose
#         Drink
```

```
#         Conc
#         IVDoseTCA
#         PODoseTCA
#         IVDoseTCOH
#         PODoseTCOH
# Other State Variables and Global Parameters:
#         ACh
#         CC
#         VCh
#         MWTCE
#         BW
#         TChng
#         kAS
#         kTSD
#         kAD
#         kTD
#         kASTCA
#         kASTCOH
# Temporary variables used:
#         none
# Temporary variables assigned:
#         kIV - rate into CVen
#         kIA - rate into CArt
#         kPV - rate into portal vein
#         kStom - rate into stomach
#         kDrink - incorporated into RAO
#         RAO - rate into gut (oral absorption - both gavage and drinking water)
#         CInh - inhalation exposure concentration
#         kIVTCA - rate into blood
#         kStomTCA - rate into stomach
#         kPOTCA - rate into liver (oral absorption)
#         kIVTCOH - rate into blood
#         kStomTCOH - rate into stomach
#         kPOTCOH - rate into liver (oral absorption)
# Notes:
# For oral dosing, using "Spikes" for instantaneous inputs
# Inhalation Concentration (mg/L)
#         CInh uses Conc when open chamber (CC=0) and
#         ACh/VCh when closed chamber CC>0.
#*******************************************************************************

#### TCE DOSING
## IV route
    kIV = (IVDose * BW) / TChng;# IV infusion rate (mg/hr)
                                    # (IVDose constant for duration TChng)
    kIA = (IADose * BW) / TChng;        # IA infusion rate (mg/hr)
    kPV = (PVDose * BW) / TChng;        # PV infusion rate (mg/hr)
    kStom = (PDose * BW) / TChng;# PO dose rate (into stomach) (mg/hr)

## Oral route
# Amount of TCE in stomach -- for oral dosing only (mg)
    dt(AStom) = kStom - AStom * (kAS + kTSD);

# Amount of TCE in duodenum -- for oral dosing only (mg)
    dt(ADuod) = (kTSD * AStom) - (kAD + kTD) * ADuod;
# Rate of absorption from drinking water
    kDrink = (Drink * BW) / 24.0; #Ingestion rate via drinking water (mg/hr)

# Total rate of absorption including gavage and drinking water
    RAO = kDrink + (kAS * AStom) + (kAD * ADuod);
## Inhalation route
    CInh = (CC > 0 ? ACh/VCh : Conc*MWTCE/24450.0); # in mg/l

#### TCA Dosing
          kIVTCA = (IVDoseTCA * BW) / TChng;  # TCA IV infusion rate (mg/hr)
          kStomTCA = (PODoseTCA * BW) / TChng; # TCA PO dose rate into stomach
          dt(AStomTCA) = kStomTCA - AStomTCA * kASTCA;
          kPOTCA = AStomTCA * kASTCA;  # TCA oral absorption rate (mg/hr)

#### TCOH Dosing
          kIVTCOH = (IVDoseTCOH * BW) / TChng;#TCOH IV infusion rate (mg/hr)
          kStomTCOH = (PODoseTCOH * BW) / TChng; # TCOH PO dose rate into stomach
    dt(AStomTCOH) = kStomTCOH - AStomTCOH * kASTCOH;
          kPOTCOH = AStomTCOH * kASTCOH;# TCOH oral absorption rate (mg/hr)

#*******************************************************************************
#***                         TCE Model                                       ***
#*******************************************************************************
# State Variables with dynamics:
#         ARap,               # Amount in rapidly perfused tissues
#         ASlw,               # Amount in slowly perfused tissues
#         AFat,               # Amount in fat
#         AGut,               # Amount in gut
#         ALiv,               # Amount in liver
#         AInhResp,
#         AResp,
#         AExhResp,
#         AKid,               # Amount in Kidney -- currently in Rap tissue
#         ABld,               # Amount in Blood -- currently in Rap tissue
#         ACh,                # Amount of TCE in closed chamber
# Input Variables:
#         none
# Other State Variables and Global Parameters:
#         VRap
#         PRap
#         VSlw
#         PSlw
#         VFat
#         PFat
#         VGut
#         PGut
#         VLiv
#         PLiv
#         VRespLum
#         VRespEff
#         FracLungSys
#         VKid
#         PKid
#         VBld
#         VMAXClara
#         KMClara
#         PB
#         Rodents
#         VCh
#         kLoss
```

```
#         VMAX
#         KM
#         VMAXDCVG
#         KMDCVG
#         VMAXKidDCVG
#         KMKidDCVG
# Temporary variables used:
#         QM
#         QFat
#         QGutLiv
#         QSlw
#         QRap
#         QKid
#         kIV
#         QCnow
#         CInh
#         QP
#         RAO
# Temporary variables assigned:
#         QM
#         CRap
#         CSlw
#         CFat
#         CGut
#         CLiv
#         CInhResp
#         CResp
#         CExhResp
#         ExhFactor
#         CMixExh
#         CKid
#         CVRap
#         CVSlw
#         CVFat
#         CVGut
#         CVLiv
#         CVTB
#         CVKid
#         CVen
#         RAMetLng
#         CArt_tmp
#         CArt
#         CAlv
#         RAMetLiv1
#         RAMetLiv2
#         RAMetKid
# Notes:
#*******************************************************************************
#

#****Blood (venous)*************************************************************
# Tissue Concentrations (mg/L)
          CRap = ARap/VRap;
          CSlw = ASlw/VSlw;
          CFat = AFat/VFat;
          CGut = AGut/VGut;
          CLiv = ALiv/VLiv;
          CKid = AKid/VKid;
# Venous Concentrations (mg/L)
          CVRap = CRap / PRap;
          CVSlw = CSlw / PSlw;
          CVFat = CFat / PFat;
          CVGut = CGut / PGut;
          CVLiv = CLiv / PLiv;
          CVKid = CKid / PKid;
# Concentration of TCE in mixed venous blood (mg/L)
          CVen = ABld/VBld;
# Dynamics for blood
    dt(ABld) = (QFat*CVFat + QGutLiv*CVLiv + QSlw*CVSlw +
                    QRap*CVRap + QKid*CVKid + kIV) - CVen * QCnow;

#****Gas exchange and Respiratory Metabolism*********************************
#
    QM = QP/0.7;    # Minute-volume
    CInhResp = AInhResp/VRespLum;
    CResp = AResp/VRespEff;
    CExhResp = AExhResp/VRespLum;
    dt(AInhResp) = (QM*CInh + DResp*(CResp-CInhResp) - QM*CInhResp);
    RAMetLng = VMAXClara * CResp/(KMClara + CResp);
    dt(AResp) = (DResp*(CInhResp + CExhResp - 2*CResp) - RAMetLng);
    CArt_tmp = (QCnow*CVen + QP*CInhResp)/(QCnow + (QP/PB));
    dt(AExhResp) = (QM*(CInhResp-CExhResp) + QP*(CArt_tmp/PB-CInhResp) +
                    DResp*(CResp-CExhResp));
    CMixExh = (CExhResp > 0 ? CExhResp : 1e-15); # mixed exhaled breath

# Concentration in alveolar air (mg/L)
          # Correction factor for exhaled air to account for
          # absorption/desorption/metabolism in respiratory tissue
          # = 1 if DResp = 0
          ExhFactor_den = (QP * CArt_tmp / PB + (QM-QP)*CInhResp);
          ExhFactor = (ExhFactor_den > 0) ? (
                    QM * CMixExh / ExhFactor_den) : 1;
          # End-exhaled breath (corrected for absorption/
          #         desorption/metabolism in respiratory tissue)
          CAlv = CArt_tmp / PB * ExhFactor;
# Concentration in arterial blood entering circulation (mg/L)
          CArt = CArt_tmp + kIA/QCnow;  # add inter-arterial dose

#****Other dynamics for inhalation/exhalation ********************************
# Dynamics for amount of TCE in closed chamber
    dt(ACh) = (Rodents * (QM * CMixExh - QM * ACh/VCh)) - (kLoss * ACh);

#**** Non-metabolizing tissues ***********************************************
# Amount of TCE in rapidly perfused tissues (mg)
    dt(ARap) = QRap * (CArt - CVRap);
# Amount of TCE in slowly perfused tissues
    dt(ASlw) = QSlw * (CArt - CVSlw);
# Amount of TCE in fat tissue (mg)
    dt(AFat) = QFat*(CArt - CVFat);
# Amount of TCE in gut compartment (mg)
    dt(AGut) = (QGut * (CArt - CVGut)) + RAO;

#**** Liver ******************************************************************
# Rate of TCE oxidation by P450 to TCA, TCOH, and other (DCA) in liver (mg/hr)
```

```
          RAMetLiv1 = (VMAX * CVLiv) / (KM + CVLiv);
# Rate of TCE metabolized to DCVG in liver (mg)
          RAMetLiv2 = (VMAXDCVG * CVLiv) / (KMDCVG + CVLiv);
# Dynamics for amount of TCE in liver (mg)
    dt(ALiv) = (QLiv * (CArt - CVLiv)) + (QGut * (CVGut - CVLiv))
                              - RAMetLiv1 - RAMetLiv2 + kPV; # added PV dose

#**** Kidney ********************************************************************
# Rate of TCE metabolized to DCVG in kidney (mg) #
          RAMetKid = (VMAXKidDCVG * CVKid) / (KMKidDCVG + CVKid);
# Amount of TCE in kidney compartment (mg)
    dt(AKid) = (QKid * (CArt - CVKid)) - RAMetKid;

#*******************************************************************************
#***                        TCOH Sub-model                                   ***
#*******************************************************************************
# State Variables with dynamics:
#         ABodTCOH
#         ALivTCOH
# Input Variables:
#         none
# Other State Variables and Global Parameters:
#         ABileTCOG
#         kEHR
#         VBodTCOH
#         PBodTCOH
#         VLiv
#         PLivTCOH
#         VMAXTCOH
#         KMTCOH
#         VMAXGluc
#         KMGluc
#         kMetTCOH - hepatic metabolism of TCOH (e.g., to DCA)
#         FracOther
#         FracTCA
#         StochTCOHTCE
#         StochTCOHGluc
#         FracLungSys
# Temporary variables used:
#         QBod
#         QGutLiv
#         QCnow
#         kPOTCOH
#         RAMetLiv1
#         RAMetLng
# Temporary variables assigned:
#         CVBodTCOH
#         CVLivTCOH
#         CTCOH
#         RAMetTCOHTCA
#         RAMetTCOHGluc
#         RAMetTCOH
#         RARecircTCOG
# Notes:
#*******************************************************************************
#**** Blood (venous=arterial) ***************************************************
# Venous Concentrations (mg/L)
          CVBodTCOH = ABodTCOH / VBodTCOH / PBodTCOH;
          CVLivTCOH = ALivTCOH / VLiv / PLivTCOH;
          CTCOH = (QBod * CVBodTCOH + QGutLiv * CVLivTCOH + kIVTCOH)/QCnow;

#**** Body **********************************************************************
# Amount of TCOH in body
    dt(ABodTCOH) = QBod * (CTCOH - CVBodTCOH);

#**** Liver *********************************************************************

# Rate of oxidation of TCOH to TCA (mg/hr)
          RAMetTCOHTCA = (VMAXTCOH * CVLivTCOH) / (KMTCOH + CVLivTCOH);
# Amount of glucuronidation to TCOG (mg/hr)
          RAMetTCOHGluc = (VMAXGluc * CVLivTCOH) / (KMGluc + CVLivTCOH);
# Amount of TCOH metabolized to other (e.g., DCA)
          RAMetTCOH = kMetTCOH * ALivTCOH;
# Amount of TCOH-Gluc recirculated (mg)
          RARecircTCOG = kEHR * ABileTCOG;
# Amount of TCOH in liver (mg)
    dt(ALivTCOH) = kPOTCOH + QGutLiv * (CTCOH - CVLivTCOH)
                    - RAMetTCOH - RAMetTCOHTCA - RAMetTCOHGluc
                    + ((1.0 - FracOther - FracTCA) * StochTCOHTCE *
                    (RAMetLiv1 + FracLungSys*RAMetLng))
                    + (StochTCOHGluc * RARecircTCOG);

#*******************************************************************************
#***                        TCA Sub-model                                    ***
#*******************************************************************************
# State Variables with dynamics:
#         APlasTCA
#         ABodTCA
#         ALivTCA
#         AUrnTCA
#         AUrnTCA_sat
#         AUrnTCA_collect
# Input Variables:
#         TCAUrnSat
#         UrnMissing
# Other State Variables and Global Parameters:
#         VPlas
#         MWTCA
#         kDissoc
#         BMax
#         kMetTCA -- hepatic metabolism of TCA (e.g., to DCA)
#         VBod
#         PBodTCA
#         PLivTCA
#         kUrnTCA
#         FracTCA
#         StochTCATCE
#         StochTCATCOH
#         FracLungSys
# Temporary variables used:
#         kIVTCA
#         kPOTCA
#         QBodPlas
#         QGutLivPlas
```

```
#         QCPlas
#         RAMetLiv1
#         RAMetTCOHTCA
#         RAMetLng
# Temporary variables assigned:
#         CPlasTCA
#         CPLasTCAMole
#         a, b, c
#         CPlasTCAFreeMole
#         CPlasTCAFree
#         APlasTCAFree
#         CPlasTCABnd
#         CBodTCAFree
#         CLivTCAFree
#         CBodTCA
#         CLivTCA
#         CVBodTCA
#         CVLivTCA
#         RUrnTCA
#         RAMetTCA
# Notes:
#*****************************************************************************
#**** Plasma *****************************************************************
# Concentration of TCA in plasma (umoles/L)
          CPlasTCA = (APlasTCA<1.0e-15 ? 1.0e-15 : APlasTCA/VPlas);
# Concentration of free TCA in plasma in (umoles/L)
          CPlasTCAMole = (CPlasTCA / MWTCA) * 1000.0;
          a = kDissoc+BMax-CPlasTCAMole;
          b = 4.0*kDissoc*CPlasTCAMole;
          c = (b < 0.01*a*a ? b/2.0/a : sqrt(a*a+b)-a);
          CPlasTCAFreeMole = 0.5*c;
# Concentration of free TCA in plasma (mg/L)
          CPlasTCAFree = (CPlasTCAFreeMole * MWTCA) / 1000.0;
          APlasTCAFree = CPlasTCAFree * VPlas;
# Concentration of bound TCA in plasma (mg/L)
          CPlasTCABnd = (CPlasTCA<CPlasTCAFree ? 0 : CPlasTCA-CPlasTCAFree);
# Concentration in body and liver
          CBodTCA = (ABodTCA<0 ? 0 : ABodTCA/VBod);
          CLivTCA = (ALivTCA<1.0e-15 ? 1.0e-15 : ALivTCA/VLiv);
# Total concentration in venous plasma (free+bound)
          CVBodTCAFree = (CBodTCA / PBodTCA);     # free in equilibrium
          CVBodTCA = CPlasTCABnd + CVBodTCAFree;
          CVLivTCAFree = (CLivTCA / PLivTCA);
          CVLivTCA = CPlasTCABnd + CVLivTCAFree;  # free in equilibrium
# Rate of urinary excretion of TCA
          RUrnTCA = kUrnTCA * APlasTCAFree;
# Dynamics for amount of total (free+bound) TCA in plasma (mg)
    dt(APlasTCA) = kIVTCA + (QBodPlas*CVBodTCA) + (QGutLivPlas*CVLivTCA)
                            - (QCPlas * CPlasTCA) - RUrnTCA;

#**** Body *******************************************************************
# Dynamics for amount of TCA in the body (mg)
    dt(ABodTCA) = QBodPlas * (CPlasTCAFree - CVBodTCAFree);

#**** Liver ******************************************************************
# Rate of metabolism of TCA
          RAMetTCA = kMetTCA * ALivTCA;

# Dynamics for amount of TCA in the liver (mg)
    dt(ALivTCA) = kPOTCA + QGutLivPlas*(CPlasTCAFree - CVLivTCAFree)
                              - RAMetTCA + (FracTCA * StochTCATCE *
                              (RAMetLiv1 + FracLungSys*RAMetLng))
                              + (StochTCATCOH * RAMetTCOHTCA);

#**** Urine ******************************************************************
# Dynamics for amount of TCA in urine (mg)
    dt(AUrnTCA) = RUrnTCA;
    dt(AUrnTCA_sat) = TCAUrnSat*(1-UrnMissing)* RUrnTCA;
                    # Saturated, but not missing collection times
    dt(AUrnTCA_collect) = (1-TCAUrnSat)*(1-UrnMissing)*RUrnTCA;
                    # Not saturated and not missing collection times

#*****************************************************************************
#***                        TCOG Sub-model                                 ***
#*****************************************************************************
# State Variables with dynamics:
#         ABodTCOG
#         ALivTCOG
#         ABileTCOG
#         AUrnTCOG
#         AUrnTCOG_sat
#         AUrnTCOG_collect
# Input Variables:
#         TCOGUrnSat
#         UrnMissing
# Other State Variables and Global Parameters:
#         VBodTCOH
#         VLiv
#         PBodTCOG
#         PLivTCOG
#         kUrnTCOG
#         kBile
#         StochGlucTCOH
# Temporary variables used:
#         QBod
#         QGutLiv
#         QCnow
#         RAMetTCOHGluc
#         RARecircTCOG
# Temporary variables assigned:
#         CVBodTCOG
#         CVLivTCOG
#         CTCOG
#         RUrnTCOG
#         RBileTCOG
# Notes:
#*****************************************************************************
#**** Blood (venous=arterial) ************************************************
# Venous Concentrations (mg/L)
          CVBodTCOG = ABodTCOG / VBodTCOH / PBodTCOG;
          CVLivTCOG = ALivTCOG / VLiv / PLivTCOG;
          CTCOG = (QBod * CVBodTCOG + QGutLiv * CVLivTCOG)/QCnow;
#**** Body *******************************************************************
# Amount of TCOG in body
          RUrnTCOG = kUrnTCOG * ABodTCOG;
```

```
    dt(ABodTCOG) = QBod * (CTCOG - CVBodTCOG) - RUrnTCOG;
          RUrnTCOGTCOH = RUrnTCOG*StochTCOHGluc; #(vrisk)
#**** Liver ***********************************************************************
# Amount of TCOG in liver (mg)
          RBileTCOG = kBile * ALivTCOG;
    dt(ALivTCOG) = QGutLiv * (CTCOG - CVLivTCOG)
                   + (StochGlucTCOH * RAMetTCOHGluc) - RBileTCOG;

#**** Bile *************************************************************************
# Amount of TCOH-Gluc excreted into bile (mg)
    dt(ABileTCOG) = RBileTCOG - RARecircTCOG;

#**** Urine ************************************************************************
# Amount of TCOH-Gluc excreted in urine (mg)
    dt(AUrnTCOG) = RUrnTCOG;
    dt(AUrnTCOG_sat) = TCOGUrnSat*(1-UrnMissing)*RUrnTCOG;
                   # Saturated, but not missing collection times
    dt(AUrnTCOG_collect) = (1-TCOGUrnSat)*(1-UrnMissing)*RUrnTCOG;
                   # Not saturated and not missing collection times

#*****************************************************************************
#***                        DCVG Sub-model                                 ***
#*****************************************************************************
# State Variables with dynamics:
#         ADCVGmol
# Input Variables:
#         none
# Other State Variables and Global Parameters:
#         kDCVG
#         FracKidDCVC         # Fraction of kidney DCVG going to DCVC in first pass
#         VDCVG
# Temporary variables used:
#         RAMetLiv2
#         RAMetKid
# Temporary variables assigned:
#         RAMetDCVGmol
#         CDCVGmol
# Notes:
#         Assume negligible GGT activity in liver as compared to kidney,
#         supported by in vitro data on GGT (even accounting for 5x
#         greater liver mass relative to kidney mass), as well as lack
#         of DCVC detected in blood.
#         "FracKidDCVC" Needed to account for "first pass" in
#         kidney (TCE->DCVG->DCVC without systemic circulation of DCVG).
#*****************************************************************************
# Rate of metabolism of DCVG to DCVC
          RAMetDCVGmol = kDCVG * ADCVGmol;
# Dynamics for DCVG in blood
    dt(ADCVGmol) = (RAMetLiv2 + RAMetKid*(1-FracKidDCVC)) / MWTCE
                   - RAMetDCVGmol;
# Concentration of DCVG in blood (in mmoles/l)
          CDCVGmol = ADCVGmol / VDCVG;

#*****************************************************************************
#***                        DCVC Sub-model                                 ***
#*****************************************************************************
# State Variables with dynamics:
#         ADCVC
#         AUrnNDCVC
# Input Variables:
#         none
# Other State Variables and Global Parameters:
#         MWDCVC
#         FracKidDCVC
#         StochDCVCTCE
#         kNAT
#         kKidBioact
#         StochN
# Temporary variables used:
#         RAMetDCVGmol
#         RAMetKid
# Temporary variables assigned:
#         RAUrnDCVC
# Notes:
#         Cannot detect DCVC in blood, so assume all is locally generated
#         and excreted or bioactivated in kidney.
#*****************************************************************************
# Amount of DCVC in kidney (mg)
    dt(ADCVC) = RAMetDCVGmol * MWDCVC
                   + RAMetKid * FracKidDCVC * StochDCVCTCE
                   - ((kNAT + kKidBioact) * ADCVC);
# Rate of NAcDCVC excretion into urine (mg)
          RAUrnDCVC = kNAT * ADCVC;
# Dynamics for amount of N Acetyl DCVC excreted (mg)
     dt(AUrnNDCVC) = StochN * RAUrnDCVC;
          RUrnNDCVC = StochN * RAUrnDCVC; #(vrisk)
#*****************************************************************************
#***                        Total Mass Balance                             ***
#*****************************************************************************
#**** Mass Balance for TCE *********************************************************
# Total intake from inhalation (mg)
          RInhDose = QM * CInh;
    dt(InhDose) = RInhDose;
# Amount of TCE absorbed by non-inhalation routes (mg)
    dt(AO) = RAO + kIV + kIA + kPV; #(vrisk)
# Total dose
          TotDose = InhDose + AO; #(vrisk)
# Total in tissues
          TotTissue = #(vrisk)
                   ARap + ASlw + AFat + AGut + ALiv + AKid + ABld + #(vrisk)
                   AInhResp + AResp + AExhResp; #(vrisk)
# Total metabolized
    dt(AMetLng) = RAMetLng; #(vrisk)
    dt(AMetLiv1) = RAMetLiv1; #(vrisk)
    dt(AMetLiv2) = RAMetLiv2; #(vrisk)
    dt(AMetKid) = RAMetKid; #(vrisk)
          ATotMetLiv = AMetLiv1 + AMetLiv2; #(vrisk)
          TotMetab = AMetLng + ATotMetLiv + AMetKid; #(vrisk)
          AMetLivOther = AMetLiv1 * FracOther; #(vrisk)
          AMetGSH = AMetLiv2 + AMetKid; #(vrisk)
# Amount of TCE excreted in feces (mg)
          RAExc = kTD * ADuod; #(vrisk)
    dt(AExc) = RAExc; #(vrisk)
# Amount exhaled (mg)
```

```
          RAExh = QM * CMixExh;
    dt(AExh) = RAExh;
# Mass balance
          TCEDiff = TotDose - TotTissue - TotMetab; #(vrisk)
          MassBalTCE = TCEDiff - AExc - AExh; #(vrisk)

#**** Mass Balance for TCOH ************************************************
# Total production/intake of TCOH
    dt(ARecircTCOG) = RARecircTCOG; #(vrisk)
    dt(AOTCOH) = kPOTCOH + kIVTCOH; #(vrisk)
          TotTCOHIn = AOTCOH + ((1.0 - FracOther - FracTCA) * #(vrisk)
                    StochTCOHTCE * (AMetLiv1 + FracLungSys*AMetLng)) + #(vrisk)
                    (StochTCOHGluc * ARecircTCOG); #(vrisk)
          TotTCOHDose = AOTCOH + ((1.0 - FracOther - FracTCA) * #(vrisk)
                    StochTCOHTCE * (AMetLiv1 + FracLungSys*AMetLng)); #(vrisk)
# Total in tissues
          TotTissueTCOH = ABodTCOH + ALivTCOH; #(vrisk)
# Total metabolism of TCOH
    dt(AMetTCOHTCA) = RAMetTCOHTCA; #(vrisk)
    dt(AMetTCOHGluc) = RAMetTCOHGluc; #(vrisk)
    dt(AMetTCOHOther) = RAMetTCOH; #(vrisk)
          TotMetabTCOH = AMetTCOHTCA + AMetTCOHGluc + AMetTCOHOther; #(vrisk)
# Mass balance
          MassBalTCOH = TotTCOHIn - TotTissueTCOH - TotMetabTCOH; #(vrisk)

#**** Mass Balance for TCA *************************************************
# Total production/intake of TCA
    dt(AOTCA) = kPOTCA + kIVTCA; #(vrisk)
          TotTCAIn = AOTCA + (FracTCA*StochTCATCE*(AMetLiv1 + #(vrisk)
                    FracLungSys*AMetLng)) + (StochTCATCOH*AMetTCOHTCA); #(vrisk)
# Total in tissues
          TotTissueTCA = APlasTCA + ABodTCA + ALivTCA; #(vrisk)
# Total metabolism of TCA
    dt(AMetTCA) = RAMetTCA; #(vrisk)
# Mass balance
          TCADiff = TotTCAIn - TotTissueTCA - AMetTCA; #(vrisk)
          MassBalTCA = TCADiff - AUrnTCA; #(vrisk)

#**** Mass Balance for TCOG ************************************************
# Total production of TCOG
          TotTCOGIn = StochGlucTCOH * AMetTCOHGluc; #(vrisk)
# Total in tissues
          TotTissueTCOG = ABodTCOG + ALivTCOG + ABileTCOG; #(vrisk)
# Mass balance
          MassBalTCOG = TotTCOGIn - TotTissueTCOG - #(vrisk)
                    ARecircTCOG - AUrnTCOG; #(vrisk)

#**** Mass Balance for DCVG ************************************************
# Total production of DCVG
    dt(ADCVGIn) = (RAMetLiv2 + RAMetKid*(1-FracKidDCVC)) / MWTCE; #(vrisk)
# Metabolism of DCVG
    dt(AMetDCVG) = RAMetDCVGmol; #(vrisk)
# Mass balance
          MassBalDCVG = ADCVGIn - ADCVGmol - AMetDCVG; #(vrisk)

#**** Mass Balance for DCVC ************************************************
# Total production of DCVC
    dt(ADCVCIn) = RAMetDCVGmol * MWDCVC  #(vrisk)
                    + RAMetKid * FracKidDCVC * StochDCVCTCE;#(vrisk)
# Bioactivation of DCVC
    dt(ABioactDCVC) = (kKidBioact * ADCVC);#(vrisk)
# Mass balance
          AUrnNDCVCequiv = AUrnNDCVC/StochN;
          MassBalDCVC = ADCVCIn - ADCVC - ABioactDCVC - AUrnNDCVCequiv;#(vrisk)

#*****************************************************************************
#***                        Dynamic Outputs                                ***
#*****************************************************************************
# Amount exhaled during exposure (mg)
    dt(AExhExp) = (CInh > 0 ? RAExh : 0);

#*****************************************************************************
#***                        Dose Metrics                                   ***
#*****************************************************************************
#**** AUCs in mg-hr/L unless otherwise noted ********************************
#AUC of TCE in arterial blood
    dt(AUCCBld) = CArt; #(vrisk)
#AUC of TCE in liver
    dt(AUCCLiv) = CLiv; #(vrisk)
#AUC of TCE in kidney
    dt(AUCCKid) = CKid; #(vrisk)
#AUC of TCE in rapidly perfused
    dt(AUCCRap) = CRap; #(vrisk)
#AUC of TCOH in blood
    dt(AUCCTCOH) = CTCOH; #(vrisk)
#AUC of TCOH in body
    dt(AUCCBodTCOH) = ABodTCOH / VBodTCOH; #(vrisk)
#AUC of free TCA in the plasma (mg/L * hr)
    dt(AUCPlasTCAFree) = CPlasTCAFree; #(vrisk)
#AUC of total TCA in plasma (mg/L * hr)
    dt(AUCPlasTCA) = CPlasTCA; #(vrisk)
#AUC of TCA in liver (mg/L * hr)
    dt(AUCLivTCA) = CLivTCA; #(vrisk)
#AUC of total TCOH (free+gluc) in TCOH-equiv in blood (mg/L * hr)
    dt(AUCTotCTCOH) = CTCOH + CTCOGTCOH; #(vrisk)
#AUC of DCVG in blood (mmol/L * hr) -- NOTE moles, not mg
    dt(AUCCDCVG) = CDCVGmol; #(vrisk)
};
################ End of Dynamics ####################################


CalcOutputs{

#**** Static outputs for comparison to data ********************************
# TCE
          RetDose = ((InhDose-AExhExp) > 0 ? (InhDose - AExhExp) : 1e-15);
          CAlvPPM = (CAlv < 1.0e-15 ? 1.0e-15 : CAlv * (24450.0 / MWTCE));
          CInhPPM = (ACh< 1.0e-15 ? 1.0e-15 : ACh/VCh*24450.0/MWTCE);
                    # CInhPPM Only used for CC inhalation
          CArt = (CArt < 1.0e-15 ? 1.0e-15 : CArt);
          CVen = (CVen < 1.0e-15 ? 1.0e-15 : CVen);
          CBldMix = (CArt+CVen)/2;
          CFat = (CFat < 1.0e-15 ? 1.0e-15 : CFat);
          CGut = (CGut < 1.0e-15 ? 1.0e-15 : CGut);
```

```
          CRap = (CRap < 1.0e-15 ? 1.0e-15 : CRap);
          CSlw = (CSlw < 1.0e-15 ? 1.0e-15 : CSlw);
          CHrt = CRap;
          CKid = (CKid < 1.0e-15 ? 1.0e-15 : CKid);
          CLiv = (CLiv < 1.0e-15 ? 1.0e-15 : CLiv);
          CLung = CRap;
          CMus = (CSlw < 1.0e-15 ? 1.0e-15 : CSlw);
          CSpl = CRap;
          CBrn = CRap;
          zAExh = (AExh < 1.0e-15 ? 1.0e-15 : AExh);
          zAExhpost = ((AExh - AExhExp) < 1.0e-15 ? 1.0e-15 : AExh - AExhExp);
# TCOH
          CTCOH = (CTCOH < 1.0e-15 ? 1.0e-15 : CTCOH);
          CBodTCOH = (ABodTCOH < 1.0e-15 ? 1.0e-15 : ABodTCOH/VBodTCOH);
          CKidTCOH = CBodTCOH;
          CLivTCOH = (ALivTCOH < 1.0e-15 ? 1.0e-15 : ALivTCOH/VLiv);
          CLungTCOH = CBodTCOH;
# TCA
          CPlasTCA = (CPlasTCA < 1.0e-15 ? 1.0e-15 : CPlasTCA);
          CBldTCA = CPlasTCA*TCAPlas;
          CBodTCA = (CBodTCA < 1.0e-15 ? 1.0e-15 : CBodTCA);
          CLivTCA = (CLivTCA < 1.0e-15 ? 1.0e-15 : CLivTCA);
          CKidTCA = CBodTCA;
          CLungTCA = CBodTCA;
          zAUrnTCA = (AUrnTCA < 1.0e-15 ? 1.0e-15 : AUrnTCA);
          zAUrnTCA_sat = (AUrnTCA_sat < 1.0e-15 ? 1.0e-15 : AUrnTCA_sat);
          zAUrnTCA_collect = (AUrnTCA_collect < 1.0e-15 ? 1.0e-15 :
AUrnTCA_collect);
# TCOG
          zABileTCOG = (ABileTCOG < 1.0e-15 ? 1.0e-15 : ABileTCOG);
          #  Concentrations are in TCOH-equivalents
          CTCOG = (CTCOG < 1.0e-15 ? 1.0e-15 : CTCOG);
          CTCOGTCOH = (CTCOG < 1.0e-15 ? 1.0e-15 : StochTCOHGluc*CTCOG);
          CBodTCOGTCOH = (ABodTCOG < 1.0e-15 ? 1.0e-15 :
StochTCOHGluc*ABodTCOG/VBodTCOH);
          CKidTCOGTCOH = CBodTCOGTCOH;
          CLivTCOGTCOH = (ALivTCOG < 1.0e-15 ? 1.0e-15 :
StochTCOHGluc*ALivTCOG/VLiv);
          CLungTCOGTCOH = CBodTCOGTCOH;
          AUrnTCOGTCOH = (AUrnTCOG < 1.0e-15 ? 1.0e-15 : StochTCOHGluc*AUrnTCOG);
          AUrnTCOGTCOH_sat = (AUrnTCOG_sat < 1.0e-15 ? 1.0e-15 :
StochTCOHGluc*AUrnTCOG_sat);
          AUrnTCOGTCOH_collect = (AUrnTCOG_collect < 1.0e-15 ? 1.0e-15 :
StochTCOHGluc*AUrnTCOG_collect);
# Other
          CDCVGmol = (CDCVGmol < 1.0e-15 ? 1.0e-15 : CDCVGmol);
          CDCVGmol0 = CDCVGmol; #(v1.2.3.2)
          CDCVG_NDtmp = CDFNormal(3*(1-CDCVGmol/CDCVGmolLD));
                    # Assuming LD = 3*sigma_blank, Normally distributed
          CDCVG_ND = ( CDCVG_NDtmp < 1.0 ? ( CDCVG_NDtmp >= 1e-100 ? -
log(CDCVG_NDtmp) : -log(1e-100)) : 1e-100 );
             #(v1.2.3.2)
          zAUrnNDCVC =(AUrnNDCVC < 1.0e-15 ? 1.0e-15 : AUrnNDCVC);
          AUrnTCTotMole = zAUrnTCA / MWTCA + AUrnTCOGTCOH / MWTCOH;
          TotCTCOH = CTCOH + CTCOGTCOH;
          TotCTCOHcomp = CTCOH + CTCOG; # ONLY FOR COMPARISON WITH HACK
          ATCOG = ABodTCOG + ALivTCOG; # ONLY FOR COMPARISON WITH HACK
# Misc
          CVenMole = CVen / MWTCE;
          CPlasTCAMole = (CPlasTCAMole < 1.0e-15 ? 1.0e-15 : CPlasTCAMole);
          CPlasTCAFreeMole = (CPlasTCAFreeMole < 1.0e-15 ? 1.0e-15 :
CPlasTCAFreeMole);

#**** Additional Dose Metrics ************************************************
#

          TotTCAInBW = TotTCAIn/BW;#(vrisk)

# Scaled by BW^3/4
          TotMetabBW34 = TotMetab/BW75;#(vrisk)
          AMetGSHBW34 = AMetGSH/BW75;#(vrisk)
          TotDoseBW34 = TotDose/BW75;#(vrisk)
          AMetLiv1BW34 = AMetLiv1/BW75;#(vrisk)
          TotOxMetabBW34 = (AMetLng+AMetLiv1)/BW75;#(vrisk)
          AMetLngBW34 = AMetLng/BW75; #(vrisk)
          ABioactDCVCBW34 = ABioactDCVC/BW75;#(vrisk)
          AMetLivOtherBW34 = AMetLivOther/BW75; #(vrisk)

# Scaled by tissue volume
          AMetLiv1Liv = AMetLiv1/VLiv; #(vrisk)
          AMetLivOtherLiv = AMetLivOther/VLiv; #(vrisk)
          AMetLngResp = AMetLng/VRespEfftmp; #(vrisk)
          ABioactDCVCKid = ABioactDCVC/VKid;#(vrisk)

#**** Fractional Volumes

          VFatCtmp = VFat/BW; #(vrisk)
          VGutCtmp = VGut/BW; #(vrisk)
          VLivCtmp = VLiv/BW; #(vrisk)
          VRapCtmp = VRap/BW; #(vrisk)
          VRespLumCtmp = VRespLum/BW; #(vrisk)
          VRespEffCtmp = VRespEfftmp/BW; #(vrisk)
          VKidCtmp = VKid/BW; #(vrisk)
          VBldCtmp = VBld/BW; #(vrisk)
          VSlwCtmp = VSlw/BW; #(vrisk)
          VPlasCtmp = VPlas/BW; #(vrisk)
          VBodCtmp = VBod/BW; #(vrisk)
          VBodTCOHCtmp = VBodTCOH/BW; #(vrisk)


};
```

B. SYSTEMATIC REVIEW OF EPIDEMIOLOGIC STUDIES ON CANCER AND TCE EXPOSURE

B.1. INTRODUCTION

The epidemiologic evidence on TCE is large with over 50 studies and includes occupational cohort studies, case-control studies, both nested within a cohort (nested case-control study) or population-based, and geographic-based studies. The analysis of epidemiologic studies on cancer and TCE serves to document essential design features, exposure assessment approaches, statistical analyses, and potential sources of confounding and bias. These studies are described below and reviewed according to criteria to assess: (1) their ability to inform weight of evidence evaluation for TCE exposure and a cancer hazard and (2) their utility for examination using meta-analysis approaches. A secondary goal of the qualitative review is to provide transparency on study strengths and weaknesses, providing background for inclusion or exclusion of individual studies for quantitative treatment using meta-analysis approaches. Individual study qualities are discussed according to specific criteria in Sections B.2.1 to B.2.8., and rationale for studies examined using meta-analysis approaches, the systematic review, contained in Section B.2.9. Appendix C contains a full discussion of the meta-analysis, its analytical methodology, including sensitivity analyses, and findings. This analysis supports discussion of site-specific cancer observations in Chapter 4 where a presentation may be found of study findings with assessment and discussion of observations according to a study's weight of evidence and potential for alternative explanations, including bias and confounding.

B.2. METHODOLOGIC REVIEW OF EPIDEMIOLOGIC STUDIES ON CANCER AND TCE

Epidemiologic studies considered in this analysis assess the relationship between TCE exposure and cancer, and are identified using several sources and their utility for characterizing hazard and quantitative treatment is based on recommendations in NRC (2006). A thorough search of the literature was carried out through December 2010 without restriction on year of publication or language using the following approaches: a search of the bibliographic databases PubMed (http://www.ncbi.nlm.nih.gov/ pubmed/), TOXNET (http://toxnet.nlm.nih.gov/), and EMBASE (http://www.embase.com/) using the terms "trichloroethylene cancer epidemiology" and ancillary terms, "degreasers," "aircraft, aerospace or aircraft maintenance workers," "metal workers," and "electronic workers," "trichloroethylene and cohort," or "trichloroethylene and case-control;" bibliographies of reviews of the TCE epidemiologic literature such as those of the Institute of Medicine (IOM, 2003), NRC (2009, 2006), and Scott and Chiu (2006) and review of bibliographies of individual studies for relevant studies not identified in the previous two

approaches. The search strategy identified studies that were either published or available on-line (in press). NRC (2006) noted "a full review of the literature should identify all published studies in which there was a possibility that TCE was investigated, even though results per se may not have been reported."

Additional steps of U.S. EPA staff to identify studies not published in the literature included contacting primary investigators for case-control studies of liver, kidney and lymphoma and occupation, asking for information on analyses examining TCE uniquely and a review of ATSDR or state health department community health surveys or statistics reviews for information on TCE exposure and cancer incidence or mortality.

The breadth of the available epidemiologic database on TCE and cancer is wide compared to that available for other chemicals assessed by U.S. EPA. However, few studies were designed with the sole, or primary, objective of this report—to characterize the magnitude of underlying association, if such exists, between TCE and cancer. Yet, many studies in the body of evidence can provide information for identifying cancer hazard and dose-response inferences. The weight a study contributes to the overall evidence on TCE and cancer depends on a number of characteristics regarding the design, exposure assessment, and analysis approaches. Epidemiologic studies were most informative for analysis if they approached ideals described below, as evaluated using objective criteria for identifying a cancer hazard.

Seventy-five studies potentially relevant to health assessment of TCE exposure and cancer and identified from the above comprehensive search are presented in Tables B-1, B-2, and B-3. The studies vary widely in their approaches to study design, exposure assessment, and statistical analysis; for these reasons, studies vary in their usefulness for identifying cancer hazard. Studies are reviewed according to a set of a priori guidelines of their utility for assessing TCE exposure and cancer according to the below criteria. Studies approaching criteria ideals contribute greater weight in the weight of evidence analysis than studies with significant deficiencies. These criteria are not meant to be used to "accept" or "reject" a particular study for identifying cancer hazard. Rather, they are to be used as measurement tools for evaluating a study's ability to identify TCE exposure and cancer outcomes. Studies suitable for meta-analysis treatment are selected according to specific criteria identified in Section B.2.9.4. Individual study descriptions and abstract sheets according to these criteria are found in Section B.3. Appendix C describes meta-analysis methods and findings.

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Aircraft and aerospace workers			
Radican et al. (2008), Blair et al. (1998)	Civilian aircraft-maintenance workers with at least 1 yr in 1952–1956 at Hill Air Force Base, Utah. Vital status (VS) to 1990 (Blair et al., 1998) or 2000 (Radican et al., 2008); cancer incidence 1973–1990 (Blair et al., 1998).	14,457 (7,204 ever exposed to TCE). Incidence (Blair et al., 1998) and mortality rates (Radican et al., 2008; Blair et al., 1998) of nonchemical exposed subjects.	Most subjects (n = 10,718) with potential exposure to 1–25 solvents. Cumulative TCE assigned to individual subjects using JEM. Exposure-response patterns assessed using cumulative exposure, continuous or intermittent exposures, and peak exposure. TCE replaced in 1968 with 1,1,1-trichloroethane and was discontinued in 1978 in vapor degreasing activities. Median TCE exposures were about 10 ppm for rag and bucket; 100–200 ppm for vapor degreasing. Poisson regression analyses controlled for age, calendar time, sex (Blair et al., 1998), or Cox proportional hazard model for age and race.
Krishnadasan et al. (2007)	Nested case-control study within a cohort of 7,618 workers employed for between 1950 and 1992, or who had started employment before 1980 at Boeing/Rockwell/ Rocketdyne (SSFL [the UCLA cohort of (Morgenstern et al., 1997)]). Cancer incidence 1988–1999.	326 prostate cancer cases, 1,805 controls. Response rate: Cases, 69%; Controls, 60%.	JEM for TCE, hydrazine, PAHs, benzene, and mineral oil constructed from company records, walk-through, or interviews. Lifestyle factors obtained from living subjects through mail and telephone surveys. Conditional logistic regression controlled for cohort, age at diagnosis, physical activity, SES and other occupational exposure (benzene, PAHs, mineral oil, hydrazine).
Zhao et al. (2005); Ritz et al. (1999a)	Aerospace workers with >2 yrs of employment at Rockwell/ Rocketdyne (now Boeing) and who worked at SSFL, Ventura, California, from 1950 to 1993 (the UCLA cohort of (Morgenstern et al., 1997)). Cancer mortality as of December 31, 2001. Cancer incidence 1988–2000 for subjects alive as of 1988.	6,044 (2,689 with high cumulative exposure to TCE). Mortality rates of subjects in lowest TCE exposure category. 5,049 (2,227 with high cumulative exposure to TCE). Incidence rates of subjects in lowest TCE exposure category.	JEM for TCE, hydrazine, PAHs, mineral oil, and benzene. IH ranked each job title ranked for presumptive TCE exposure as high (3), medium (2), low (1), or no (0) exposure for 3 time periods (1951–1969, 1970–1979, 1980–1989). Cumulative TCE score: low (≤3), medium (>3–12), high (>12) assigned to individual subjects using JEM. Cox proportional hazard, controlled for time, since 1st employment, SES, age at diagnosis, and hydrazine.

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Boice et al. (2006b)	Aerospace workers with >6 months employment at Rockwell/ Rocketdyne (SSFL and nearby facilities) from 1948 to 1999 (IEI cohort, IEI [2005]). VS to 1999.	41,351, 1,642 male hourly test stand mechanics (1,111 with potential TCE exposure). Mortality rates of U.S. population and California population. Internal referent groups including male hourly nonadministrative Rocketdyne workers; male hourly, nonadministrative SSFL workers; and test stand mechanics with no potential exposure to TCE.	Potential TCE exposure assigned to test stands workers only whose tasks included the cleaning or flushing of rocket engines (engine flush) (n = 639) or for general utility cleaning (n = 472); potential for exposure to large quantities of TCE was much greater during engine flush than when TCE used as a utility solvent. JEM for TCE and hydrazine without semiquantitative intensity estimates. Exposure to other solvents not evaluated due to low potential for confounding (few exposed, low exposure intensity, or not carcinogenic). Exposure metrics included employment duration, employment decade, years worked with potential TCE exposure, and years worked with potential TCE exposure via engine cleaning, weighted by number of tests. Lifetable (SMR); Cox proportional hazard controlling for birth year, hire year, and hydrazine exposure.
Boice et al. (1999)	Aircraft-manufacturing workers with at least 1 yr >1960 at Lockheed Martin (Burbank, California). VS to 1996.	77,965 (2,267 with potential routine TCE exposures and 3,016 with routine or intermittent TCE exposure). Mortality rates of U.S. population (routine TCE exposed subjects) and non-exposed internal referents (routine and intermittent TCE exposed subjects).	12% with potential routine mixed solvent exposure and 30% with route or intermittent solvent exposure. JEM for potential TCE exposure on: (1) routine basis; or (2) intermittent or routine basis without semiquantitative intensity estimate. Exposure-response patterns assessed by any exposure or duration of exposure and internal control group. Vapor degreasing with TCE before 1966 and perchloroethylene, afterwards. Lifetable analyses (SMR); Poisson regression analysis adjusting for birth date, starting employment date, finishing employment date, sex, and race.
Morgan et al. (1998)	Aerospace workers with >6 months 1950–1985 at Hughes (Tucson, Arizona). VS to 1993.	20,508 (4,733 with TCE exposures). Mortality rates of U.S. population for overall TCE exposure; mortality rates of all-other cohort subjects (internal referents) for exposure-response analyses.	TCE exposure intensity assigned using JEM. Exposure-response patterns assessed using cumulative exposure (low vs. high) and job with highest TCE exposure rating (peak, medium/high exposure vs. no/low exposure). "High exposure" job classification defined as >50 ppm. Vapor degreasing with TCE 1952–1977, but limited IH data <1975. Limited IH data before 1975 and medium/ low rankings likely misclassified given temporal changes in exposure intensity not fully considered (NRC, 2006).
Costa et al. (1989)	Aircraft manufacturing workers employed 1954–1981 at plant in Italy. VS to 1981.	8,626 subjects Mortality rates of the Italian population.	No exposure assessment to TCE and job titles grouped into one of four categories: blue- and white-collar workers, technical staff, and administrative clerks. Lifetable (SMR).

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Garabrant et al. (1988)	Aircraft manufacturing workers >4 yrs employment and who had worked at least 1 d at San Diego, California, plant 1958–1982. VS to 1982.	14,067 Mortality rates of U.S. population.	TCE exposure assessment for 70 of 14,067 subjects; 14 cases of esophageal cancer and 56 matched controls. For these 70 subjects, company work records identified 37% with job title with potential TCE exposure without quantitative estimates. Lifetable (SMR).
Cohorts identified from biological monitoring (U-TCA)			
Hansen et al. (2001)	Workers biological monitored using U-TCA and air-TCE, 1947–1989. Cancer incidence from 1964 to 1996.	803 total Cancer incidence rates of the Danish population.	712 with U-TCA, 89 with air-TCE measurement records, 2 with records of both types. U-TCA from 1947 to 1989; air TCE measurements from 1974. Historic median exposures estimated from the U-TCA concentrations were: 9 ppm for 1947–1964, 5 ppm for 1965–1973, 4 ppm for 1974–1979, and 0.7 ppm for 1980–1989. Air TCE measurements from 1974 onward were 19 ppm (mean) and 5 ppm (median). Overall, median TCE exposure to cohort as extrapolated from air TCE and U-TCA measurements was 4 ppm (arithmetic mean, 12 ppm). Exposure metrics: year 1st employed, employment duration, mean exposure, cumulative exposure. Exposure metrics: employment duration, average TCE intensity, cumulative TCE, period 1st employment. Lifetable analysis (SIR).
Anttila et al. (1995)	Workers biological monitored using U-TCA, 1965–1982. VS 1965–1991 and cancer incidence 1967–1992.	3,974 total (3,089 with U-TCA measurements). Mortality and cancer incidence rates of the Finnish population.	Median U-TCA, 63 µmol/L for females and 48 µmol/L for males; mean U-TCA was 100 µmol/L. Average 2.5 U-TCA measurements per individual. Using the Ikeda et al. (1972) relationship for TCE exposure to U-TCA, TCE exposures were roughly 4 ppm (median) and 6 ppm (mean). Exposure metrics: years since 1st measurement. Lifetable analysis (SMR, SIR).
Axelson et al. (1994)	Workers biological monitored using U-TCA, 1955–1975. VS to 1986 and cancer incidence 1958–1987.	1,4,21 males Mortality and cancer incidence rates of Swedish male population.	Biological monitoring for U-TCA from 1955 and 1975. Roughly ¾ of cohort had U-TCA concentrations equivalent to <20 ppm TCE. Exposure metrics: duration exposure, mean U-TCA. Lifetable analysis (SMR, SIR).

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Other cohorts			
Clapp and Hoffman (2008)	Deaths between 1969 and 2001 among employees >5 yrs employment duration at an IBM facility (Endicott, New York).	360 deaths Proportion of deaths among New York residents during 1979 to 1998.	No exposure assessment to TCE. PMR analysis.
Sung et al. (2008; 2007)	Female workers 1st employed 1973–1997 at an electronics (RCA) manufacturing factory (Taoyuan, Taiwan). Cancer incidence 1979–2001 (Sung et al., 2007). Childhood leukemia 1979–2001 among first born of female subjects in (Sung et al., 2008)	63,982 females and 40,647 females with 1st live born offspring. Cancer incidence rates of Taiwan population (Sung et al., 2007). Childhood leukemia incidence rates of first born live births of Taiwan population (Sung et al., 2008).	No exposure assessment. Chlorinated solvents including TCE and perchloroethylene found in soil and groundwater at factory site. Company records indicated TCE not used 1975–1991 and perchloroethylene 1975–1991 and perchloroethylene after 1981. No information for other time periods. Exposure-response using employment duration. Lifetable analysis (SMR, SIR) (Sung et al., 2007; Chang et al., 2005; Chang et al., 2003) or Poisson regression adjusting for maternal age, education, sex, and birth year (Sung et al., 2008).
Chang et al. (2005; 2003)	Male and female workers employed 1978–1997 at electronics factory as studied by Sung et al. (2007). VS from 1985 to 1997 and cancer incidence 1979–1997.	86,868 total Incidence (Chang et al., 2005) or mortality (Chang et al., 2003) rates Taiwan population.	
ATSDR (2004a)	Workers 1952–1980 at the View-Master factory (Beaverton, Oregon).	616 deaths 1989–2001 Proportion of deaths between 1989 and 2001 in Oregon population.	No exposure information on individual subjects. TCE and other VOCs detected in well water at the time of the plant closure in 1998 were TCE, 1,220–1,670 µg/L; 1,1-DCE, up to 33 µg/L; and, perchloroethylene up to 56 µg/L. PMR analysis.
Raaschou-Nielsen et al. (2003)	Blue-collar workers employed >1968 at 347 Danish TCE-using companies. Cancer incidence through 1997.	40,049 total (14,360 with presumably higher level exposure to TCE). Cancer incidence rates of the Danish population.	Employers had documented TCE usage but no information on individual subjects. Blue-collar vs. white-collar workers and companies with <200 workers were variables identified as increasing the likelihood for TCE exposure. Subjects from iron and metal, electronics, painting, printing, chemical, and dry cleaning industries. Median exposures to TCE were 40–60 ppm for the years before 1970, 10–20 ppm for 1970–1979, and approximately 4 ppm for 1980–1989. Exposure metrics: employment duration, year 1st employed, and # employees in company. Lifetable (SIR).

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Ritz (1999a)	Male uranium-processing plant workers >3 months employment 1951–1972 at DOE facility (Fernald, Ohio). VS 1951–1989, cancer.	3,814 white males monitored for radiation (2,971 with potential TCE exposure). Mortality rates of the U.S. population; non-TCE exposed internal controls for TCE exposure-response analyses.	JEM for TCE, cutting fluids, kerosene, and radiation generated by employees and industrial hygienists. Subjects assigned potential TCE according to intensity: light (2,792 subjects), moderate (179 subjects), heavy (no subjects). Lifetable (SMR) and conditional logistic regression adjusted for pay status, date first hire, radiation.
Henschler et al. (1995)	Male workers >1 yr 1956–1975 at cardboard factory (Arnsberg region, Germany). VS to 1992.	169 exposed; 190 unexposed. Mortality rates from German Democratic Republic (broad categories) or RCC incidence rates from Danish population, German Democratic, or non-TCE exposed subjects.	Walk-through surveys and employee interviews used to identify work areas with TCE exposure. TCE exposure assigned to renal cancer cases using workman's compensation files. Lifetable (SMR, SIR) or Mantel-Haenszel.
Greenland et al. (1994)	Cancer deaths, 1969–1984, among pensioned workers employed <1984 at GE transformer manufacturing plant (Pittsfield, Massachusetts), and who had job history record; controls were noncancer deaths among pensioned workers.	512 cases, 1,202 controls. Response rate: Cases, 69%; Controls, 60%.	Industrial hygienist assessment from interviews and position descriptions. TCE (no/any exposure) assigned to individual subjects using JEM. Logistic regression.
Sinks et al. (1992)	Workers employed 1957–1980 at a paperboard container manufacturing and printing plant (Newnan, Georgia). VS to 1988. Kidney and bladder cancer incidence through 1990.	2,050 total Mortality rates of the U.S. population, bladder and kidney cancer incidence rates from the Atlanta-SEER registry for the years 1973–1977.	No exposure assessment to TCE; analyses of all plant employees including white- and blue-collar employees. Assignment of work department in case-control study based upon work history; Material Safety Data Sheets identified chemical usage by department. Lifetable (SMR, SIR) or conditional logistic regression adjusted for hire date and age at hire, and using 5- and 10-yr lagged employment duration.
Blair et al. (1989)	Workers employed 1942–1970 in U.S. Coast. VS to 1980.	3,781 males of whom 1,767 were marine inspectors (48%). Mortality rates of the U.S. population. Mortality rates of marine inspectors also compared to that of noninspectors.	No exposure assessment to TCE. Marine inspectors worked in confined spaces and had exposure potential to multiple chemicals. TCE was identified as one of 10 potential chemical exposures. Lifetable (SMR) and directly adjusted RRs.

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Shannon et al. (1988)	Workers employed ≥6 months at GE lamp manufacturing plant, 1960–1975. Cancer incidence from 1964 to 1982.	1,870 males and females, 249 (13%) in coiling and wire-drawing area. Cancer incidence rates from Ontario Cancer Registry.	No exposure assessment to TCE. Workers in coiling and wire drawing (CWD) had potential exposure to many chemicals including metals and solvents. A 1955-dated engineering instruction sheet identified TCE used as degreasing solvent in CWD. Lifetable (SMR).
Shindell and Ulrich (1985)	Workers employed >3 months at a TCE manufacturing plant 1957–1983. VS to 1983.	2,646 males and females Mortality rates of the United States population.	No exposure assessment to TCE; job titles categorized as either white- or blue-collar. Lifetable analysis (SMR).
Wilcosky et al. (1984)	Respiratory, stomach, prostate, lymphosarcoma, and lymphatic leukemia cancer deaths 1964–1972 among 6,678 active and retired production workers at a rubber plant (Akron, Ohio); controls were a 20% age-stratified random sample of the cohort.	183 cases (101 respiratory, 33 prostate, 30 stomach, 9 lymphosarcoma and 10 lymphatic leukemia cancer deaths).	JEM without quantitative intensity estimates for 20 exposures including TCE. Exposure metric: ever held job with potential TCE exposure.

DOE = U.S. Department of Energy; IEI = International Epidemiology Institute; Los Angeles; VS = vital status.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Bladder			
Pesch et al. (2000a)	Histologically confirmed urothelial cancer (bladder, ureter, renal pelvis) cases from German hospitals (five regions) in 1991–1995; controls randomly selected from residency registries matched on region, sex, and age.	1,035 cases 4,298 controls Cases, 84%; controls, 71%	Occupational history using job title or self-reported exposure. JEM and JTEM to assign exposure potential to metals and solvents (chlorinated solvents, TCE, perchloroethylene). Lifetime exposure to TCE exposure examined as 30^{th}, 60^{th}, and 90^{th} percentiles (medium, high, and substantial) of exposed control exposure index. Duration used to examine occupational title and job task duties and defined as 30^{th}, 60^{th}, and 90^{th} percentiles (medium, long, and very long) of exposed control durations. Logistic regression with covariates for age, study center, and smoking.
Siemiatycki (1994), (1991)	Male bladder cancer cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	484 cases 533 population controls; 740 other cancer controls Cases, 78%; controls, 72%	JEM to assign 294 exposures including TCE on semiquantitative scales categorized as any or substantial exposure. Other exposure metrics included exposure duration in occupation or job title. Logistic regression adjusted for age, ethnic origin, SES, smoking, coffee consumption, and respondent status [occupation or job title] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, and respondent status (TCE).
Brain			
De Roos et al. (2001); Olshan et al. (1999)	Neuroblastoma cases in children of <19 yrs selected from Children's Cancer Group and Pediatric Oncology Group with diagnosis in 1992–1994; population controls (random digit dialing) matched to control on birth date.	504 cases 504 controls Cases, 73%; controls, 74%	Telephone interview with parent using questionnaire to assess parental occupation and self-reported exposure history and judgment-based attribution of exposure to chemical classes (halogenated solvents) and specific solvents (TCE). Exposure metric was any potential exposure. Logistic regression with covariate for child's age and material race, age, and education.
Heineman et al. (1994)	White, male cases, age >30 yrs, identified from death certificates in 1978–1981; controls identified from death certificates and matched for age, year of death, and study area.	300 cases 386 controls Cases, 74%; controls, 63%	In-person interview with next-of-kin; questionnaire assessing lifetime occupational history using job title and JEM of Gomez et al. (1994). Cumulative exposure metric (low, medium, or high) based on weighted probability and duration. Logistic regression with covariates for age and study area.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Colon and rectum			
Goldberg et al. (2001); Siemiatycki (1991)	Male colon cancer cases, 35–75 yrs, from 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	497 cases 533 population controls and 740 cancer controls Cases, 82%; controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, ethnic origin, birthplace, education, income, parent's occupation, smoking, alcohol consumption, tea consumption, respondent status, heating source SES, smoking, coffee consumption, and respondent status [occupation, some chemical agents] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, and respondent status [TCE].
Dumas et al. (2000); Simeiatycki (1991)	Male rectal cancer cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	292 cases 533 population controls and 740 other cancer controls Cases, 78%; controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, respondent status, cigarette smoking, beer consumption, and BMI [TCE] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, ethnic origin, and beer consumption [TCE].
Fredriksson et al. (1989)	Colon cancer cases aged 30–75 yrs identified through the Swedish Cancer Registry among patients diagnosed in 1980–1983; population-based controls were frequency-matched on age and sex and were randomly selected from a population register.	329 cases 658 controls Not available	Mailed questionnaire assessing occupational history with telephone interview follow-up. Self-reported exposure to TCE defined as any exposure. Mantel-Haenszel stratified on age, sex, and physical activity.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Esophagus			
Parent et al. (2000a), Siemiatycki (1991)	Male esophageal cancer cases, 35–75 yrs, diagnosed in 19 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	292 cases 533 population controls; 740 subjects with other cancers Cases, 78%; controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, respondent status, cigarette smoking, beer consumption, and BMI [solvents] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, ethnic origin, and beer consumption [TCE].
Lymphoma			
Purdue et al. (2011);	Cases aged 20–74 with histologically-confirmed NHL (B-cell diffuse and follicular, T-cell, lymphoreticular) without HIV in 1998–2000 and identified from four SEER areas (Los Angeles County and Detroit metropolitan area, random sample; Seattle_Puget Sound and Iowa, all consecutive cases); population controls aged 20–74 yrs with no previous diagnosis of HIV infection or NHL, identified through: (1) if >65 yrs of age, random digit dialing; or (2) if ≥65 yrs, identified from Medicare eligibility files and stratified on geographic area, age, and race.	1,321 cases 1,057 controls Cases, 76%; controls, 78%	In-person interview using questionnaire or computer-assisted personal interview questionnaire specific for jobs held for >1 yr since the age of 16 yrs, hobbies, and medical and family history. For occupational history, 32 job- or industry-specific interview modules asked for detailed information on individual jobs and focused on solvents exposure, including TCE, assessment by expert industrial hygienist blinded to case and control status by levels of probability, frequency, and intensity. Exposure metric of overall exposure, average weekly exposure, years exposed, average exposure intensity, and cumulative exposure. Logistic regression adjusted for sex, age, race, education, and SEER site.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Gold et al.(2011)	Cases aged 35–74 with histologically-confirmed multiple myeloma in 2000–2002 and identified from SEER areas (Detroit, Seattle-Puget Sound); population controls.	181 cases 481 controls Cases, 71%; controls, 52%	In-person interview using computer-assisted personal interview questionnaire for jobs held ≥1 yr since 1941 (cases) or 1946 (controls) and since age 18 yrs. For occupational history, 20 occupations, job- or industry-specific interview modules asked for detailed information on individual jobs held at least 2 yrs and focused on solvents exposure, including TCE, assessment by expert industrial hygienist blinded to case and control status by levels of probability, duration, and cumulative exposure. Logistic regression adjusted for sex, age, race, education, and SEER site.
Cocco et al. (2010)	Cases aged ≥17 yrs with lymphoma (B-cell, T-cell, CLL, multiple myeloma, Hodgkin) in 1998–2004 and residents of referral areas from seven European countries (Czech Republic, Finland, France, Germany, Ireland, Italy, and Spain); hospital (four participating countries) or population controls (all others); controls from: (1) Germany and Italy selected by random digit dialing from general population and matched (individually in German and group-based in Italy) to cases by sex, age and residence area, and; (2) for all other countries, matched hospital controls with diagnoses other than cancer, infectious diseases and immundeficient diseases.	2,348 cases 2,462 controls Cases, 88%; controls, 81% hospital and 52% population	In-person interviews using same structured questionnaire translated to the local language for information on sociodemographic factors, lifestyle, health history, and all full-time job held ≥1 yr. Assessment by industrial hygienists in each participating center to 43 agents, including TCE, by confidence, exposure intensity, and exposure frequency. Exposure metric of overall TCE exposure and cumulative TCE exposure for subjects assessed with high degree of confidence (defined as low, medium, and high). Logistic regression adjusted for age, gender, education and study center.
German centers: Seidler et al. (2007); Mester et al. (2006); Becker et al. (2004)	NHL and Hodgkin lymphoma cases aged 18–80 yrs identified through all hospitals and ambulatory physicians in six regions of Germany between 1998 and 2003; population controls were identified from population registers and matched on age, sex, and region.	710 cases 710 controls Cases, 87%; controls, 44%	In-person interview using questionnaire assessing personal characteristics, lifestyle, medical history, UV light exposure, and occupational history of all jobs held for ≥1 yr. Exposure of a prior interest were assessed using job task-specific supplementary questionnaires. JEM used to assign cumulative quantitative TCE exposure metric, categorized according to the distribution among the control persons (50^{th} and 90^{th} percentile of the exposed controls). Conditional logistic regression adjusted for age, sex, region, smoking, and alcohol consumption.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Wang et al. (2009)	Cases among females aged 21 and 84 yrs with NHL in 1996–2000 and identified from Connecticut Cancer Registry; population-based female controls: (1) if <65 yrs of age, having Connecticut address stratified by 5-yr age groups identified from random digit dialing; or (2) >65 yrs of age, by random selection from Centers for Medicare and Medicaid Service files.	601 cases 717 controls Cases, 72%; controls, 69% (<65 yrs), 47% (>65 yrs)	In-person interview with using questionnaire assessment specific jobs held for >1 yr. Intensity and probability of exposure to broad category of organic solvents and to individual solvents, including TCE, estimated using JEM (Dosemeci et al., 1999; Gómez et al., 1994) and assigned blinded. Exposure metric of any exposure, exposure intensity (low, medium/high), and exposure probability (low, medium/high). Logistic regression adjusted for age, family history of hematopoietic cancer, alcohol consumption and race.
Costantini et al. (2008); Miligi et al. (2006)	Cases aged 20–74 with NHL, including CLL, all forms of leukemia, or multiple myeloma (MM) in 1991–1993 and identified through surveys of hospital and pathology departments in study areas and in specialized hematology centers in eight areas in Italy; population-based controls stratified by 5-yr age groups and by sex selected through random sampling of demographic or of National Health Service files.	1,428 NHL + CLL, 586 Leukemia, 263, MM 1,278 controls (leukemia analysis) 1,100 controls (MM analysis) Cases, 83%; controls, 73%	In-person interview primarily at interviewee's home (not blinded) using questionnaire assessing specific jobs, extra occupational exposure to solvents and pesticides, residential history, and medical history. Occupational exposure assessed by job-specific or industry-specific questionnaires. JEM used to assign TCE exposure and assessed using intensity (two categories) and exposure duration (two categories). All NHL diagnoses and 20% sample of all cases confirmed by panel of three pathologists. Logistic regression with covariates for sex, age, region, and education. Logistic regression for specific NHL included an additional covariate for smoking.
Persson and Fredriksson (1999); Combined analysis of NHL cases in Persson et al. (1993); Persson et al. (1989)	Histologically confirmed cases of B-cell NHL, age 20–79 yrs, identified in two hospitals in Sweden: Oreboro in 1964–1986 (Persson et al., 1989) and in Linkoping between 1975 and 1984 (Persson et al., 1993); controls were identified from previous studies and were randomly selected from population registers.	199 NHL cases, 479 controls Cases, 96% (Oreboro), 90% (Linkoping); controls, not reported	Mailed questionnaire to assess self reported occupational exposures to TCE and other solvents. Mantel-Haenszel χ^2.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Nordstrom et al. (1998)	Histologically-confirmed cases in males of hairy-cell leukemia reported to Swedish Cancer Registry in 1987–1992 (includes one case latter identified with an incorrect diagnosis date); population-based controls identified from the National Population Registry and matched (1:4 ratio) to cases for age and county.	111 cases 400 controls Cases, 91%; controls, 83%	Mailed questionnaire to assess self reported working history, specific exposure, and leisure time activities. Univariate analysis for chemical-specific exposures (any TCE exposure).
Fritschi and Siemiatycki (1996a); Siemiatycki (1991)	Male NHL cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	215 cases 533 population controls (Group 1) and 1,900 subjects with other cancers (Group 2) Cases, 83%; controls, 71%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales). Exposure metric defined as any or substantial exposure. Logistic regression adjusted for age, proxy status, income, and ethnicity (solvents) or Mantel-Haenszel stratified by age, BMI, and cigarette smoking (TCE).
Hardell et al. (1994; 1981)	Histologically-confirmed cases of NHL in males, age 25–85 yrs, admitted to Swedish (Umea) hospital between 1974 and 1978; living controls (1:2 ratio) from the National Population Register, matched to living cases on sex, age, and place of residence; deceased controls from the National Registry for Causes of Death, matched (1:2 ratio) to dead cases on sex, age, place of residence, and year of death.	105 cases 335 controls Response rate not available	Self-administered questionnaire assessing self-reported solvent exposure; phone follow-up with subject, if necessary. Mantel-Haenszel χ^2.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Persson et al. (1993); Persson et al. (1989)	Histologically confirmed cases of Hodgkin lymphoma, age 20–80 yrs, identified in two hospitals in Sweden: Oreboro in 1964–1986 (Persson et al., 1989) and in Linkoping between 1975 and 1984 (Persson et al., 1993); controls randomly selected from population registers.	54 cases (1989 study); 31 cases (1993 study) 275 controls (1989 study); 204 controls (1993 study) Response rate not available	Mailed questionnaire to assess self reported occupational exposures to TCE and other solvents. Logistic regression with adjustment for age and other exposure; unadjusted Mantel-Haenszel χ^2.
Childhood leukemia			
Shu et al. (2004; 1999)	Childhood leukemia cases, <15 yrs, diagnosed between 1989 and 1993 by a Children's Cancer Group member or affiliated institute; population controls (random digit dialing), matched for age, race, and telephone area code and exchange.	1,842 cases 1,986 controls Cases, 92%; controls, 77%	Telephone interview with mother, and whenever available, fathers using questionnaire to assess occupation using job-industry title and self-reported exposure history. Questionnaire included questions specific for solvent, degreaser, or cleaning agent exposures. Logistic regression with adjustment for maternal or paternal education, race, and family income. Analyses of paternal exposure also included age and sex of the index child.
Costas et al. (2002); MDPH (1997b)	Childhood leukemia (<19 yrs of age) diagnosed in 1969–1989 and who were resident of Woburn, Massachusetts; controls randomly selected from Woburn public School records, matched for age.	19 cases 37 controls Cases, 91%; controls, not available	Questionnaire administered to parents separately assessing demographic and lifestyle characteristics, medical history information, environmental and occupational exposure, and use of public drinking water in the home. Hydraulic mixing model used to infer delivery of TCE and other solvents water to residence. Logistic regression with composite covariate, a weighted variable of individual covariates.
McKinney et al. (1991)	Incident childhood leukemia and NHL cases, 1974–1988, ages not identified, from three geographical areas in England; controls randomly selected from children of residents in the three areas and matched for sex and birth health district.	109 cases 206 controls Cases, 72%; controls, 77%	In-person interview with questionnaire with mother to assess maternal occupational exposure history, and with father and mother, as surrogate, to assess paternal occupational exposure history. No information provided in paper whether interviewer was blinded as to case and control status. Matched pair design using logistic regression for univariate and multivariate analysis.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Lowengart et al. (1987)	Childhood leukemia cases aged <10 yrs and identified from the Los Angeles (California) Cancer Surveillance Program in 1980–1984; controls selected from random digit dialing or from friends of cases and matched on age, sex, and race.	123 cases 123 controls Cases, 79%; controls, not available	Telephone interview with questionnaire to assess parental occupational and self-reported exposure history. Matched (discordant) pair analysis.
Melanoma			
Fritschi and Siemiatycki (1996b); Siemiatycki (1991)	Male melanoma cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	103 cases 533 population controls and 533 other cancer controls Cases, 78%; controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, and ethnic origin (TCE) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, and ethnic origin (TCE).
Prostate			
Aronson et al. (1996); Siemiatycki (1991)	Male prostate cancer cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	449 cases 533 population controls (Group 1) and other cancer cases from same study (Group 2) Cases, 81%; controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales). Logistic regression adjusted for age, ethnic origin, SES, Quetlet, and respondent status (occupation) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, ethnic origin, and respondent status (TCE).

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Renal cell			
Moore et al. (2010)	Cases aged 20–74 yrs from four European countries (Czech Republic, Poland, Russia, Romania) with histologically-confirmed kidney cancer in 1999–2003; hospital controls with diagnoses unrelated to smoking or genitourinary disorders in 1998–2003 and frequency matched by sex, age, and study center.	1,097 cases (825 renal cell carcinomas) 1,184 controls Cases, 90–99%; controls, 90.3–96%	In-person interview using questionnaire for information on lifestyle habits, smoking, antopometric measures, personal and family medical history, and occupational history. Specialized job-specific questionnaire for specific jobs or industries of interest focused on solvents exposure, including.TCE, with exposure assignment by expert blinded to case and control status by frequency, intensity and confidence of TCE exposure. Exposure metric of overall exposure, duration (total hours, years) and cumulative exposure. Logistic regression adjusted for sex, age, and study center. BMI, hypertension, smoking, and residence location also included in initial models but did not alter ORs by >10%.
Charbotel et al. (2009; 2006)	Cases from Arve Valley region in France identified from local urologists files and from area teaching hospitals; age- and sex-matched controls chosen from file of same urologist as who treated case or recruited among the patients of the case's general practitioner.	87 cases 316 controls Cases, 74%; controls, 78%	Telephone interview with case or control, or, if deceased, with next-of-kin (22% cases, 2% controls). Questionnaire assessing occupational history, particularly, employment in the screw cutting jobs, and medical history. Semiquantitative TCE exposure assigned to subjects using a task/TCE-Exposure Matrix designed using information obtained from questionnaires and routine atmospheric monitoring of workshops or biological monitoring (U-TCA) of workers carried out since the 1960s. Cumulative exposure, cumulative exposure with peaks, and TWA. Conditional logistic regression with covariates for tobacco smoking and BMI.
Brüning et al. (2003)	Histologically-confirmed cases 1992–2000 from German hospitals (Arnsberg); hospital controls (urology department) serving area, and local geriatric department, for older controls, matched by sex and age.	134 cases 401 controls Cases, 83%; controls, not available	In-person interviews with case or next-of-kin; questionnaire assessing occupational history using job title. Exposure metrics included longest job held, JEM of Pannett et al. (1985) to assign cumulative exposure to TCE and perchloroethylene, and exposure duration. Logistic regression with covariates for age, sex, and smoking.
Pesch et al. (2000b)	Histologically-confirmed cases from German hospitals (five regions) in 1991–1995; controls randomly selected from residency registries matched on region, sex, and age.	935 cases 4,298 controls Cases, 88%; controls, 71%	In-person interview with case or next-of-kin; questionnaire assessing occupational history using job title (JEM approach), self-reported exposure, or job task (JTEM approach) to assign TCE and other exposures. Logistic regression with covariates for age, study center, and smoking.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Parent et al. (2000a); Siemiatycki (1991)	Male RCC cases, age 35–75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979–1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing.	142 cases 533 population controls (Group 1) and other cancer controls (excluding lung and bladder cancers) (Group 2) Cases, 82%; controls, 71%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (about 300 exposures on semiquantitative scales); TCE defined as any or substantial exposure. Mantel-Haenszel stratified by age, BMI, and cigarette smoking (TCE) or logistic regression adjusted for respondent status, age, smoking, and BMI (occupation, job title).
Dosemeci et al. (1999)	Histologically-confirmed cases, 1988–1990, white males and females, 20–85 yrs, from Minnesota Cancer Registry; controls stratified for age and sex using random digit dialing, 21–64 yrs, or from HCFA records, 64–85 yrs.	438 cases 687 controls Cases, 87%; controls, 86%	In-person interviews with case or next-of-kin; questionnaire assessing occupational history of TCE using job title and JEM of Gomez et al. (1994). Exposure metric was any TCE exposure. Logistic regression with covariates for age, smoking, hypertension, and BMI.
Vamvakas et al. (1998)	Cases who underwent nephrectomy in 1987–1992 in a hospital in Arnsberg region of Germany; controls selected accident wards from nearby hospital in 1992.	58 cases 84 controls Cases, 83%; controls, 75%	In-person interview with case or next-of-kin; questionnaire assessing occupational history using job title or self-reported exposure to assign TCE and perchloroethylene exposure. Logistic regression with covariates for age, smoking, BMI, hypertension, and diuretic intake.
Multiple or other sites			
Lee et al. (2003)	Liver, lung, stomach, colorectal cancer deaths in males and females between 1966 and 1997 from two villages in Taiwan; controls were cardiovascular and cerebral-vascular disease deaths from same underlying area as cases.	53 liver, 39 stomach, 26 colorectal, and 41 lung cancer cases; 286 controls Response rate not reported	Residence as recorded on death certificate. Mantel-Haenszel stratified by age, sex, and time period.
Kernan et al. (1999)	Pancreatic deaths, 1984–1993, in 24 states; noncancer death and non-pancreatic disease death controls, frequency matched to cases by age, gender, race, and state.	63,097 pancreatic cancer cases 252,386 noncancer population controls Response rate not reported	Usual occupation and industry on death certificate coded to standardized occupation codes and industry codes for 1980 U.S. census. Potential exposure to 11 chlorinated hydrocarbons, including TCE, assessed using JEM of Gomez et al. (1994). Logistic regression adjusted for age, marital status, gender, race, and metropolitan and residential status.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Siemiatycki (1991)	Male cancer cases, 1979–1985, 35–75 yrs, diagnosed in 16 Montreal-area hospitals, histologically confirmed; cancer controls identified concurrently; age-matched, population-based controls identified from electoral lists and random digit dialing.	857 lung and 117 pancreatic cancer cases 533 population controls (Group 1) and other cancer cases from same study (Group 2) Cases, 79% (lung), 71% (pancreas); controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); TCE defined as any or substantial exposure. Mantel-Haenszel stratified on age, income, index for cigarette smoking, ethnic origin, and respondent status (lung cancer) and age, income, index for cigarette smoking, and respondent status (pancreatic cancer).

HCFA = Health Care Financing Administration; NCI =; UV = ultra-violet

Table B-3. Geographic-based studies assessing cancer and TCE exposure

Reference	Description	Analysis approach	Exposure assessment
Broome County, New York studies			
ATSDR (2006a, 2008b)	Total, 22 site-specific, and childhood cancer incidence from 1980 to 2001 among residents in two areas in Endicott, New York.	SIR among all subjects (ATSDR, 2006a) or among white subjects only (ATSDR, 2008b) with expected numbers of cancers derived using age-specific cancer incidence rates for New York State, excluding New York City. Limited assessment of smoking and occupation using medical and other records in lung and kidney cancer subjects (ATSDR, 2008b).	Two study areas, Eastern and Western study areas, identified based on potential for soil vapor intrusion exposures as defined by the extent of likely soil vapor contamination. Contour lines of modeled VOC soil vapor contamination levels based on exposure model using GIS mapping and soil vapor sampling results taken in 2003. The study areas were defined by 2000 Census block boundaries to conform to model predicted areas of soil vapor contamination. TCE was the most commonly found contaminant in indoor air in Eastern study area at levels ranging from 0.18 to 140 $\mu g/m^3$, with tetrachloroethylene, cis-1,2-dichloroethene, 1,1,1-trichloroethane, 1,1-DCE, 1,1-dichloroethane, and Freon 113 detected at lower levels. Perchloroethylene was most common contaminant in indoor air in Western study area with other VOCs detected at lower levels.
Maricopa County, Arizona studies			
Aickin et al. (1992); Aickin (2004)	Cancer deaths, including leukemia, 1966–1986, and childhood (≤19 yrs old) leukemia incident cases (1965–1986), Maricopa County, Arizona.	Standardized mortality rate ratio from Poisson regression modeling. Childhood leukemia incidence data evaluated using Bayes methods and Poisson regression modeling.	Location of residency in Maricopa County, Arizona, at the time of death as surrogate for exposure. Some analyses examined residency in West Central Phoenix and cancer. Exposure information is limited to TCE concentration in two drinking water wells in 1982.
Pima County, Arizona studies			
ADHS (1995, 1990)	Cancer incidence in children (≤19 yrs old) and testicular cancer in 1970–1986 and 1987–1991, Pima County, Arizona.	Standardized incidence RR from Poisson regression modeling using method of Aickin et al. (1992). Analysis compares incidence in Tucson Airport Area to rate for rest of Pima County.	Location of residency in Pima, County, Arizona, at the time of diagnosis or death as surrogate for exposure. Exposure information is limited to monitoring since 1981 and include VOCs in soil gas samples (TCE, perchloroethylene, 1,1-DCE, 1,1,1-trichloroacetic acid); PCBs in soil samples, and TCE in municipal water supply wells.

Table B-3. Geographic-based studies assessing cancer and TCE exposure (continued)

Reference	Description	Analysis approach	Exposure assessment
Other			
Coyle et al. (2005)	Incident breast cancer cases among men and women, 1995–2000, reported to Texas Cancer Registry.	Correlation study using rank order statistics of mean average annual breast cancer rate among women and men and atmospheric release of 12 hazardous air pollutants.	Reporting to EPA Toxic Release Inventory the number of pounds released for 12 hazardous air pollutants, (carbon tetrachloride, formaldehyde, methylene chloride, styrene, tetrachloroethylene, TCE, arsenic, cadmium, chromium, cobalt, copper, and nickel).
Morgan and Cassady (2002)	Incident cancer cases, 1988–1989, among residents of 13 census tracts in Redlands area, San Bernardino County, California.	SIR for all cancer sites and 16 site-specific cancers; expected numbers using incidence rates of site-specific cancer of a four-county region between 1988 and 1992.	TCE and perchlorate detected in some county wells; no information on location of wells to residents, distribution of contaminated water, or TCE exposure potential to individual residents in studied census tracts.
Vartiainen et al. (1993)	Total cancer and site-specific cancer cases (lymphoma sites and liver) from 1953 to 1991 in two Finnish municipalities.	SIR with expected number of cancers and site-specific cancers derived from incidence of the Finnish population.	Monitoring data from 1992 indicated presence of TCE, tetrachloroethylene and 1,1,1-trichloroethane in drinking water supplies in largest towns in municipalities. Residence in town used to infer exposure to TCE.
Cohn et al. (1994b); Fagliano et al. (1990)	Incident leukemia and NHL cases, 1979–1987, from 75 municipalities and identified from the New Jersey State Cancer Registry. Histological type classified using WHO scheme and the classification of NIH Working Formulation Group for grading NHL.	Logistic regression modeling adjusted for age.	Monitoring data from 1984 to 1985 on TCE, trihalomethanes, and VOCs concentrations in public water supplies, and historical monitoring data conducted in 1978–1984.
Mallin (1990)	Incident bladder cancer cases and deaths, 1978–1985, among residents of nine northwestern Illinois counties.	SIR and SMR by county of residence and zip code; expected numbers of bladder cancers using age-race-sex specific incidence rates from SEER or bladder cancer mortality rates of the U.S. population from 1978 to 1985.	Exposure data are lacking for the study population with the exception of noting one of two zip code areas with observed elevated bladder cancer rates also had groundwater supplies contaminated with TCE, perchloroethylene, and other solvents.
Isacson et al. (1985)	Incident bladder, breast, prostate, colon, lung, and rectal cancer cases reported to Iowa cancer registry between 1969 and 1981.	Age-adjusted site-specific cancer incidence in Iowa towns with populations of 1,000–10,000 and who were serviced by a public drinking water supply.	Monitoring data of drinking water at treatment plant in each Iowa municipality with populations of 1,000–10,000 used to infer TCE and other VOC concentrations in finished drinking water supplies.

Category A: Study Design

- Clear articulation of study objectives or hypothesis. The ideal is a clearly stated hypothesis or study objectives and the study is designed to achieve the identified objectives.
- Selection and characterization in cohort studies of exposure and control groups and of cases and controls (case-control studies) is adequate. The ideal is for selection of cohort and referents from the same underlying population and differences between these groups are due to TCE exposure or level of TCE exposure and not to physiological, health status, or lifestyle factors. Controls or referents are assumed to lack or to have background exposure to TCE. These factors may lead to a downward bias including one of which is known as "healthy worker bias," often introduced in analyses when mortality or incidence rates from a large population such as the U.S. population are used to derive expected numbers of events. The ideal in case-control studies is cases and controls are derived from the same population and are representative of all cases and controls in that population. Any differences between controls and cases are due to exposure to TCE itself and not to confounding factors related to both TCE exposure and disease. Additionally, the ideal is for controls to be free of any disease related to TCE exposure. In this latter case, potential bias is toward the null hypothesis.

Category B: Endpoint Measured

- Levels of health outcome assessed. Three levels of health outcomes are considered in assessing the human health risks associated with exposure to TCE: biomarkers of effects and susceptibility, morbidity, and mortality. Both morbidity as enumerated by incidence and mortality as identified from death certificates are useful indicators in risk assessment for hazard identification. The ideal is for accurate and predictive indicator of disease. Incidence rates are generally considered to provide an accurate indication of disease in a population and cancer incidence is generally enumerated with a high degree of accuracy in cancer registries. Death certifications are readily available and have complete national coverage but diagnostic accuracy is reduced and can vary by specific diagnosis. Furthermore, diagnostic inaccuracies can contribute to death certificates as a poor surrogate for disease incidence. Incidence, when obtained from population-based cancer registries, is preferred for identifying cancer hazards.
- Changes in diagnostic coding systems for lymphoma, particularly NHL. Classification of lymphomas today is based on morphologic, immunophenotypic, genotypic, and clinical features and is based upon the WHO classification, introduced in 2001, and incorporation of WHO terminology into International Classification of Disease (ICD)-0-3. ICD Versions 7 and earlier had rubrics for general types of lymphatic and hematopoietic cancer, but no categories for distinguishing specific types of cancers, such as acute leukemia. Epidemiologic studies based on causes of deaths as coded using these older ICD classifications typically grouped together lymphatic neoplasms instead of examining individual types of cancer or specific cell types. Before the use of immunophenotyping, these grouping of ambiguous diseases such as NHL and Hodgkin lymphoma may be have misclassified. Lymphatic tumors coding, starting in 1994 with the introduction of the Revised European-American Lymphoma classification, the basis of the current WHO

classification, was more similar to that presently used. Misclassification of specific types of cancer, if unrelated to exposure, would have attenuated estimate of RR and reduced statistical power to detect associations. When the outcome was mortality, rather than incidence, misclassification would be greater because of the errors in the coding of underlying causes of death on death certificates (IOM, 2003). Older studies that combined all lymphatic and hematopoietic neoplasms must be interpreted with care.

Category C: TCE-Exposure Criteria

- Adequate characterization of exposure. The ideal is for TCE exposure potential known for each subject and quantitative assessment (job-exposure-matrix approach) of TCE exposure assessment for each subject as a function of job title, year exposed, duration, and intensity. Consideration of job task as additional information supplementing job title strengthens assessment increases specificity of TCE assignment. The assessment approach is accurate for assigning TCE intensity (TCE concentration or a TWA) to individual study subjects and estimates of TCE intensity are validated using monitoring data from the time period. The objective for cohort and case-controls studies is to differentiate TCE exposed subjects from subjects with little or no TCE exposure. A variety of dose-metrics may be used to quantify or classify exposures for an epidemiologic study. They include precise summaries of quantitative exposure, concentrations of biomarkers, cumulative exposure, and simple qualitative assessments of whether exposure occurred (yes or no). Each method has implicit assumptions and potential problems that may lead to misclassification. Exposure assessment approaches in which it was unclear that the study population was actually exposed to TCE are considered inferior since there may be a lower likelihood or degree of exposure to study subjects compared to approaches that assign known TCE exposure potential to each subject.

Category D: Follow-up (Cohort)

- Loss to follow-up. The ideal is complete follow-up of all subjects; however, this is not achievable in practice, but it seems reasonable to expect loss to follow-up not to exceed 10%. The bias from loss to follow-up is indeterminate. Random loss may have less effect than if subjects who are not followed have some significant characteristics in common.
- Follow-up period allows full latency period for over 50% of the cohort. The ideal to follow all study subjects until death. Short of the ideal, a sufficient follow-up period to allow for cancer induction period or latency over 15 or 20 years is desired for a large percentage of cohort subjects.

Category E: Interview Type (Case-control)

- Interview approach. The ideal interviewing technique is face-to-face by trained interviewers with >90% of interviews with cases and control subjects conduced face-to-

face. The effect on the quality of information from other types of data collection is unclear, but telephone interviews and mail-in questionnaires probably increase the rate of misclassification of subject information. The bias is toward the null hypothesis if the proportion of interview by type is the same for case and control, and of indeterminate direction otherwise.

- Blinded interviewer. The ideal is for the interviewer to be unaware whether the subject is among the cases or controls and the subject to be unaware of the purpose and intended use of the information collected. Although desirable for case-control studies, blinding is usually not possible to fully accomplish because subject responses during the interview provide clues as to subject status. In face-to-face and telephone interviews, potential bias may arise from the interviewer expects regarding the relationship between exposure and cancer incidence. The potential for bias from face-to-face interviews is probably less than with mail-in interviews. Some studies have assigned exposure status in a blinded manner using a JEM and information collected in the unblinded interview. The potential for bias in this situation is probably less with this approach than for nonblinded assignment of exposure status.

Category F: Proxy Respondents

- Proxy respondents. The ideal is for data to be supplied by the subject because the subject generally would be expected to be the most reliable source; <10% of either total cases or total controls for case-control studies. A subject may be either deceased or too ill to participate, however, making the use of proxy responses unavoidable if those subjects are to be included in the study. The direction and magnitude of bias from use of proxies is unclear, and may be inconsistent across studies.

Category G: Sample Size

- The ideal is for the sample size is large enough to provide sufficient statistical power to ensure that any elevation of effect in the exposure group, if present, would be found, and to ensure that the confidence bounds placed on RR estimates can be well-characterized.

Category H: Analysis Issues

- Control for potentially confounding factors of importance in analysis. The ideal in cohort studies is to derive expected numbers of cases based on age-sex- and time-specific cancer rates in the referent population and in case-control studies by matching on age and sex in the design and then adjusting for age in the analysis of data. Age and sex are likely correlated with exposure and are also risk factors for cancer development. Similarly, other factors such as cigarette smoking and alcohol consumption are risk factors for several site-specific cancers reported as associative with TCE exposure. To be a confounder of TCE, exposure to the other factor must be correlated, and the association of the factor with the site-specific cancer must be causal. The expected effect from controlling for confounders is to move the estimated RR estimate closer to the true value.

- Statistical methods are appropriate. The ideal is that conclusions are drawn from the application of statistical methods that are appropriate to the problem and accurately interpreted.
- Evaluation of exposure-response. The ideal is an examination of a linear exposure-response as assessed with a quantitative exposure metric such as cumulative exposure. Some studies, absent quantitative exposure metrics, examine exposure response relationships using a semiquantitative exposure metric or by duration of exposure. A positive dose-response relationship is usually more convincing of an association as causal than a simple excess of disease using TCE dose-metric. However, a number of reasons have been identified for a lack of linear exposure-response finding and the failure to find such a relationship means little from an etiological viewpoint and does not minimize an observed association with overall TCE exposure.
- Documentation of results. The ideal is for analysis observations to be completely and clearly documented and discussed in the published paper, or provided in supplementary materials accompanying publication.

B.2.1. Study Designs and Characteristics

The epidemiologic designs investigating TCE exposure and cancer include cohort studies of occupationally exposure populations, population case-control studies, and geographic studies of residents in communities with TCE in water supplies or ambient air. Analytical epidemiologic studies, which include case-control and cohort designs, are generally relied on for identifying a causal association between human exposure and adverse health effects (U.S. EPA, 2005b) due to their clear ability to show exposure precedes disease occurrence. In contrast, ecologic studies such as health surveys of cancer incidence or mortality in a community during a specified time period (i.e., geographic-based studies identified in Table B-3, provide correlations between rates of cancer and exposure measured at the geographic level).

An epidemiologic study's ability to inform a question on TCE and cancer depends on clear articulation of study objective or hypothesis and adequate selection of exposed and control group in cohort studies and cases and controls in case-control studies are important. As the body of evidence on TCE has grown over the past 20 years, so has the number of studies with clearly articulated hypothesis. All Nordic cohort studies (Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994) are designed to examine cancer and TCE, albeit some with limited statistical power, as are recent cohort studies of U.S. occupationally exposed populations (Radican et al., 2008; Boice et al., 2006b; Zhao et al., 2005; Boice et al., 1999; Ritz, 1999a). Exposure assessment approaches in these studies distinguished subjects with varying potentials for TCE exposure, and in some cases, assigned a semiquantitative TCE exposure surrogate to individual study subjects. Three case-control studies nested in cohorts, furthermore, examined TCE exposure and site-specific cancer, albeit a subject's potential and overall prevalence of TCE exposure greatly varied between these studies (Krishnadasan et al., 2007; Greenland et al., 1994; Wilcosky et al., 1984). Typically, studies of all workers at a plant or

manufacturing facility (Clapp and Hoffman, 2008; 2008; 2007; Chang et al., 2005; 2004a; Chang et al., 2003; Sinks et al., 1992; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Shannon et al., 1988; Shindell and Ulrich, 1985) are not designed to evaluate cancer and TCE specifically, given their inability to identify varying TCE exposure potential for individual study subjects; rather, such studies evaluate the health status of the entire population working at that facility. Bias associated with exposure misclassification is greater in these studies, and for this and other reasons more fully discussed below, they are of limited utility for informing evaluations on TCE exposure and cancer.

Recent case-control studies with hypotheses specific for TCE exposure include the kidney cancer case-control studies of Vamvakas et al. (1998), Brüning et al. (2003), and Charbotel et al. (2009; 2006). More common, population-based, case-control studies assess occupational exposure to organic solvents, using a JEM approach for exposure assessment to examine organic solvent categories (i.e., aliphatic hydrocarbons, or specific solvents such as TCE). The case-control studies of Costas et al. (2002) and Lee et al. (2003) were also designed to examine possible association with contaminated drinking water containing TCE and other solvents detected at lower concentrations. The hypothesis of Siemiatycki (1991) and ancillary publications (Goldberg et al., 2001; Dumas et al., 2000; Parent et al., 2000a; Fritschi and Siemiatycki, 1996a; Siemiatycki et al., 1994) explored possible association between 20 site-specific cancers and occupational title or chemical exposures, including TCE exposure, using a contemporary exposure assessment approach for more focused research investigation.

Cases and control selection in most population-based case-control studies of TCE exposure are considered a random sample and representative of the source population [Gold et al., 2011; Cocco et al., 2010; Moore et al., 2010; Charbotel et al., 2009; Seidler et al., 2007; Charbotel et al., 2006; Miligi et al., 2006; Shu et al., 2004; Brüning et al., 2003; Lee et al., 2003; Costas et al., 2002; De Roos et al., 2001; Pesch et al., 2000a, 2000b; Dosemeci et al., 1999; Kernan et al., 1999; Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994; Heineman et al., 1994; McKinney et al., 1991; Lowengart et al., 1987Siemiatycki et al., 1991 (and related publications: Siemiatycki et al., 1994; Aronson et al., 1996; Fritschi and Siemiatycki 1996b; Dumas et al., 2000; Parent et al., 2000b; Goldberg et al., 2001, and Fritschi and Siemiatycki, 1996a)].

Case and control selection in Vamvakas et al. (1998), a study conducted in the Arnsberg area of Germany, is subject to criticism regarding possible selection bias resulting from differences in selection criteria, cases worked in small industries and controls from a wider universe of industries; differences in age, controls being younger than cases with possible lower exposure potentials; and temporal difference in case and control selection, controls selected only during the last year of the study period with possible lower exposure potential if exposure has decreased over period of the study (NRC, 2006). The potential for selection bias in Brüning et al. (2003), another study in the same area as Vamvakas et al. (1998) but of later period of

observation, was likely reduced compared to Vamvakas et al. (1998) due to the broader region of southern Germany from which cases were identified and interviewing cases and controls during the same time. One case-control study nested in a cohort (Greenland et al., 1994) included subjects whose deaths were reported to and known by the employer, e.g., occurred among vested or pensioned employees or among currently employees. A 10–15-year employment period was required for subjects in this study to receive a pension; deaths among employees who left employment before this time were not known to the employer and not included the study. Survivor bias, a selection bias, may be introduced by excluding nonpensioned workers or those who leave employment before becoming vested in a company's retirement plan is more likely than in a study of all employees with complete follow-up. The use of pensioned deaths as controls, as was done in this study, would reduce potential bias if both cases and control had the same likelihood of becoming pensioned. That is, the probability for becoming a pensioned worker is similar for all deaths and unrelated to the likelihood of exposure or magnitude of exposure and disease. No information was available in (Greenland et al., 1994) to evaluate this assumption.

Geographic-based and ecological studies of TCE contaminated water supplies typically focus on estimating cancer or other disease rates in geographically circumscribed populations who are geospatially located with a source containing TCE, e.g., a hazardous waste site, well water, or air. These studies are often less informative for studying cancer due to their inability to estimate incidence rate ratios, essential for causal inferences, inferior exposure assessment approach, and to possible selection biases. Ecological studies also are subject to bias known as "ecological fallacy" since variables of exposure and outcome measured on an aggregate level do not represent association at the individual level. Consideration of this bias is important for diseases with more than one risk factor, such as the site-specific cancers evaluated in this assessment.

B.2.2. Outcomes Assessed in TCE Epidemiologic Studies

The epidemiologic studies consider at least three levels of health outcomes in their examinations of human health risks associated with exposure to TCE: biomarkers of effects and susceptibility, morbidity, and mortality (NRC, 2006). Few susceptibility biomarkers have been examined and these are not specific to TCE (NRC, 2006). By far, the bulk of the literature on cancer and TCE exposure is of cancer morbidity (Gold et al., 2011; Purdue et al., 2011; Cocco et al., 2010; Moore et al., 2010; Charbotel et al., 2009; Wang et al., 2009; Sung et al., 2008; Seidler et al., 2007; ATSDR, 2006a; Charbotel et al., 2006; Miligi et al., 2006; Coyle et al., 2005; Aickin, 2004; Shu et al., 2004; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Costas et al., 2002; Morgan and Cassady, 2002; De Roos et al., 2001; Hansen et al., 2001; Dumas et al., 2000; Pesch et al., 2000a, 2000b; Dosemeci et al., 1999; Persson and Fredrikson, 1999; Nordström et al., 1998; Vamvakas et al., 1998; ADHS, 1995; Anttila et al., 1995; Axelson et al., 1994;. Cohn

et al., 1994b;; Hardell et al., 1994; Persson et al., 1993; Vartiainen et al., 1993; McKinney et al., 1991; Siemiatycki, 1991; ADHS, 1990; Fredriksson et al., 1989; Shannon et al., 1988; Lowengart et al., 1987; Isacson et al., 1985), mortality (Clapp and Hoffman, 2008; Radican et al., 2008; Boice et al., 2006b; ATSDR, 2004a; Lee et al., 2003; Boice et al., 1999; Kernan et al., 1999; Ritz, 1999a; Morgan et al., 1998; Greenland et al., 1994; Heineman et al., 1994; Aickin et al., 1992; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Shindell and Ulrich, 1985; Wilcosky et al., 1984), or both (Sung et al., 2007; Chang et al., 2005; Zhao et al., 2005; Chang et al., 2003; Blair et al., 1998; Henschler et al., 1995; Sinks et al., 1992).

Mortality is readily identified from death certificates; however, diagnostic accuracy from death certificates varies by the specific diagnosis (Brenner and Gefeller, 1993). Incident cancer cases are enumerated more accurately by tumor registries and by hospital pathology records and cases identified from these sources are considered to have less bias resulting from disease misclassification than cause or underlying cause of death as noted on death certificates. Studies of incidence are preferred, particularly for examining association with site-specific cancers having high 5-year survival rates or which may be misclassified on death certificate. Misclassification of the cause of death as noted on death certificates attenuates statistical power through errors of outcome identification. This nondifferential misclassification of outcome in cohort studies will lead to attenuation of rate ratios, although the magnitude of is difficult to predict (NRC, 2006). Cancer registries are used for cases diagnosed in more recent time periods and cohorts whose entrance dates are 30 or 40 years may miss many incident cancers and reduced statistical power as a consequence. Two studies examine both cancer incidence and mortality (Zhao et al., 2005; Blair et al., 1998). The lapse of ≥20 years in Blair et al. (1998) and 38 years in Zhao et al. (2005) between date of cohort identification and cancer incidence ascertainment suggests these studies are missing cases and limits incidence examinations.

B.2.3. Disease Classifications Adopted in TCE Epidemiologic Studies

Disease coding and changes over time are important in epidemiologic evaluations, particularly in evaluation of heterogeneity or consistency of observations from a body of evidence. The ICD, published by WHO, is used to code underlying and contributing cause of death on death certificates and is updated periodically, adding to diagnostic inconsistency for cross-study comparisons (NRC, 2006). Tumor registries use the International Classification of Diseases-Oncology (ICD-O) for coding the site and the histology of neoplasms, principally obtained from a pathology report.

The epidemiologic studies of TCE exposure have used a number of different classification systems (Scott and Chiu, 2006). A number of studies classified neoplasms according to ICD-O (Gold et al., 2011; Purdue et al., 2011; Moore et al., 2010; Chang et al., 2005; Costas et al., 2002; Siemiatycki, 1991) or to ICD-9 (Zhao et al., 2005; Kernan et al., 1999; Ritz, 1999a; Nordström et al., 1998). Other ICD revisions used in recent studies include ICDA-8

(Blair et al., 1998; Greenland et al., 1994; Blair et al., 1989), ICD-7 (Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994), or several ICD revisions, whichever was in effect at the date of death (Radican et al., 2008; Morgan et al., 2000; Boice et al., 1999; Morgan et al., 1998; Garabrant et al., 1988). In this latter case, changes in disease classification over revisions are not harmonized or recoded to a common classification; and diagnostic inconsistencies and disease misclassification errors leads to a greater likelihood for bias in these studies. Greatest weight is placed on studies where all cases or deaths are classified using current classification systems. However, association in studies adopting older revisions, ICD 7 (Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994), for example, is noteworthy given the narrow consideration of lymphoid neoplasms compared to contemporary classification systems. Consistency examinations of the overall body of evidence using meta-analysis methods and examination of heterogeneity will need to consider study differences in coding in interpreting findings.

A major shift in thinking occurred around 1995 with the Revised European-American Lymphoma (REAL) classification of grouping diseases of the blood and lymphatic tissues along their cell lines compared to previous approaches to group lymphomas by a cell's physical characteristics. It was increasing recognized that some NHLs and corresponding lymphoid leukemias were different phases (solid and circulating) of the same disease entity (Morton et al., 2007). Many concepts of contemporary knowledge of lymphomas are incorporated in the WHO Classification of Neoplastic Diseases of the Hematopoietic and Lymphoid Tissues, an international consensus scheme for classifying leukemia and lymphoma now in use and the predecessor to REAL (IARC, 2001). Both the ICD-O, 3rd edition, and ICD-10 have adopted the WHO classification framework.

The only study coding NHLs using the WHO classification is (Cocco et al., 2010). Other NHL studies have adopted older lymphoma classification systems, either the NCI's Working Formulation (Costantini et al., 2008; Miligi et al., 2006) or other systems coding lymphomas according to NCI's Working Formulation (i.e., International Classification of Disease-Oncology, 2nd Edition (Gold et al., 2011; Purdue et al., 2011; Wang et al., 2009)]) that divided lymphomas into low-grade, intermediate-grade and high grade, with subgroups based on cell type and presentation, or Rappaport (Hardell et al., 1994; 1981), with groupings based on microscopic morphology (Lymphoma Information Network, 2008). Both Purdue et al. (2011) and Gold et al. (2011) provide equivalent ICD-O-3 morphology codes (http://www.seer.cancer.gov/tools/conversion/ICDO2-3manual.pdf, accessed April 6, 2011,). Lowengart et al. (1987), Persson et al. (1993; 1989), McKinney et al. (1991), and Persson and Fredriksson (1999) do not provide information in their published articles on lymphomas classification systems used in these studies.

Implications of classification changes are most significant for NHL. As noted by the IOM (2003), in Revision 7 and earlier editions of the ICD, all lymphatic and hematopoietic

neoplasms were grouped together instead of treated as individual types of cancer (such as Hodgkin lymphoma) or specific cell types (such as acute lymphocytic leukemia). One limitation of this treatment was the amalgamation of these relatively rare cancers would increase the apparent sample size but could also result in diluted estimates of effect if etiologic heterogeneity of different lymphoma subtypes existed (i.e., different sites of cancer were not associated in similar ways with the exposures of interest). Additionally, immunophenotyping was not available, leading to decreased ability to distinguish ambiguous diseases, and diagnoses of these cancers may have been misclassified; for example, NHL may have been grouped with other lymphatic and hematopoietic cancers to increase statistical power or misclassified as Hodgkin lymphoma, for example. Examination of distinct lymphoma subtypes is expected to reduce disease misclassification bias. Five case-control studies on NHL include analysis of lymphoma subtype and TCE exposure (Gold et al., 2011; Purdue et al., 2011; Cocco et al., 2010; Costantini et al., 2008; Miligi et al., 2006).

A change in liver cancer coding occurred between ICDA-8 and ICD-9 and is important to consider in examinations of liver cancer observations across the TCE studies. With ICD-9, liver cancer "not specified as primary or secondary" was moved from the grouping of secondary malignant neoplasms and added to the larger class of malignant liver neoplasms. Thus, a similar grouping of liver cancer causes is necessary to cross-study comparisons. For example, an examination of liver cancer, based on ICDA-8, would need to include codes for liver and intrahepatic bile duct (code 155) and liver, not specified as primary or secondary (code 197.8), but, for ICD-9, would include liver and intrahepatic bile duct (code 155) only. The effect of adding "liver cancer, not specified as primary or secondary" to the larger liver and intrahepatic bile duct category in ICD-9 was a twofold increase in the overall liver cancer mortality (Percy et al., 1990).

B.2.4. Exposure Classification

Adequacy of exposure assessment approaches and their supporting data are a critical determinant of a study's contribution in a weight-of-evidence evaluation (Checkoway et al., 1989). Exposure assessment approaches in studies of TCE and cancer vary greatly. At one extreme, studies assume subjects are exposed by residence in a defined geographic area (ATSDR, 2008b, 2006a; Coyle et al., 2005; Aickin, 2004; Lee et al., 2003; Morgan and Cassady, 2002; ADHS, 1995; Cohn et al., 1994b; Vartiainen et al., 1993; Aickin et al., 1992; ADHS, 1990; Isacson et al., 1985) or by employment in a plant or job title (Clapp and Hoffman, 2008; Sung et al., 2008; Sung et al., 2007; Chang et al., 2005; ATSDR, 2004a; Chang et al., 2003; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Shannon et al., 1988; Shindell and Ulrich, 1985). This is a poor exposure surrogate given potential for TCE exposure can vary in these broad categories depending on job function, year, use of personal protection, and, for residential exposure, pollutant fate and transport, water system distribution characteristics, percent of time

per day in residence, presence of mitigation devices, drinking water consumption rates, and showering times. Another example comprises measurement from a subset of workers with jobs where TCE is routinely used to infer TCE exposure and TCE intensity to all subjects. In both examples, exposure misclassification potential may be extensive and with a downward bias in risk estimates.

At the other extreme and preferred given a reduced likelihood for misclassification bias, quantitative exposure assessment based upon a subject's job history, job title, and monitoring data are used to develop estimates of TCE intensity and cumulative exposure (quantitative exposure metrics or measures) and is known as JEM approaches. Peak exposure is also well characterized. Addition to JEM approaches of information on job tasks (JTEM) associated with exposure such as that done by Pesch et al. (2000a, 2000b) is expected to reduce potential exposure misclassification. In between these two extremes, semiquantitative estimates of low, medium, and high TCE exposure are assigned to subjects. Twenty-one studies assigned a quantitative or semiquantitative TCE surrogate metrics to individual subjects using a JEM, JTEM, or expert knowledge: (Siemiatycki, 1991) (and related publications (Goldberg et al., 2001; Dumas et al., 2000; Parent et al., 2000a; Aronson et al., 1996; Fritschi and Siemiatycki, 1996a, b; Siemiatycki et al., 1994); Blair et al. (1998) and follow-up by Radican et al. (2008); Morgan et al. (1998), Vamvakas et al. (1998), Kernan et al. (1999), Ritz (1999a), Pesch et al. (2000a, 2000b), Brüning et al. (2003), Zhao et al. (2005), Miligi et al. (2006), Charbotel et al. (2009; 2006), Krishnadansen et al. (2007), Seidler et al. (2007), Costantini et al. (2008), Wang et al. (2009), Cocco et al. (2010), Gold et al. , Moore et al. (2010), and Purdue et al. (2011).

Thirteen other studies assigned a qualitative TCE surrogate metric (ever exposed or never exposed), less preferred to a semi-quantitative exposure surrogate given greater likelihood for error associated exposure misclassification, using general job classification of job title by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, JEMs, water distribution models, for cohort studies, or obtained from subjects using questionnaire for case-control studies. The 13 studies were: Wilcosky et al. (1984), Lowengart et al. (1987), McKinney et al. (1991), Greenland et al. (1994), Hardell et al. (1994), Nordstrom et al. (1998), Shu et al. (1999), Boice et al. (2006b; 1999), Dosemeci et al. (1999), Persson and Fredriksson (1999), Costas et al. (2002), and Raaschou-Nielsen et al. (2003). Without quantitative measures, however, it is not possible to quantify exposure difference between groupings nor is it possible to compare similarly named categories across studies. Exposure misclassification for dichotomous exposure defined in these studies, if nondifferential, would downward bias resulting risk estimates.

Zhao et al. (2005), Krishnadansen et al. (2007), and Boice et al. (2006b) are studies with overlap in some subjects, but with different exposure assessment approaches, more fully discussed in Section B.3.1.1, with implication on study ability to identify cancer hazard. While these studies used job title to assign TCE exposure potential, Zhao et al. (2005) and

Krishnadansen et al. (2007) developed a semiquantitative estimate of TCE exposure potential, whereas Boice et al. (2006b) classified subjects as either "exposed" or "unexposed" using a qualitative surrogate. These studies, furthermore, identify TCE exposure potentially differently for possibly similar job titles. For example, jobs as instrument mechanics, inspectors, test stand engineers, and research engineers are identified with medium potential exposure in Zhao et al. (2005) and Krishnadansen et al. (2007); however, these job titles were considered in Boice et al. (2006b) as having background exposure and were combined with unexposed subjects, the referent population in Cox Proportional Hazard analyses.

Three Nordic cohorts have TCE exposure as indicated from biological markers, assigning TCE exposure to subjects using either concentration of TCA in urine or TCE in blood (Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994). The utility of a biomarker depends on it selectivity and the exposure situation. Urinary TCA (U-TCA) is a nonselective marker since other chlorinated solvents besides TCE are metabolized to TCA and resultant urinary elimination. If TCE is the only exposure, urinary TCE may be a useful marker; however, in setting with mixed exposure, urinary TCA may serve as an integrated exposure marker of several chlorinated solvents. The Nordic studies used the linear relationship found for average inhaled TCE vs. U-TCA: TCE (mg/m^3) = 1.96; U-TCA (mg/L) = 0.7 for exposures <375 mg/m^3 (69.8 ppm) (Ikeda et al., 1972). This relationship shows considerable variability among individuals, which reflects variation in urinary output and activity of metabolic enzymes. Therefore, the estimated inhalation exposures are only approximate for individuals but can provide reasonable estimates of group exposures. There is evidence of nonlinear formation of U-TCA above about 400 mg/m^3 or 75 ppm of TCE. The half-life of U-TCA is about 100 hours. Therefore, the U-TCA value represents roughly the weekly average of exposure from all sources, including skin absorption. The Ikeda et al. (1972) relationship can be used to convert urinary values into approximate airborne concentration, which can lead to misclassification if tetrachloroethylene and 1,1,1-trichloroethane are also being used because they also produce U-TCA. In most cases, the Ikeda et al. (1972) relationship provides a rough upper boundary of exposure to TCE.

B.2.5. Follow-up in TCE Cohort Studies

Cohort studies are most informative if vital status is ascertained for all cohort subjects and if the period of time for disease ascertainment is sufficient to allow for long latencies, particularly for cancer detection and death, in the case of mortality studies. Inability to ascertain vital status for all subjects, or, conversely, subjects who are loss-to-follow-up, can affect the validity of observations and lead to biased results. Both power and rate ratios estimated in cohort studies can be underestimated due to bias introduced if the follow-up period was not long enough to account for latency (NRC, 2006). The probability of loss to follow-up may be related to exposure, disease, or both. The multiple-stage process of cancer development occurs over

decades after first exposure and studies with full latent periods are considered to provide greater weight to the evaluation compared to cohort studies with shortened follow-up period and lower percentage of subjects whose vital status was known on the date follow-up ended. Vital status ascertainment for over 90% of all cohort studies and long mean follow-up periods, about 15 years of longer, characterized many occupational cohort studies on TCE and cancer (Blair et al., 1998; Anttila et al., 1995; Costa et al., 1989; Garabrant et al., 1988) and the follow-up study of Radican et al. (2008; Boice et al., 2006b; Zhao et al., 2005; Raaschou-Nielsen et al., 2003; Boice et al., 1999; Ritz, 1999a; Morgan et al., 1998). Information is lacking in two biomarker studies (Hansen et al., 2001; Axelson et al., 1994), additionally, to estimate the mean follow-up period for TCE-exposed subjects; although Hansen et al. (2001) state "some workers were followed for as long as 50 years after their exposure, which allowed the detection of cancers with long latency periods." Other studies of TCE and cancer did not identify a latent period, information for calculating a latent period, or contained other deficiencies in follow-up criteria (Sung et al., 2007; Chang et al., 2005; Henschler et al., 1995; Sinks et al., 1992; Blair et al., 1989; Costa et al., 1989; Shannon et al., 1988; Wilcosky et al., 1984). PMR studies, based only on deaths and which lack information on person-year structure as cohort studies, by definition, do not contain information on cancer latent periods or follow-up (Clapp and Hoffman, 2008; ATSDR, 2004a).

B.2.6. Interview Approaches in Case-Control Studies of Cancer and TCE Exposure

Interview approaches and the percentage of subjects with information obtained from proxy or next-of-kin respondents need consideration in interpreting population and hospital-based, case-control studies in light of possible biases. Biases resulting from proxy respondent or from low participation related to mailed questionnaires are not relevant to cohort or geographic studies since information is obtained from local, national, or corporate records. Both face-to-face and telephone interviews are common and valid approaches used in population or hospital-based case-control studies. Important to each is the use of a structured questionnaires combined with intensive training as ways to minimize a high potential for biases often associated with mailed questionnaires (Blatter et al., 1997; Schlesselman, 1982). Studies with information limited to job title, type of business and dates of employment and aided with computer or job-exposure-matrix approaches are preferred to studies of job title only; the added approaches can reduce exposure misclassification bias and improve disease risk estimates (Stewart et al., 1996). Moreover, interview with respondents other than the individual case or control, through proxy or next-of-kin respondents, may also introduce bias in case-control studies. Proxy respondents are used when cases or control are either too sick to respond or if deceased. This bias would dampen observed associations if proxy respondents did not fully provide accurate information. Boyle et al. (1992), for example, in their study of several site-specific cancers and occupational exposures found low sensitivity, or correct reporting, for occupational exposure to solvents among proxy

respondents. The weight-of-evidence analysis on TCE and cancer, for this reason, places greatest weight on observations from studies which obtain information on personal, medical, and occupational histories from each case and control with lesser weight is placed on studies where ≥10% of interviews are with proxy respondents.

Many of the more recent case-control studies include face-to-face (Gold et al., 2011; Purdue et al., 2011; Cocco et al., 2010; Moore et al., 2010; Wang et al., 2009; Seidler et al., 2007; Miligi et al., 2006; Brüning et al., 2003; Costas et al., 2002; Pesch et al., 2000a, 2000b; Dosemeci et al., 1999; Vamvakas et al., 1998; McKinney et al., 1991; Siemiatycki, 1991) or telephone (Charbotel et al., 2009; Charbotel et al., 2006; Shu et al., 2004; Shu et al., 1999; Lowengart et al., 1987) interviews. Few of these studies included interviewers who were blinded or did not know the identity of who is a case and who is a control. Although desirable for case-control studies, blinding is usually not possible to fully accomplish because subject responses during the interview provide clues as to subject status. For this reason, the lack of blinded interviewers is not considered a serious limitation. More importantly, most studies assigned exposure to cases and controls in a blinded manner

Information obtained from mailed questionnaire predominantly characterized older Nordic studies (Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994; Persson et al., 1993; Fredriksson et al., 1989; Persson et al., 1989; Hardell et al., 1981). One case-control study did not ascertain information from a questionnaire or through interviews, instead using occupation coded on death certificates to infer TCE exposure potential (Kernan et al., 1999). In all studies except Costas et al. (2002) and Kernan et al. (1999), assignment of potential TCE exposure to cases and controls, to different degrees depending on each study, is based on self-reported information on job title, and in some cases, to specific chemicals.

More common to the case-control studies on TCE and cancer was possible bias related to a higher percentage of proxy interviews. Seven studies (Gold et al., 2011; Purdue et al., 2011; Moore et al., 2010; Wang et al., 2009; Pesch et al., 2000a, 2000b; Dosemeci et al., 1999) excluded subjects with proxy interviews and the percentage of proxy interview among subjects in one other study is <10% (Nordström et al., 1998). Charbotel et al. (2009; 2006) furthermore presents analyses for data they considered as better quality, including higher confidence exposure information and excluding proxy respondents, in addition to analyses using both living and proxy respondents. A consideration of proxy interviews in studies of childhood cancers, which include an examination of paternal occupational exposure, is needed given a greater likelihood for bias if fathers are not directly interviewed and the father's occupational information is provided only by the child's mother. A good practice is for statistical analyses examining paternal occupational exposure to include only cases and controls with direct information provided by the fathers, such as De Roos et al. (2001), the only childhood cancer study (neuroblastoma) to exclude the use of proxy information.

B.2.7. Sample Size and Approximate Statistical Power

Cancer is generally considered a rare disease compared to more common health outcomes such as cardiovascular disease. Of all site-specific cancers, endocrine cancers of the breast prostate and lung cancer are most common, with age-adjusted incidence rates of 126 per 100,000 women (breast), 163 per 100,000 men (prostate), and 63.9 per 100,000 men and women (lung) (Ries et al., 2008). Several site-specific cancers including kidney cancer, liver cancer, and NHL that are of interest to TCE are rarer and consideration of study size and the influence on statistical power are factors for judging a study's validity and assessment of a study's contribution to the overall weight of evidence for identifying a hazard. For example, the age-adjusted incidence rates of NHL, liver and intrahepatic bile duct cancer, and kidney, renal, and pelvis cancer in the United States population are 19.5 per 100,000, 6.4 per 100,000, and 13.2 per 100,000; rates vary by sex and race. Age-adjusted mortality rates for these cancers are lower: 7.3 per 100,000 (NHL), 5.0 per 100,000 (liver and intrahepatic bile duct), 4.2 per 100,000 (kidney and renal pelvis). Rates of the childhood cancer, acute lymphocytic leukemia, are even lower: 1.6 (incidence) and 0.5 (mortality) per 100,000 (Ries et al., 2008).

Only very large cohort or case-control studies would have a sufficient number of cases and statistical power to estimate excess risks and exposure-response relationships (NRC, 2006). Observations from studies with large numbers of TCE-exposed subjects, given consideration of exposure conditions and other criteria discussed in this section, can provide useful information on hazard and may provide quantitative information on possible upper bound TCE cancer risks. Alternatively, studies of small numbers of subjects or cases and controls, typically, studies with statistical power <80% to detect risk of a magnitude of ≤2, are not likely to provide useful evidence for or against the hypothesis that TCE is a human carcinogen.

Studies with either a large number of TCE-exposed subjects or with large numbers of total deaths, cancer deaths, or cancer cases among TCE-exposed subjects are the cohort studies of Blair et al. (1998), Raaschou-Nielsen et al. (2003), and Zhao et al. (2005), and the case-control studies of Pesch et al. (2000a, 2000b), Shu et al. (2004; 1999) [paternal exposure assessment, only]), Wang et al. (2009) and Cocco et al. (2010), with ≥50 TCE-exposed cases. The cohorts of Boice et al. (2006b; 1999) and Morgan et al. (1998), like that of Blair et al. (1998), comprised over 10,000 subjects both with and without potential TCE exposure; however, the number of subjects and the percentage of the larger cohort identified with TCE exposure in these studies was less than that in Blair et al. (1998); 23% of all subjects in Morgan et al. (1998), 3% in Boice et al. (1999), 2% in Boice et al. (2006b) compared to 50% in Blair et al. (1998). Moreover, although the cohorts of Garabrant et al. (1988), Chang et al. (2005) and Sung et al. (2007) are also of population sizes >10,000, these studies of employees at one manufacturing facility lack assignment of potential TCE exposure to individual subjects and include subjects with varying exposure potential, some of whom are likely with very low to no exposure potential to TCE. Rate ratios estimated from cohorts that include unexposed subjects would be underestimated,

although the magnitude of this bias cannot be calculated given the absence in individual studies of information on the percentage of subjects lacking potential TCE exposure.

Examination of the statistical power or ability to detect a rate ratio magnitude for site-specific cancer in an epidemiologic study informs weight-of-evidence evaluations and provides perspective on a study's validity and robustness of observations. Although statistical power calculations are traditionally carried out during the design phase for sample size estimation, examination of a study's statistical power post hoc is one of several tools to evaluate a study's validity; however, such calculations must be interpreted in context of exposure conditions in the study. Given the lower average exposure concentrations in the cohort studies and in population case-control studies, an assumption of low RRs is plausible. Approximate statistical power to detect a RR of 2.0 with $\alpha = 0.05$ was calculated for site-specific cancers in cohort and geographic-based studies according to the methods of Beaumont and Breslow (1981), as suggested by NRC (2006), and are found in Table B-4. Approximate statistical power was calculated for kidney, NHL, and liver cancers as examples. Radican et al. (2008), the previous follow-up of this cohort by Blair et al. (1998), and Raaschou-Nielsen et al. (2003) have over 80% statistical power to detect RR of 2.0 for kidney and liver cancers and NHL and overall TCE exposure. However, while these studies may appear sufficient for examining overall TCE exposure and RRs of 2.0, they have a greatly reduced ability to detect underlying risks of this magnitude in analyses using rank-ordered exposure- or duration-response analyses. Other studies with fewer TCE-exposed subjects and of similar or lower exposure conditions as Blair et al. (1998) will decreased statistical power to detect most site-specific cancer risks of <2.0. Statistical power in Morgan et al. (1998) and Boice et al. (1999) approaches that in Blair et al. (1998) and Raaschou-Nielsen et al. (2003). As further identified in Table B-4, Garabrant et al. (1988) and Morgan and Cassady (2002) each had over 80% statistical power to detect RRs of 2.0 for liver and kidney cancer and reflects the number of subjects in each of these studies. However, underlying risk in both studies and other studies such as these which lack characterization of TCE exposure to individual subjects is likely lower than 2.0 because of inclusion of subjects with varying exposure potential, including low exposure potential. Case-control studies such as Charbotel et al. (2006) and Brüning et al. (2003) examine higher level exposure to TCE than average exposure in the population case-control studies, and although these two studies contain fewer subjects than population case-control studies such as Cocco et al. (2010), a higher statistical power is expected related to the different and higher exposure conditions and to the higher prevalence of exposure.

Overall, except for a few studies noted above, the body of evidence has limited statistical power for evaluating low level cancer risk and TCE. For this reason, studies reporting statistically significant association between TCE and site-specific cancer are noteworthy if positive biases such as confounding are minimal.

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2

Exposure group		NHL	Kidney	Liver	Reference
Cohort studies—incidence					
Aerospace workers (Rocketdyne)					Zhao et al. (2005)
	Any exposure to TCE	Not reported	Not reported	Not reported	
	Low cumulative TCE score	Referent	Referent	Referent	
	Medium cumulative TCE score	97.0	43.8	Not reported	
	High TCE score	58.2	18.7	Not reported	
All employees at electronics factory (Taiwan)					Chang et al. (2005)
	Males	Not reported	Not reported	16.9	
	Females	Not reported	92.1[a]	15.4	
Danish blue-collar worker with TCE exposure					Raaschou-Nielsen et al. (2003)
	Any exposure, all subjects	100.0	100.0	100.0	
	Employment duration, males				
	<1 yr	98.4	96.6	85.2	
	1–4.9 yrs	99.4	98.4	92.7	
	≥5 yrs	97.7	97.0	93.1	
	Employment duration, females				
	<1 yr	40.3	30.1	27.3	
	1–4.9 yrs	48.4	37.1	34.1	
	≥5 yrs	39.6	31.9	30.5	

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2 (continued)

Exposure group		NHL	Kidney	Liver	Reference
Biologically-monitored Danish workers					Hansen et al. (2001)
	Any TCE exposure	37.9	47.9	35.7	
	Cumulative exposure (Ikeda)		Not reported	Not reported	
	<17 ppm-yr	17.9			
	≥17 ppm-yr	20.3			
	Mean concentration (Ikeda)		Not reported	Not reported	
	<4 ppm	21.0			
	4+ ppm	23.6			
	Employment duration		Not reported	Not reported	
	<6.25 yr	18.3			
	≥6.25	20.1			
Aircraft maintenance workers from Hill Air Force Base					Blair et al. (1998)
	TCE subcohort	Not reported	Not reported	Not reported	
	Males, cumulative exposure				
	0	Referent	Referent	Referent	
	<5 ppm-yr	79.5	67.8	58.2	
	5–25 ppm-yr	63.1	49.4	44.7	
	>25 ppm-yr	70.8	58.4	47.4	
	Females, cumulative exposure				
	0	Referent	Referent	Referent	
	<5 ppm-yr	28.2	0 cases	0 cases	
	5–25 ppm-yr	0 cases	0 cases	0 cases	
	>25 ppm-yr	34.1		0 cases	
Biologically-monitored Finnish workers					Anttila et al. (1995)
	All subjects	53.8	70.4	56.5	
	Mean air-TCE (Ikeda extrapolation)				
	<6 ppm	36.8	Not reported	23.2	
	6+ ppm	25.6	Not reported	17.4	

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2 (continued)

Exposure group		NHL	Kidney	Liver	Reference
Cardboard manufacturing workers in Arnsberg, Germany					Henschler et al. (1995)
	Exposed workers	Not reported	16.3	Not reported	
Biologically-monitored Swedish workers					Axelson et al. (1994)
	Any TCE exposure, males	43.5	59.6	40.1	
	Any TCE exposure, females	Not reported	Not reported	Not reported	
Cardboard manufacturing workers, Atlanta area, Georgia					Sinks et al. (1992)
	All subjects	Not reported	27.9	Not reported	
Cohort studies—mortality					
Aerospace workers (Rocketdyne)					
	Any TCE (utility/engine flush)	56.0	43.5	42.6	Boice et al. (2006b)
	Any exposure to TCE	Not reported	Not reported	Not reported	Zhao et al. (2005)
	Low cumulative TCE score	Referent	Referent	Referent	
	Medium cumulative TCE score	97.0	57.6	Not reported	
	High TCE score	55.4	26.4	Not reported	
View-Master employees					ATSDR (2004a)
	Males	40.9	17.3	23.4	
	Females	74.1	24.1	0 deaths	
All employees at electronics factory (Taiwan)					Chang et al. (2003)
	Males	49.8	0 deaths	16.9	
	Females	79.0	37.5	15.4	
United States uranium-processing workers (Fernald)					Ritz (1999a)
	Any TCE exposure				
	Light TCE exposure, >2 yrs duration	91.6[b]	59.7[c]	10.1	
	Modified TCE exposure, >2 yrs duration	20.9[b]	0 deaths[c]	0.08	
Aerospace workers (Lockheed)					Boice et al. (1999)
	Routine exposure	88.4	71.3	72.9	
	Duration of exposure, routine-intermittent				
	0 yrs	Referent	Referent	Referent	
	<1 yr	81.7	66.3	73.6	

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2 (continued)

Exposure group		NHL	Kidney	Liver	Reference
	1–4 yrs	73.5	60.3	63.5	
	≥5 yrs	78.5	63.8	67.3	
	p for trend				
Aerospace workers (Hughes)					Morgan et al. (1998)
	TCE subcohort	42.6, 79.6[d]	65.5	65.6	
	Low intensity (<50 ppm)	22.1	33.3	34.7	
	High intensity (>50 ppm)	31.8	50.1	49.2	
Aircraft maintenance workers (Hill Air Force Base, Utah)					Blair et al. (1998)
	TCE subcohort	92.7	81.5	87.9	
	Males, cumulative exposure				
	0				
	<5 ppm-yr	62.1	50.7	61.4	
	5–25 ppm-yr	43.1	37.1	44.7	
	>25 ppm-yr	54.8	44.9	52.8	
	Females, cumulative exposure				
	0				
	<5 ppm-yr	18.2	0 deaths	0 deaths	
	5–25 ppm-yr	0 deaths	8.4	0 deaths	
	>25 ppm-yr	22.0	11.5	19.1	
	TCE subcohort	99.9	94.4	99.7	Radican et al. (2008)
	Males, cumulative exposure				
	0				
	<5 ppm-yr	83.0	43.8	59.4	
	5–25 ppm-yr	64.9	53.0	70.6	
	>25 ppm-yr	75.7	33.4	50.9	
	Females, cumulative exposure				
	0				
	<5 ppm-yr	38.9	0 deaths	25.9	
	5–25 ppm-yr	0 deaths	12.4	0 deaths	

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2 (continued)

Exposure group		NHL	Kidney	Liver	Reference
	>25 ppm-yr	49.2	21.1	32.2	
Cardboard manufacturing workers in Arnsberg, Germany					Henschler et al. (1995)
	TCE exposed workers	19.6[b]	16.0	Not reported	
Cardboard manufacturing workers, Atlanta area, Georgia		45.3[b]	17.3	Not reported	Sinks et al. (1992)
Coast Guard employees (US)					Blair et al. (1989)
	Marine inspectors	31.8	31.8	38.6	
Aircraft manufacturing plant employees (Italy)					Costa et al. (1989)
	All subjects	94.1[b]	Not reported	63.1	
Aircraft manufacturing plant employees (San Diego, California)					Garabrant et al. (1988)
	All subjects	95.1[e], 74.2[f]	90.9	77.9	
Geographic-based studies					
Residents in two study areas in Endicott, New York		90.8	41.7	31.8	ATSDR (2006a)
Residents of 13 census tracts in Redlands, California		100	100.0	98.7	Morgan and Cassady (2002)
Finnish residents					Vartiainen et al. (1993)
	Residents of Hausjarvi	98.8	Not reported	84.2	
	Residents of Huttula	98.7	Not reported	83.2	

[a]Kidney cancer and other urinary organs, excluding bladder, as reported in Sung et al. (2008).
[b]All cancers of hematopoietic and lymphatic tissues.
[c]Bladder and kidney cancer, as reported in NRC (2006).
[d]Based on number of observed cases of NHL reported in Mandel et al. (2006).
[e]Lymphosarcoma and reticulosarcoma.
[f]Other lymphatic and hematopoietic tissue neoplasms.

B.2.8. Statistical Analysis and Result Documentation

Appropriate analysis approaches characterize most cohort and case-control studies on TCE cancer. Many studies clearly documented statistical analyses, evaluated possible confounding factors, and included an examination of exposure-response. In occupational cohort studies, potential confounding factors other than age, sex, race, and calendar year are, generally, not evaluated. Expected numbers of outcomes (deaths or incident cancers) were calculated using life table analysis and an external comparison group, national or regional population mortality or incidence rates (Sung et al., 2007; 2006b; Chang et al., 2005; ATSDR, 2004a; Chang et al., 2003; Raaschou-Nielsen et al., 2003; Boice et al., 1999; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Henschler et al., 1995; Axelson et al., 1994; Sinks et al., 1992; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Shannon et al., 1988; Shindell and Ulrich, 1985). Risk ratios are also presented in some cohort studies using proportional hazard and logistic regression statistical methods using mortality or incidence rates of non-TCE exposed cohort subjects as referent or internal controls (Radican et al., 2008; Boice et al., 1999; Ritz, 1999a; Blair et al., 1998). Use of a non-TCE exposed referent group employed at the same facility as exposed generally reduces downward bias or bias having potential associations masked by a healthy worker work or other factors such as smoking that may be more similar within an occupational cohort than between the cohort and the general population. However, the advantage is minimized if subjects with lower TCE exposure potential are included in the referent group as in Boice et al. (2006b). One referent group (the Santa Susanna Field Laboratory [SSFL] group) of Boice et al. (2006b) included individuals with low TCE potential, a treatment different from the overlapping study of Zhao et al. (2005) whose exposure assessment adopted a semi-quantitative approach, grouping subjects identified with low TCE exposure potential separately from subjects with no TCE exposure potential. A second referent group of all Rocketdyne workers in Boice et al. (2006b) for whom TCE exposure potential was not examined may, also, have potential for greater than background exposure since TCE use was widespread and rocket engine cleaning occurred at other locations besides at test sites (Morgenstern, 1998). The inclusion of nonexposed subjects in the low-exposure group can obscure resultant associations due to misclassification bias (Stewart and Correa-Villaseor, 1991).

Cohort studies additionally evaluate a limited number of other factors associated with employment which could be easily obtained from company and other records such as hire date, time since first employment, SES or pay status, and termination date (2006b; Zhao et al., 2005; Boice et al., 1999; Greenland et al., 1994), and three studies (Boice et al., 2006b; Zhao et al., 2005; Ritz, 1999a) included a limited evaluation of smoking using information collected by survey on smoking patterns from a subgroup of subjects. Neither analysis of Morgan et al. (1998) nor Zhao et al. (2005) control for race, although Morgan et al. (1998) stated that "data concerning race were too sparse to use." The direction of any bias introduced depends on proportion of nonwhites in the referent (internal) group compared to TCE-exposed and on

differences between racial groups in site-specific cancer incidence and mortality rates. Blair et al. (1998), furthermore, presumed all subjects of unknown race were white, an assumption with little associated error as shown later by Radican et al. (2008) whose RR estimates were adjusted for race in follow-up analysis of this cohort.

The case-control studies on TCE are better able than cohort studies to evaluate other possible confounders besides age and sex using logistic regression approaches since such information can be obtained directly through interview and questionnaires. The case-control studies of Hardell et al. (1994), Nordstrom et al. (1998), and Persson and Fredriksson (1999) lack evaluation of possible confounding factors other than age, sex, and other demographic information used to match control subjects to case subjects. RCC case-control studies included evaluation of suggested risk factors for RCC such as smoking (Charbotel et al., 2006; Brüning et al., 2003; Pesch et al., 2000b; Vamvakas et al., 1998; Siemiatycki, 1991), weight, or obesity (Charbotel et al., 2006; Dosemeci et al., 1999), and diuretics (Dosemeci et al., 1999; Vamvakas et al., 1998). Moore et al. (2010) examined the effect on RCC by smoking in univariate analyses and reported a change in their OR of <10% compared to that for TCE and RCC. They concluded that smoking was not a confounder of the observed association with TCE. NHL and childhood leukemia case-control studies included evaluation and control for possible confounding due to smoking (Seidler et al., 2007; Costas et al., 2002; Siemiatycki, 1991), alcohol consumption (Seidler et al., 2007; Costas et al., 2002), and education (Costantini et al., 2008; Miligi et al., 2006), although etiological factors for these cancers are not well identified other than a suggestion of a role of immune function and some infectious agents in NHL (Alexander et al., 2007b). Smoking was not controlled in other NHL case-control studies; however, neither smoking nor alcohol is a strong risk factor for NHL (Besson et al., 2006; Morton et al., 2005).

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Mineral oils such as cutting fluids or hydrazine common to some job titles with potential TCE exposure as machinists, metal workers, and test stand mechanics are included as covariates in statistical analyses of Zhao et al. (2005), Boice et al. (2006b), and Charbotel et al. (2009; 2006) or evaluated as a single exposure for cases and controls in Moore et al., 2010 (Moore et al., 2010) and Karami et al. (Karami et al., 2011; 2010). Two other kidney case-control studies of TCE exposure examined the effect of cutting oil as a single occupational exposure on kidney cancer risk (Karami et al., 2011; Brüning et al., 2003). In Brüning et al. (2003), cutting oil exposure did not appear highly correlated with TCE exposure as only five cases reported exposure to cutting oils compared to 25 cases reporting TCE exposure. Karami et al. (2011), who examined mineral oil or cutting fluid exposure among cases and controls in Moore et al. (2010), reported an OR of 0.8 (95% CI: 0.6, 1,1) and 1.1 (95% CI: 0.8, 1.4), for cutting oil mists or other mineral oil mists respectively, and provides little evidence for confounding in Moore et al. (2010) by cutting or mineral oil exposures. Moreover, cutting oils and mineral oils have not been associated with kidney cancer in other cohort or case-control studies (Mirer, 2010; NIOSH, 1998). In all other studies, exposure to cutting oils or to hydrazine did not greatly affect magnitude of risk estimates for TCE exposure.

Geographical studies do not examine possible confounding factors other than sex, age and calendar year. These studies are generally health surveys using publically-available records such as death certificates and lack information on other risk factors such as smoking and exposure to viruses, important to Lee et al. (2003), introduces uncertainties for informing evaluations of TCE and cancer.

B.2.9. Systematic Review for Identifying Cancer Hazards and TCE Exposure

The epidemiological studies on cancer and TCE are reviewed systematically and transparently using criteria to identify studies for meta-analysis. Section B.3 contains a description of and comment on 79 studies of varying qualities for identifying cancer hazard, a question complementary but separate from that examined using meta-analysis. This section identifies of the studies reviewed, studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on JEMs, biomarker monitoring, or industrial hygiene data indicating a high probability of TCE use) and were judged to have met the inclusion criteria identified below. Lack of inclusion of an individual study in the meta-analysis does not necessarily imply an inability to identify cancer hazard. Not all questions associated with identifying a cancer hazard are addressed using meta-analyses and the 79 studies with varying abilities approached, to sufficient degrees, the standards of epidemiologic design and analysis, identified in the beginning of Section B.2.

The NRC (2006) suggested U.S. EPA conduct a new meta-analysis of the epidemiologic data on TCE to synthesize the epidemiologic data on TCE exposure. Meta-analysis approaches are feasible for examining cancers of the liver, kidney, and NHL given most studies presented risks for these sites in their published papers and these cancer sites are of interest given observations in the animal studies. Examination of site-specific cancers other than kidney cancer, liver cancer, and NHL, such as for childhood leukemia, bladder cancer, esophageal cancer, or cervical cancer is more difficult and not recommended due to fewer available high-quality studies. NRC (2006) specifically suggested EPA to:

1. Document essential design features, exposure, and results from the epidemiologic studies—Information on study design, exposure assessment approach, statistical analysis, and other aspects important to interpreting observations in a weight of evidence evaluation for individual studies is found in Section B.3 and site-specific estimated RRs or measures of association are presented in Chapter 4;

2. Analyze the epidemiologic studies to discriminate the amount of exposure experience by the study population; exclude studies in meta-analysis based on objective criteria (e.g., studies in which it was unclear that the study population was exposed)—Section B.3. describes exposure assessment approach for individual studies and inclusion criteria for identifying studies for meta-analysis are identified below;

3. Classify studies in terms of objective characteristics, such as on the basis of the study's design characteristics or documentation of exposure—Section B.3. groups studies by study design, analytical designs and geographic-based designs, with discussion of factors important to study design, endpoint measured, exposure assessment approach, study size, and statistical analysis methods including adjustment for potential confounding exposures;

4. Assess statistical power of each study—Table B-4 presents power calculations for cohort studies;

5. Combine case-control and cohort studies in the analysis, unless it introduces substantial heterogeneity—Appendix C discusses the meta-analysis statistical methods and findings;

6. Testing of heterogeneity (e.g., fixed or random effect models)—Appendix C discusses the meta-analysis statistical methods and findings;

7. Perform a sensitivity analysis in which each study is excluded from the analysis to determine whether any study significantly influences the finding—Appendix C discusses the meta-analysis statistical methods and findings.

Studies selected for inclusion in the meta-analysis met the following criteria: (1) cohort or case-control designs; (2) evaluation of incidence or mortality; (3) adequate selection in cohort studies of exposure and control groups and of cases and controls in case-control studies; (4) TCE exposure potential inferred to each subject and quantitative assessment of TCE exposure for each subject by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, JEMs, water distribution models, or obtained from subjects using questionnaire (case-control studies); and (5) RR estimates for kidney cancer, liver cancer, or NHL adjusted, at minimum, for possible confounding of age, sex, and race. Table B-5 in Section B.2.9.4 identifies studies included in the meta-analysis and studies that did not meet the inclusion criteria and the primary reasons for their deficiencies.

B.2.9.1. Cohort Studies

The cohort studies (Radican et al., 2008; Sung et al., 2008; Krishnadasan et al., 2007; Sung et al., 2007; Boice et al., 2006b; Chang et al., 2005; Zhao et al., 2005; Chang et al., 2003; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Boice et al., 1999; Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998; Anttila et al., 1995; Henschler et al., 1995; Axelson et al., 1994; Greenland et al., 1994; Sinks et al., 1992; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Shannon et al., 1988; Shindell and Ulrich, 1985; Wilcosky et al., 1984), with data on the incidence or morality of site-specific cancer in relation to TCE exposure range in size (803 (Hansen et al., 2001) to 86,868 (Chang et al., 2005; Chang et al., 2003)), and were conducted in Denmark, Sweden, Finland, Germany, Taiwan, and the United States (see Table B-1). Three case-control studies nested within cohorts (Krishnadasan et al., 2007; Greenland et al., 1994; Wilcosky et al., 1984) are considered as cohort studies because the summary risk estimate from a nested case-control study, the OR, was estimated from incidence density sampling and is considered an unbiased estimate of the hazard ratio, similar to an RR estimate from a cohort study. Two studies of deaths within a cohort were included in the group, but these studies lacked information on the person-year structure (i.e., both are PMR studies, and did not satisfy the meta-

analysis inclusion criteria for analytical study design [(Clapp and Hoffman, 2008; ATSDR, 2004a)]).

Cohort and nested case-control study designs are analytical epidemiologic studies and are generally relied on for identifying a causal association between human exposure and adverse health effects (Zhou et al., 2003). Some subjects in the Hansen et al. study are also included in a study reported by Raaschou-Nielsen et al. (2003); however, any contribution from the former to the latter are minimal given the large differences in cohort sizes of these studies (Raaschou-Nielsen et al., 2003; Hansen et al., 2001). Similarly, some females in Chang et al. (2005; 2003), a large cohort of 70,735 female and 16,133 male subjects, are included in Sung et al. (2007), a cohort of 63,982 female electronic workers from the same factory who were followed an additional 4-year period than subjects in Chang et al. (2005; 2003). Cancer observations for female subjects in these studies are considered as equivalent since they are derived from essentially the same population. Krishnadasan et al. (2007) is a nested case-control study of prostate cancer with cases and controls drawn from subjects in a large cohort of aerospace workers as subjects in Zhao et al. (2005), who did not report on prostate cancer, and met all of the inclusion criteria except that for reporting an RR estimate for cancer of the kidney, liver or NHL.

Eleven of the cohort studies met all five inclusion criteria: the cohorts of Blair et al. (1998) and its further follow-up by Radican et al. (2008), Morgan et al. (1998), Boice et al. (2006b; 1999) and Zhao et al. (2005) of aerospace workers or aircraft mechanics; Axelson et al. (1994), Anttila et al. (1995), Hansen et al. (2001), and Raaschou-Nielsen et al. (2003) of Nordic workers in multiple industries with TCE exposure; and Greenland et al. (1994) of electrical manufacturing workers. All 11 cohort studies adopted statistical methods, e.g., life table analysis, Poisson regression analysis, or Cox Proportional Hazard analysis, that met epidemiologic standards, and were able to control for age, race, sex, and calendar time trends in cancer rates. Statistical analyses in Boice et al. (1999) adjusted for demographic variable such as age, race, and sex, and also included date of first employment and terminating date of employments, which may have decreased the statistical power of their analyses due to collinearity between age, first and last employment dates. Statistical analyses in Zhao et al. (2005) and Boice et al. (2006b) adjusted for potential effects by other occupational exposures on cancer and both Raaschou-Nielsen et al. (2003) and Zhao et al. (2005) examined possible confounding by smoking on TCE exposure and cancer risks using indirect approaches.

Of the 11 studies, 2 studies reported risk estimates for both site-specific cancer incidence and mortality (Zhao et al., 2005; Blair et al., 1998), 4 studies reported risk estimates for cancer incidence only (Krishnadasan et al., 2007; Raaschou-Nielsen et al., 2003; Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994), and four studies reported risk estimates for mortality only (Radican et al., 2008; 2006b; Boice et al., 1999; Morgan et al., 1998). Incidence ascertainment in two cohorts began 21 (Blair et al., 1998) and 38 years (Zhao et al., 2005) after

the inception of the cohort. Specifically, Zhao et al. (2005) note "results may not accurately reflect the effects of carcinogenic exposure that resulted in nonfatal cancers before 1988." Because of the issues concerning case ascertainment raised by this incomplete coverage, incidence observations must be interpreted in light of possible bias reflecting incomplete ascertainment of incident cases. Furthermore, use of an internal referent population, nonexposed subjects drawn from the same or nearby facilities as exposed workers, in Blair et al. (1998) and Radican et al. (2008) for overall TCE exposure, and in Blair et al. (1998), Morgan et al. (1998), Boice et al. (1999), Zhao et al. (2005), Boice et al. (2006b), and Radican et al. (2008) for rank-ordered TCE exposure is expected to reduce bias associated with the healthy worker effect. Morgan et al. (1998) presents risk estimates for overall TCE exposure comparing mortality in their TCE subcohort to that expected using mortality rate of the U.S. population in an Environmental Health Strategies Final Report and sent to U.S. EPA by Paul Cammer, Ph.D., on behalf of the Trichloroethylene Issues Group (EHS, 1997). The final report also contained risk estimates from internal analyses of rank-order TCE exposure and published as Morgan et al. (1998). Both internal cohort analyses of the rank-ordered exposure, presented in both the final report of Environmental Health Strategies (1997) and Morgan et al. (1998), and overall TCE exposure, available in the final report or upon request, are based on the same group of internal referents, nonexposed TCE subjects employed at the same facility.

Subjects in these studies had a high likelihood or potential for TCE exposure, although estimated average exposure intensity for overall TCE exposure in some cohorts was considered as <10 or 20 ppm (TWA). The exposure assessment techniques used in these cohort studies included a detailed JEM (Blair et al., 1998; Greenland et al., 1994); its follow-up by Radican et al. (2008) (2008; Boice et al., 2006b; Zhao et al., 2005; Boice et al., 1999; Morgan et al., 1998); Radican et al. (2008), biomonitoring data (Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994), or use of industrial hygiene data on TCE exposure patterns and factors that affect such exposure (Raaschou-Nielsen et al., 2003), with high probability of TCE exposure potential to individual subjects. The JEM in six studies provided rank-ordered surrogate metrics for TCE exposure (Hansen et al., 2001; Blair et al., 1998; Anttila et al., 1995; Axelson et al., 1994) and its follow-up by Radican et al. (2008; Zhao et al., 2005), a strength compared to use of duration of employment as an exposure surrogate, e.g., Boice et al. (2006b; 1999) or Raaschou-Nielsen et al. (2003), which is a poorer exposure metric given subjects may have differing exposure intensity with similar exposure duration (NRC, 2006). Rank-ordered TCE dose surrogates for low and medium exposure from the JEM of Morgan et al. (1998) are uncertain because of a lack of information on frequency of exposure-related tasks and on temporal changes (NRC, 2006); only the high category for TCE exposure is unambiguous. The nested case-control study of Greenland et al. (1994) examined TCE as one of seven exposures and potential assigned to individual cases and controls using a job-exposure-matrix approach. However, the low exposure prevalence, missing job history information for 34% of eligible subjects, and study of pensioned

workers only were other factors judged to lower this study's sensitivity for cancer hazard identification.

The remaining cohort studies (Chang et al., 2005; Chang et al., 2003; Ritz, 1999a; Henschler et al., 1995; Sinks et al., 1992; Blair et al., 1989; Costa et al., 1989; Garabrant et al., 1988; Shannon et al., 1988; Shindell and Ulrich, 1985; Wilcosky et al., 1984); Sung et al., (Sung et al., 2008; 2007) less satisfactorily meet inclusion criteria. These studies, while not meeting the meta-analysis inclusion criteria, can inform the hazard analysis although their findings are weighted less than for observations in the other studies, and observations may have alternative causes. Reasons for study insufficiencies varied. Nine studies do not assign TCE exposure potential to individual subjects (Clapp and Hoffman, 2008; Sung et al., 2008; Sung et al., 2007; Chang et al., 2005; ATSDR, 2004a; Chang et al., 2003; Sinks et al., 1992; Costa et al., 1989; Garabrant et al., 1988; Shindell and Ulrich, 1985) all subjects are presumed as "exposed" because of employment in the plant or facility although individual subjects would be expected to have differing exposure potentials.

TCE exposure potential is ambiguous in both Wilcosky et al. (1984) and Ritz (1999a), two studies of low potential, low intensity TCE exposure compared to studies using exposure assessment approaches supported by information on job titles, tasks, and industrial hygiene monitoring data. Furthermore, high correlation in Ritz (1999a) between TCE and other exposures, particularly cutting fluids and radiation, may not have been sufficiently controlled in statistical analyses. Ritz et al. (1999a), furthermore, did not report estimated RRs for kidney or NHL separately; rather, presenting RR estimates for kidney and bladder cancer combined and for all hemato- and lymphopoietic cancers.

Two studies do not sufficiently define the underlying cohort or there is uncertainty in cancer case or death ascertainment (Henschler et al., 1995; Shindell and Ulrich, 1985). Furthermore, magnitude of observed risk in Henschler et al. (1995), ATSDR (2004a), and Clapp and Hoffman (2008) must be interpreted in a weight-of-evidence evaluation in light of possible bias introduced through use of analysis of proportion of deaths (PMR) in ATSDR (2004a) and Clapp and Hoffman (2008), or to inclusion of index kidney cancer cases in Henschler et al. (1995).

B.2.9.2. Case-Control Studies

Case-control studies on TCE exposure are of several site-specific cancers and include bladder cancer (Pesch et al., 2000a; Siemiatycki et al., 1994; Siemiatycki, 1991); brain cancer (De Roos et al., 2001; Heineman et al., 1994); childhood lymphoma or leukemia (Shu et al., 2004; Costas et al., 2002; Shu et al., 1999; McKinney et al., 1991; Lowengart et al., 1987); colon cancer (Goldberg et al., 2001; Siemiatycki, 1991), esophageal cancer (Parent et al., 2000b; Siemiatycki, 1991); liver cancer (Lee et al., 2003); lung cancer (Siemiatycki, 1991); lymphoma (Hardell et al., 1994) [NHL, Hodgkin lymphoma], (Nordström et al., 1998; Fritschi and

Siemiatycki, 1996a; Siemiatycki, 1991), [hairy cell leukemia], (Persson and Fredrikson, 1999) [NHL], (Miligi et al., 2006) [NHL and CLL], (Seidler et al., 2007) [NHL, Hodgkin lymphoma], (Costantini et al., 2008) [leukemia types, CLL included in Miligi et al. (2006), Wang et al. (2009) [NHL], (Cocco et al., 2010) [NHL, CLL, MM]; (Gold et al., 2011) [MM]; Purdue et al. (2011) [NHL]; melanoma (Fritschi and Siemiatycki, 1996a; Siemiatycki, 1991); rectal cancer (Dumas et al., 2000; Siemiatycki, 1991); RCC, a form of kidney cancer (Moore et al., 2010; Charbotel et al., 2009; Charbotel et al., 2006; Brüning et al., 2003; Parent et al., 2000a; Pesch et al., 2000b; Dosemeci et al., 1999; Vamvakas et al., 1998; Siemiatycki, 1991); pancreatic cancer (Siemiatycki, 1991); and prostate cancer (Aronson et al., 1996; Siemiatycki, 1991). No case-control studies of reproductive cancers (breast or cervix) and TCE exposure were found in the peer-reviewed literature.

Several of the above publications are studies of cases and controls drawn from the same underlying population with a common control series. Miligi et al. (2006) and Costantini et al. (2008) presented observations from the Italian multicenter lymphoma population case-control study; Miligi et al. (2006) on occupation or specific solvent exposures and NHL, and who also included CLL and Hodgkin lymphoma in the overall NHL category, and Costantini et al. (2008) who examined leukemia subtypes, and included CLL as a separate disease outcome. Seidler et al. (2007) analyzed independently the German subjects of the six European country, multicenter lymphoma population case-control study (EPILYMPH study) of Cocco et al. (2010). Each study adopted a different approach to calculate cumulative exposure and apparent inconsistency in their conclusions may reflect the slightly different ranking of cases and controls in each study (personal communication from Pierluigi Cocco to Cheryl Siegel Scott). Gold et al. (2011) and Purdue et al. (2011) presented observations from the NCI-SEER population case-control studies and share a common control series; Purdue et al. (2011) of NHL in four SEER reporting areas and Gold et al. (2011) of multiple myeloma in two of the four SEER sites. Pesch et al. (2000a, 2000b), a multiple center population case- control study of urothelial cancers in Germany, presented observations on TCE and bladder cancer, including cancer of the ureter and renal pelvis, in Pesch et al. (2000a) and RCC in Pesch et al. (2000b). Siemiatycki (1991), a case-control of occupational exposures and several site-specific cancers (bladder, colon, esophagus, lung, rectum, pancreas, and prostate) and designed to generate hypotheses about possible occupational carcinogens, presents risk estimates associated with TCE exposure using Mantel-Haentszel methods. Subsequent publications examine either TCE exposure (analyses of melanoma and colon cancers) or job title/occupation (all other cancer sites) using logistic regression methods (Goldberg et al., 2001; Dumas et al., 2000; Parent et al., 2000a; Aronson et al., 1996; Fritschi and Siemiatycki, 1996b, a; Siemiatycki et al., 1994).

The population case-control studies with data on cancer incidence or mortality (Siemiatycki, 1991 [and related publications, Goldberg et al., 2001; Dumas et al., 2000; Parent et al., 2000a; Aronson et al., 1996; Fritschi and Siemiatycki, 1996b; Siemiatycki et al., 1994], Gold

et al., 2011; Purdue et al., 2011; Cocco et al., 2010; Moore et al., 2010; Wang et al., 2009; Costantini et al., 2008; Seidler et al., 2007; Charbotel et al., 2006; Miligi et al., 2006; Shu et al., 2004; Brüning et al., 2003; Lee et al., 2003; Costas et al., 2002; De Roos et al., 2001; Pesch et al., 2000a, 2000b; Dosemeci et al., 1999; Kernan et al., 1999; Persson and Fredrikson, 1999; Nordström et al., 1998; Vamvakas et al., 1998; Hardell et al., 1994; Heineman et al., 1994; McKinney et al., 1991; Lowengart et al., 1987) in relation to TCE exposure range in size, from small studies with <100 cases and control (Costas et al., 2002) to multiple-center studies large-scale studies of over 2,000 cases and controls (Costantini et al., 2008; Miligi et al., 2006; Shu et al., 2004; Pesch et al., 2000a, 2000b; Shu et al., 1999), and were conducted in Sweden, Germany, Italy, Taiwan, Canada, and the United States (see Table B-2).

Fifteen of the case-control studies met the meta-analysis inclusion criteria identified in Section B.2.9 (Purdue et al., 2011; Cocco et al., 2010; Moore et al., 2010; Charbotel et al., 2009; Wang et al., 2009; Seidler et al., 2007; Charbotel et al., 2006; Miligi et al., 2006; Brüning et al., 2003; Pesch et al., 2000b; Dosemeci et al., 1999; Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994; Siemiatycki, 1991). They were of analytical study design, cases and controls were considered to represent underlying populations and selected with minimal potential for bias; exposure assessment approaches included assignment of TCE exposure potential to individual subjects using information obtained from face-to-face, mailed, or telephone interviews; analyses methods were appropriate, well-documented, included adjustment for potential confounding exposures, with RR estimates and associated CIs reported for kidney cancer, liver cancer, or NHL. All thirteen studies evaluated TCE exposure potential to individual cases and controls and a structured questionnaire sought information on self-reported occupational history and specific exposures such as TCE. Three studies assigned TCE exposure potential to cases and controls using self-reported information (Nordström et al., 1998; Hardell et al., 1994) and two of these studies used judgment to assign potential exposure intensity (Persson and Fredrikson, 1999; Nordström et al., 1998). Persson and Fredriksson (1999) also assigned TCE exposure potential from both occupational and leisure use, the only study to do so. The 10 other studies assigned TCE exposure potential using self-reported job title and occupational history, a superior approach compared to use of a JEM supported by expert judgment and information on only self-reported information given its expect greater specificity (Purdue et al., 2011; Cocco et al., 2010; Moore et al., 2010; Charbotel et al., 2009; Wang et al., 2009; Seidler et al., 2007; Charbotel et al., 2006; Miligi et al., 2006; Brüning et al., 2003; Pesch et al., 2000b; Dosemeci et al., 1999; Siemiatycki, 1991). Pesch et al. (2000b) assigned TCE exposure potential using both JEM and JTEM. The inclusion of task information is considered superior to exposure assignment using only job title since it likely reduces potential misclassification and, for this reason, RR estimates in Pesch et al. (2000b) for TCE from a JTEM are preferred. All studies except Hardell et al. (1994) and Dosemeci et al. (1999) developed a semiquantitative or quantitative TCE exposure surrogate.

These studies to varying degrees were considered as stronger studies for weight-of evidence characterization of hazard. Both Brüning et al. (2003) and Charbotel et al. (2006), (2009) had a priori hypotheses for examining RCC and TCE exposure. Strengths of both studies are in their examination of populations with potential for high exposure intensity and in areas with high frequency of TCE usage and their assessment of TCE potential. An important feature of the exposure assessment approach of Charbotel et al. (2006) is their use of a large number of studies on biological monitoring of workers in the screw-cutting industry a predominant industry with documented TCE exposures as support. The other studies were either large multiple-center studies (Purdue et al., 2011; Cocco et al., 2010; Moore et al., 2010; Wang et al., 2009; Miligi et al., 2006; Pesch et al., 2000b); or reporting from one location of a larger international study (Seidler et al., 2007; Dosemeci et al., 1999). In contrast to Brüning et al. (2003) and Charbotel et al. (2009; 2006), two studies conducted in geographical areas with widespread TCE usage and potential for exposure to higher intensity, a lower exposure prevalence to TCE is found (any TCE exposure: 15% of cases [(Dosemeci et al., 1999); 6% of cases (Miligi et al., 2006); 13% of cases (Seidler et al., 2007); 13% of cases (Wang et al., 2009)]) and most subjects identified as exposed to TCE probably had minimal contact (3% of cases with moderate/high TCE exposure [(Miligi et al., 2006); 1% of cases with high cumulative TCE (Seidler et al., 2007); 2% of cases with high intensity, but of low probability TCE exposure (Wang et al., 2009)]). This pattern of lower exposure prevalence and intensity is common to community-based, population case-control studies (Teschke et al., 2002).

Fifteen case-control studies did not meet specific inclusion criterion (Gold et al., 2011; Costantini et al., 2008; Shu et al., 2004; Lee et al., 2003; Costas et al., 2002; Goldberg et al., 2001; Dumas et al., 2000; Parent et al., 2000a; Pesch et al., 2000a; Kernan et al., 1999; Shu et al., 1999; Vamvakas et al., 1998; Fritschi and Siemiatycki, 1996b; Siemiatycki, 1991). Costantini et al. (2008) and Gold et al. (2011) examined multiple myeloma or leukemias, not included in older NHL classification schemes, although these neoplasms are now considered as lymphomas under the WHO Lymphoma Classification. Vamvakas et al. (1998) has been subject of considerable controversy (Cherrie et al., 2001; Mandel, 2001; Green and Lash, 1999; McLaughlin and Blot, 1997; Bloemen and Tomenson, 1995; Swaen, 1995) with questions raised on potential for selection bias related to the study's controls. This study was deficient in the criterion for adequacy of case and control selection. Brüning et al. (2003), a study from the same region as Vamvakas et al. (1998), is considered a stronger study for identifying cancer hazard since it addresses many of the deficiencies of Vamvakas et al. (1998). Lee et al. (2003), in their study of hepatocellular cancer, assigns one level of exposure to all subjects in a geographic area, and inherent measurement error and misclassification bias because not all subjects are exposed uniformly. Additionally, statistical analyses in this study did not control for hepatitis viral infection, a known risk factor for hepatocellular cancer and of high prevalence in the study area. Ten of 12 studies reported RR estimates for site-specific cancers other than kidney, liver, and

NHL (Shu et al., 2004; Costas et al., 2002; Goldberg et al., 2001; Dumas et al., 2000; Parent et al., 2000b; Pesch et al., 2000a; Kernan et al., 1999; Shu et al., 1999; Aronson et al., 1996; Fritschi and Siemiatycki, 1996b; Siemiatycki et al., 1994; Garabrant et al., 1988).

B.2.9.3. Geographic-Based Studies

The geographic-based studies (ATSDR, 2008b, 2006a; Aickin, 2004; Morgan and Cassady, 2002; ADHS, 1995; Cohn et al., 1994b; Vartiainen et al., 1993; Aickin et al., 1992; ADHS, 1990; Mallin, 1990; Isacson et al., 1985) with data on cancer incidence (all studies) are correlation studies to examine cancer outcomes of residents living in communities with TCE and other chemicals detected in groundwater wells or in municipal drinking water supplies. These eight studies did not meet inclusion criteria and were deficient in a number of criteria.
All geographic-based studies are surveys of cancer rates for a defined time period among residents in geographic areas with TCE contamination in groundwater or drinking water supplies, or soil and are not of analytical designs such as cohort and case-control designs. A major shortcoming in all studies is, also, their low level of detail to individual subjects for TCE potential. The exposure surrogate is assigned to a community, town, or a geographically-defined area such as a contiguous grouping of census tracts as an aggregate level, typically based on limited number of water monitoring data from a recent time period and is a poor exposure surrogate because potential for TCE exposure can vary in these broad categories depending on job function, year, use of personal protection, and, for residential exposure, pollutant fate and transport, water system distribution characteristics, percent of time per day in residence, presence of mitigation devices, drinking water consumption rates, and showering times. Additionally, ATSDR (2008b), the only geographic-based study to examine other possible risk factors on individual subjects, reported that smoking patterns and occupational exposures may partly contribute to the observed elevated rates of kidney and renal pelvis cancer and lung cancer in subjects living in a community with contaminated groundwater and with TCE exposure potential from vapor intrusion into residences.

B.2.9.4. Recommendation of Studies for Treatment Using Meta-Analysis Approaches

All studies are initially considered for inclusion in the meta-analysis; however, as discussed throughout this section, some studies are better than others for inclusion in a quantitative examination of cancer and TCE. Twenty-six of the studies included in the meta-analysis (statistical methods and findings discussed in Appendix C) met the following five inclusion criteria: (1) cohort or case-control designs; (2) evaluation of incidence or mortality; (3) adequate selection in cohort studies of exposure and control groups and of cases and controls in case-control studies; (4) TCE exposure potential inferred to each subject and quantitative assessment of TCE exposure assessment for each subject by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, JEMs, water

distribution models, or obtained from subjects using questionnaire (case-control studies); and (5) RR estimates for kidney cancer, liver cancer, or NHL adjusted, at minimum, for possible confounding of age, sex, and race. The twenty-six studies that met these inclusion are: Siemiatycki (1991), Axelson et al. (1994), Greenland et al. (1994), Hardell et al. (1994), Anttila et al. (1995), Blair et al. (1998), Morgan et al. (1998), Nordstrom et al. (1998), Dosemeci et al. (1999), Boice et al. (2006b; 1999), Persson and Fredriksson (1999), Pesch et al. (2000b), Hansen et al. (2001), Brüning et al. (2003), Raaschou-Nielsen et al. (2003), Zhao et al. (2005), Miligi et al. (2006), Charbotel et al. (2006), Seidler et al. (2007), Radican et al. (2008), Wang et al. (2009), Cocco et al. (2010), Moore et al. (2010), and Purdue et al. (2011). Table B-5 identifies studies included in the meta-analysis and studies that did not meet the inclusion criteria and the primary reasons for their deficiencies.

Table B-5. Summary of rationale for study selection for meta-analysis

Decision outcome	Studies	Primary reason(s)
Studies recommended for meta-analysis:		
	Siemiatycki (1991); Axelson et al. (1994); Hardell (1994); Greenland et al. (1994); Anttila et al. (1995); Morgan et al. (1998); Nordstrom et al. (1998); Boice et al. (2006b; 1999); Dosemeci et al., (1999); Persson and Fredriksson, (1999); Pesch et al. (2000b); Hansen et al. (2001); Brüning et al. (2003); Raaschou-Nielsen et al. (2003); Zhao et al. (2005); Miligi et al. (2006); Charbotel et al. (2006); Radican et al. (2008) [Blair et al. (1998), incidence]; Wang et al. (2009); Cocco et al. (2010); Moore et al. (2010); Purdue et al. (2011)	Analytical study designs of cohort or case-control approaches; evaluation of cancer incidence or cancer mortality. Specifically identified TCE exposure potential to individual study subjects by reference to industrial hygiene records, individual biomarkers, JEMs, water distribution models, industrial hygiene data indicating a high probability of TCE use (cohort studies), or obtained information on TCE exposure from subjects using questionnaire (case-control studies). Reported results for kidney cancer, liver cancer, or NHL with RR estimates and corresponding CIs (or information to allow calculation).

Table B-5. Summary of rationale for study selection for meta-analysis (continued)

Decision outcome	Studies	Primary reason(s)
Studies not recommended for meta-analysis:		
	ATSDR (2004a); Clapp and Hoffman, (2008); Cohn et al. (1994b)	Weakness with respect to analytical study design (i.e., geographic-based, ecological, or PMR design)
	Wilcosky et al. (1984); Isacson et al. (1985); Shindell and Ulrich (1985); Garabrant et al. (1988); Shannon et al.(1988); Blair et al. (1989); Costa et al. (1989); ADHS (1995, 1990); Mallin (1990); Aickin et al. (1992); Sinks et al. (1992); Vartiainen et al. (1993); Morgan and Cassady (2002); Lee et al. (2003); Aickin (2004); Chang et al. (2005; 2003); Coyle et al. (2005); ATSDR (2008b, 2006a); Sung et al. (2008; 2007)	TCE exposure potential not assigned to individual subjects using JEM, individual biomarkers, water distribution models, or industrial hygiene data indicating a high probability of TCE use (cohort studies).
	Lowengart et al. (1987); Fredriksson et al. (1989); McKinney et al. (1991); Heineman et al. (1994); Siemiatycki et al. (1994); Aronson et al. (1996); Fritchi and Siemiatycki (1996b); Dumas et al. (2000); Kernan et al.(1999); Shu et al. (2004; 1999); Parent et al. (Parent et al., 2000b); Pesch et al., (2000a); De Roos et al. (2001); Goldberg et al. (2001); Costas et al. (2002); Krishnadasan et al. (2007); Costantini et al. (2008); Gold et al. (2011)	Cancer incidence or mortality reported for cancers other than kidney, liver, or NHL.
	Ritz (1999a)	Subjects monitored for radiation exposure with likelihood for potential confounding. Cancer mortality and TCE exposure not reported for kidney cancer and all hemato- and lymphopoietic cancer reported as broad category.
	Henschler et al. (1995)	Incomplete identification of cohort and index kidney cancer cases included in case series.
	Vamvakas et al. (1998)	Control selection may not represent case series with potential for selection bias.

There is some overlap between the cohorts of Zhao et al. (2005) and Boice et al. (2006b); each cohort is identified from a population of workers, but these studies differ on cohort definition, cohort identification dates, disease outcome examined, and exposure assessment approach. Zhao et al. (2005), who adopted a semiquantitative approach for TCE exposure assessment, is preferred to Boice et al. (2006b), whose TCE subcohort included subjects with a

lower likelihood for TCE exposure and duration of exposure, a poor exposure metric given that subjects may have differing exposure intensity with similar exposure duration (NRC, 2006). Additionally, a larger number of site-specific cancer deaths identified with potential TCE exposure is observed by Zhao et al. (2005) compared to Boice et al. (2006b); e.g., 95 lung cancer cases with medium or high TCE exposure (Zhao et al., 2005) and 51 lung cancer cases with any TCE exposure (Boice et al., 2006b) (see further discussion in Section B.3.1.1.1.3). Radican et al. (2008) studied the same subjects as Blair et al. (1998), adding an additional 10 years of follow-up and updating mortality. Observed site-specific cancer mortality risk estimates in Radican et al. (2008) did not change appreciably and were consistent with those reported in Blair et al. (1998) and is preferred. Blair et al. (1998) who also presented incidence RR estimates is recommended for inclusion in sensitivity analyses. Charbotel et al. (2006) is preferred to Charbotel et al. (2009), who examined kidney cancer risk and TCE exposure at the existing French occupational exposure limit for cases and controls from their earlier publication (Charbotel et al., 2009); the earlier publication contained more extensive analyses and included exposure-response analyses using several exposure metrics and multiple exposure categories. Cocco et al. (2010) is preferred to Seidler et al. (2007), whose subjects are included in the larger multicenter population case-control study. In conclusion, twenty-four studies in which there is a high likelihood for TCE exposure and judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis, are identified in a systematic review of the epidemiologic literature and for examination using meta-analysis.

B.3. INDIVIDUAL STUDY REVIEWS AND ABSTRACTS

B.3.1. Cohort Studies

B.3.1.1. Studies of Aerospace Workers

Seven papers reported on cohort studies of aerospace or aircraft maintenance and manufacturing workers in large facilities.

B.3.1.1.1. Studies of SSFL workers.

TCE exposure to workers at SSFL, an aerospace facility located nearby Los Angeles, California, operated by Rocketdyne/Atomics International, formerly a division of Boeing and currently owned by Pratt-Whitney, is subject of two research efforts: (1) the University of California at Los Angeles (UCLA) study, overseen by the California Department of Health Services and funded by the U.S. Department of Energy (DOE) (Morgenstern et al., 1999; Ritz et al., 1999; Morgenstern et al., 1997), with two publications on TCE exposure and cancer incidence (Krishnadasan et al., 2007; Zhao et al., 2005) and mortality (Zhao et al., 2005) and (2) the International Epidemiology Institute study (IEI), funded by Boeing after publication of the initial UCLA reports, of all Rocketdyne employees which included a mortality analysis of TCE exposure in a subcohort of SSFL test stand mechanics (Boice et al., 2006b). In addition to

chemical exposure, both groups examine radiation exposure and cancer among Rocketdyne workers monitored for radiation (Boice et al., 2006a; Ritz et al., 2000).

B.3.1.1.1.1. **International Epidemiology Institute study of Rocketdyne workers.**

B.3.1.1.1.1.1. **Boice et al. (2006b).**

B.3.1.1.1.1.1.1. **Author's abstract.**

Objective: The objective of this study was to evaluate potential health risks associated with testing rocket engines. **Methods**: A retrospective cohort mortality study was conducted of 8372 Rocketdyne workers employed 1948 to 1999 at the Santa Susanna Field Laboratory (SSFL). Standardized mortality ratios (SMRs) and 95% confidence intervals (CIs) were calculated for all workers, including those employed at specific test areas where particular fuels, solvents, and chemicals were used. Dose-response trends were evaluated using Cox proportional hazards models. **Results:** SMRs for all cancers were close to population expects among SSFL workers overall (SMR = 0.89; CI = 0.82-0.96) and test stand mechanics in particular (n = 1651; SMR = 1.00; CI = 0.86-1.1.6), including those likely exposure to hydrazines (n = 315; SMR = 1.09; CI = 0.75-1.52) or trichloroethylene (TCE) (n=1111; SMR = 1.00; CI = 0.83-1.19). Nonsignificant associations were seen between kidney cancer and TCE, lung cancer and hydrazines, and stomach cancer and years worked as a test stand mechanic. No trends over exposure categories were statistically significant. **Conclusion:** Work at the SSFL rocket engine test facility or as a test stand mechanic was not associated with a significant increase in cancer mortality overall or for any specific cancer.

B.3.1.1.1.1.1.2. **Study description and comment.**

Boice et al. (2006b) examined all cause, all cancer and site-specific mortality in a subcohort of 1,651 male and female test stand mechanics who had been employed on or after 1949 to 1999, the end of follow-up, for at least 6 months at SSFL. Subjects were identified from 41,345 male and female Rocketdyne workers at SSFL (n = 8.372) and two nearby facilities (32,979). Of the 1,642 male test stand mechanics, 9 females were excluded due to few numbers, personnel listing in company phone directories were used to identify test stand assignments (and infer potential specific chemical exposures) for 1,440 subjects, and of this group, 1,111 male test stand mechanics were identified with potential TCE exposure either from the cleaning of rocket engines between tests or from more generalized use as a utility degreasing solvent. Cause-specific mortality is compared to several referents: (1) morality rates of the U.S. population; (2) mortality rates of California residents; (3) hourly nonadministrative workers at SSFL and two nearby facilities; and (4) 1,598 SSFL hourly workers; however, the published paper does not

clearly present details of all analyses. For example, the referent population is not identified for the SMR analysis of the 1,111 male subjects with TCE potential exposure and analyses examining exposure duration present point estimates and p-values from tests of linear trend, but not always CIs (e.g., Boice et al. (2006b) Table 7, table footnotes).

Exposure assessment to TCE is qualitative without attempt to characterize exposure level as was done in the exposure assessment approach of Zhao et al. (2005) and Krishnadsen et al. (2007). Test stand mechanics were nonadministrative hourly positions and had the greatest potential for chemical exposures to TCE and hydrazine. Potential exposure to chemicals also existed for other subjects associated with test stand work such as instrument mechanics, inspectors, test stand engineers, and research engineers potential for chemical exposure, although Boice et al. (2006b) considered their exposure potential lower compared to that received by test stand mechanics and, thus, were not included in the cohort. Like that encountered by UCLA researchers, work history information in the personnel file was not specific to identify work location and test stand and Boice et al. (2006b) adopted ancillary information, company phone directories, as an aid to identify subjects with greater potential for TCE exposure. From these aids, investigators identified rocket stand assignment for 1,440 or 87% of the SSFL test stand mechanics. Bias is introduced through missing information on the other 211 subjects or if phone directories were not available for the full period of the study. Test stand mechanics, if exposed, had the likelihood for exposure to high TCE concentrations associated with flushing or cleaning of rocket engines; 593 of the 1,111 subjects (53%) were identified as having potential TCE exposure through rocket engine cleaning. The removal or flushing of hydrocarbon deposits in fuel jackets and in liquid oxygen dome of large engines entailed the use of 5 to 100 gallons of TCE, with TCE use starting around 1956 and ceased by the late 1960's at all test stands except one which continued until 1994. No information was provided on test stand and working conditions or the frequency of exposure-related tasks, and no atmospheric monitoring data were available on TCE. A small number of these subjects (121) also had potential exposure to hydrazines. The remaining 518 subjects in the TCE subcohort were presumed exposed to TCE as a utility solvent. Information on use of TCE as a utility solvent is lacking except that TCE as a utility solvent was discontinued in 1974 except at one test stand where it was used until 1984. These subjects have a lower likelihood of exposure compared to subjects with TCE exposure from cleaning rocket engines.

Several study design and analysis aspects limit this study for assessing risks associated with TCE exposure. Overall, exposures were likely substantially misclassified and their frequency likely low, particularly for subjects identified with TCE use as a utility solvent who comprise roughly 50% of the TCE subcohort. Analyses examining number of years employed at SSFL or worked as test stand mechanic as a surrogate for cumulative exposure has a large potential for misclassification bias due to the lack of air monitoring data and inability to account to temporal changes in TCE usage. Moreover, the exposure metric used in some dose-response

analyses is weighted by the number of workers without rationale provided and would introduce bias if the workforce changed over the period covered by this study. Some information suggests that this was likely: (1) the number of cohort subjects entering the cohort decreased over the time period of this study, as much as a 20% decrease between 1960s and 1970s, and (2) ancillary information (http://www.thewednesdayreport.com/twr/twr48v7.htm, accessed March 11, 2008; DOE Closure Project, http://www.etec.energy.gov/Reading-Room/DeSoto.html, accessed March 11, 2008). Study investigators did not carry out exposure assessment for referents and no information is provided on potential TCE exposure. If referents had more than background exposure, likely for other hourly subjects with direct association with test stand work but with a job title other than test stand mechanic, the bias introduced leads to an underestimation of risk. TCE use at SSFL was widespread and rocket engine cleaning occurred at other locations besides at test sites (Morgenstern et al., 1999), locations from which the referent population arose.

Boice JD, Marano DE, Cohen SS, Mumma MT, Blott WJ, Brill AB, Fryzek JP, Henderson BE, McLaughlin JK. (2006b). Mortality among Rocketdyne workers who tested rocket engines, 1948–1999. J Occup Environ Med 48:1070–1092.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract "objective of this study was to evaluate potential health risks associated with testing rocket engines."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	54,384 Rocketdyne workers of which 41,351 were employed on or after 1-1-1948 and for at least 6 months at SSFL or nearby facilities. Of the 41,351 subjects, 1,651 were identified as having a job title of test stand mechanic and exposure assignments could be made for 1,440 of these subjects. Site-specific mortality rates of U.S. population and of all-other Rocketdyne employees. Potential TCE exposures of all other subjects (referents) not documented but investigators assumed referents are unexposed to TCE.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality from 1948 to 12-31-1999.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Coding to ICD in use at time of death.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Qualitative exposure assessment, any TCE exposure. No quantitative information on TCE intensity by job title or to individual subjects or referents. Missing exposure potential to 12% of test stand mechanics; potential exposure hydrazine and/or TCE assigned to 1,440 of 1,651 test stand mechanics. Of 1,440 test stand mechanics, 1,111[a] identified with potential TCE exposure, 518 of the 1,111 identified as having presumed high intensity exposure from the cleaning of rocket engines. The remaining 593 subjects with potential exposure to TCE through use as "utility solvent," a job task with low likelihood or potential for TCE exposure.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	0.4% for test stand mechanic cohort (1,651 subjects).
>50% cohort with full latency	35 yrs average follow-up; 88% of 1,651 test stand mechanics >20-yr follow-up.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	TCE exposed subcohort—391 total deaths, 121 cancer deaths.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	SMR analysis restricted to male hourly test stand mechanics using U.S. population rates as referent—no adjustment of potential confounders other than age and calendar-year. Cox proportional hazard models examining TCE exposure adjusted for birth year, year of hire and potential hydrazine exposure. Race was not included in Cox proportional hazard analysis.
Statistical methods	SMR analysis and Cox proportional hazard.
Exposure-response analysis presented in published paper	Duration of exposure (employment): 2-sided tests for linear trend.
Documentation of results	All analyses are not presented in published paper. Follow-up correspondence of C Scott, U.S. EPA, to J. Boice, of 12-31-06 and 02-28-07 remain unanswered as of November 15, 2007.

[a]Zhao et al. (2005), whose study period and base population overlaps that of Boice et al. (2006b), identified a larger number of subjects with potential TCE exposures; 2,689 subjects with TCE score >3, a group having medium to high cumulative TCE exposure.

B.3.1.1.1.2. **UCLA studies of Rocketdyne workers.**

B.3.1.1.1.2.1. **Krishnadasan et al. (2007).**

B.3.1.1.1.2.1.1. **Author's abstract.**

Background To date, little is known about the potential contributions of occupational exposure to chemicals to the etiology of prostate cancer. Previous studies examining associations suffered from limitations including the reliance on mortality data and inadequate exposure assessment. **Methods** We conducted a nested case-control study of 362 cases and 1,805 matched controls to examine the association between occupational chemical exposures and prostate cancer incidence. Workers were employed between 1950 and 1992 at a nuclear energy and rocket engine-testing facility in Southern California. We obtained cancer incidence data from the California Cancer Registry and seven other state cancer registries. Data from company records were used to construct a job exposure matrix (JEM) for occupational exposures to hydrazine, trichloroethylene (TCE), polycyclic aromatic hydrocarbons (PAHs), benzene, and mineral oil. Associations between chemical exposures and prostate cancer incidence were assessed in conditional logistic regression models. **Results** With adjustment for occupational confounders, including socioeconomic status, occupational physical activity, and exposure to the other chemicals evaluated, the odds ratio for low/moderate TCE exposure was 1.3; 95%CI=0.8 to 2.1, and for high TCE exposure was 2.1; 95%CI=1.2 to 3.9. Furthermore, we noted a positive trend between increasing levels of TCE exposure and prostate cancer (p-value for trend=0.02). **Conclusion** Our results suggest that high levels of TCE exposure are associated with prostate cancer among workers in our study population.

B.3.1.1.1.2.2. **Zhao et al. (2005).**

B.3.1.1.1.2.2.1. **Author's abstract.**

Background A retrospective cohort study of workers employed at a California aerospace company between 1950 and 1993 was conducted; it examined cancer mortality from exposures to the rocket fuel hydrazine. **Methods** In this study, we employed a job exposure matrix (JEM) to assess exposures to other known or suspected carcinogens—including trichloroethylene (TCE), polycyclic aromatic hydrocarbons (PAHs), mineral oils, and benzene—on cancer mortality (1960–2001) and incidence (1988–2000) in 6,107 male workers. We derived rate-(hazard-) ratios estimates from Cox proportional hazard models with time-dependent exposures. **Results** High levels of TCE exposure were positively associated with cancer incidence of the bladder (rate ratio (RR): 1.98, 95% confidence interval (CI) 0.93–4.22) and kidney (4.90; 1.23–19.6). High levels of exposure to mineral oils increased mortality and incidence of lung cancer (1.56; 1.02–2.39 and 1.99; 1.03–3.85), and incidence of melanoma (3.32; 1.20–9.24). Mineral oil exposures also contributed to incidence and mortality of esophageal and stomach cancers and of non-Hodgkin lymphoma and leukemia when adjusting for other chemical exposures. Lagging exposure measures by 20 years changed effect estimates only minimally. No associations were observed for benzene or PAH exposures in this cohort. **Conclusions** Our findings suggest that

these aerospace workers who were highly exposed to mineral oils experienced an increased risk of developing and/or dying from cancers of the lung, melanoma, and possibly from cancers of the esophagus and stomach and non-Hodgkin lymphoma and leukemia. These results and the increases we observed for TCE and kidney cancers are consistent with findings of previous studies.

B.3.1.1.1.2.3. **Study description and comment.**

The source population for Krishnadasen et al. (2007) and Zhao et al. (2005) is the UCLA chemical cohort of 6,044 male workers with ≥2 years of employment Rocketdyne between 1950 and 1993, who engaged in rocket testing at SSFL before 1980 and who have never been monitored for radiation. Zhao et al. (2005) examined cancer mortality between 1960 and 2001, an additional 7 years from earlier analyses of the chemical subcohort (Morgenstern et al., 1999; Ritz et al., 1999), and cancer incidence (5,049 subjects) between 1988 and 2000, matching cohort subjects to names in California's Cancer Registry and eight other state cancer registries. Deaths before 1998 are coded using ICD, 9th revision, and ICD-10 after this date; ICD-0 was used to code cancer incidence with leukemia, lymphoma, and other lymphopoietic tumors grouped on the basis of morphology codes. A total of 600 cancer deaths and 691 incident cancers were identified during the study period.

Krishnadasen et al. (2007) adopted a nested case-control design to examine occupational exposure to several chemicals and prostate cancer incidence in a cohort, which included the SSFL chemically-exposed subjects and an additional 4,607 workers in the larger cohort who were enrolled in the company's radiation monitoring program. A total of 362 incident prostate cancers were identified between 1988 and 12-31-1999. Controls were randomly selected from the original cohorts using risk-set sampling and a 5:1 matching ratio on age at start of employment, age at diagnosis, and cohort.

Both studies are based on the same exposure assessment approach. Walk-through visits, interviews with managers and workers, job descriptions manual, and historical facility reports supported the development of a JEM with jobs ranked on a scale of 0 (no exposure) to 3 (highly exposure) on presumptive exposure reflecting relative intensity of that exposure over three temporal periods: 1950–1960, 1970s, 1980–1990. Of the 6,044 subjects, 2,689 had TCE exposure scores of >3 and 2,643 with an exposure score ≥3 for hydrazine. Workers with job titles indicating technical or mechanical work on rocket engines were presumed to have high hydrazine rocket fuel exposure and high TCE exposure, which was used in cleaning rocket engines and parts. Although fewer subjects had exposure to benzene (819 subjects) or mineral oil (1,499 subjects), a high percentage of these subjects were also exposed to TCE. TCE use was widespread at the facility and other mechanics, maintenance and utility workers, and machinists were presumed as having exposure. No details were provided for job titles other than rocket test stand mechanics for assigning TCE exposure intensity and historical trends in TCE usage. Air monitoring data were absent for any chemicals prior to 1985 and investigators could not link

study subjects to specific work locations and rocket-engine test stands. As a result, exposures were probably substantially misclassified, particularly those with low to moderate TCE exposure. Cumulative intensity score was the sum of the job-and time-specific intensity score and years in job. Exposure classification was assigned blinded to survival status and cause of death.

Proportional hazards modeling in calendar time with both fixed and time-dependant predictors was used by Zhao et al. (2005) to estimate exposure effects on site-specific cancer incidence and mortality for a combined exposure group of medium and high exposure intensity with workers with no to low exposure intensity as referents. Variables in the proportional hazard model included time since first employment, SES, age at diagnosis or death, and exposure to other chemical agents including benzene, polycyclic aromatic hydrocarbons (PAHs) mineral oil, and hydrazine. Krishnadasen et al. (2007) fit conditional logistic regression models to their data adjusting of cohort, age at diagnosis, occupation physical activity, SES and all other chemical exposure levels. Both publications include exposure-response analysis and present *p*-values for linear trend. Race was not controlled in either study given the lack of recording on personnel records. Smoking histories was available for only a small percentage of the cohort; for those subjects reporting smoking information, mean cumulative TCE score did not differ between smokers and nonsmokers.

This study develops semiquantitative exposure levels and is strength of the exposure assessment. However, potential for exposure misclassification exists and would be of a nondifferential direction. Rocket engine test stand mechanics had likely exposure to TCE, kerosene, and hydrazine fuels; no information is available as to exposure concentrations. Statistical analyses in both Zhao et al. (2005) and Krishnadansan et al. (2007) present risk estimates for TCE that were adjusted for these other chemical exposures. Other strengths of this study include a long follow-up period for mortality, greater than an average time of 29 years of which 16 at SSFL, use of internal referents and the examination of cancer incidence, although under ascertainment of cases is likely given only eight state cancer registries were used to identify cases and incidence ascertained after 1981, 40 years after the cohort's initial definition date.

Krishnadasan A, Kennedy N, Zhao Y, Morgenstern H, Ritz B. (2007). Nested case-control study of occupational chemical exposures and prostate cancer in aerospace and radiation workers. Am J Ind Med 50:383–390.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Nested case-control study of the UCLA chemical and radiation cohorts (Morgenstern et al., 1999, 1997) to assess occupational exposures including TCE and prostate cancer.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	4,607 radiation cohort + 6,107 Santa Susana chemical cohort (Zhao et al., 2005; Ritz et al., 1999), excluded 1,410 deaths before 1988 (date of cancer incidence follow-up). Incident prostate cancer cases identified from eight State cancer registries (California, Nevada, Arizona, Texas, Washington Florida, Arkansas, and Oregon). Controls were randomly selected from the original cohorts using risk-set sampling. 362 cases and 1,805 controls (100% participation rate).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Prostate cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	TCE exposure assigned to cases and controls based on longest job held at company as identified from personnel records. Cumulative exposure—ranked exposure intensity score for TCE by three time periods—using method of Zhao et al. (2005). Blinded ranking of exposure status.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Employment records were used to assign exposure. 734 subjects (249 cases and 485 controls, or 33% of all cases and controls) were interviewed via telephone or sent a mailed questionnaire to obtain medical history, education and personal information on physical activity level and smoking history.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No proxy interviews.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Any TCE exposure: 135 cases (37%) and 668 controls (37%). High cumulative TCE exposure: 45 cases (12%) and 124 controls (7%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Cohort, age at diagnosis, occupational physical activity, SES, other chemical exposures (benzene, PAHs, mineral oil, hydrazine). No adjustment for race due to lacking information; affect of race on OR examined using information from survey of workers still alive in 1999. Few African American workers (n = 7), TCE levels did not vary greatly with race.
Statistical methods	Crude and adjusted conditional logistic regression.
Exposure-response analysis presented in published paper	*p*-value for trend with exposure lag (0 yrs, 20 yr).
Documentation of results	Adequate.

Zhao Y, Krishnadasan A, Kennedy N, Morgenstern H, Ritz B. (2005). Estimated effects of solvents and mineral oils on cancer incidence and Mortality in a cohort of aerospace workers. Am J Ind Med 48:249–258.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From introduction "one aim of this new investigation was to determine whether these aerospace workers also developed cancers from exposures to other chemicals including trichloroethylene (TCE), polycyclic aromatic hydrocarbons (PAHs), mineral oils, and benzene."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	6,107 male workers employed for ≥2 years and before 1980 at SSFL. Internal referents (no or low TCE exposure).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence between 1988 and 2000. Mortality between 1950 and 2001.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-0 for cancer incidence. Leukemia, lymphomas, and other lymphopoietic malignancies grouped on the basis of morphology codes. Mortality: ICD-9, before 1998, and ICD-10 thereafter. Incidence: ICD-Oncology Lymphoma and leukemia grouping includes lymphosarcoma and reticulosarcoma, Hodgkin lymphoma, other malignant neoplasm of the lymphoid and histiocytic tissue, multiple myeloma and immunoproliferative neoplasms, and all leukemias except chronic lymphoid leukemia. The following incident tumors were also included: Hodgkin lymphoma, leukemia, polycythemia vera, chronic myeloproliferative disease, myelosclerosis, eosinophilic conditions, platelet diseases, and red blood cell diseases.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Cumulative exposure—ranked exposure intensity score for TCE by three time periods Blinded ranking of exposure status.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	99% follow-up for mortality (6,044 of 6,107 subjects).
>50% cohort with full latency	Average latency = 29 yrs (Ritz et al., 1999).
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	600 cancer deaths, 621 cancer cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Time since first employment, SES, age (at incidence or mortality), exposure to other carcinogens, including hydrazine. No adjustment for race. Indirectly assessment of smoking through examination of smoking distribution by chemical exposure. Mean TCE cumulative exposure scores of smokers and nonsmokers is not statistically significant different.
Statistical methods	Cox proportional hazards modeling in calendar time with both fixed and time-dependent predictors. Exposure lagged 10 and 20 yrs.
Exposure-response analysis presented in published paper	Test for monotonic trend of cumulative exposure, two-sided *p*-value for trend.
Documentation of results	Liver cancer results are not reported in published paper.

B.3.1.1.1.3. **Comment on the SSFL studies**

Rocketdyne workers at SSFL are subject of two separate and independent studies. Both research groups draw subjects from the same underlying source population, Rocketdyne workers including those at SSFL; however, the methods adopted to identify study subjects and to define TCE exposure differ with each study. A subset of SSFL workers is common to both studies; however, no information exist in final published reports (IEI, 2005; Morgenstern et al., 1999, 1997) to indicate the percentage overlap between cohorts or between observed number of site-specific events.

Notable differences in both study design and analysis including cohort identification, endpoint, exposure assessment approaches, and statistical methods exist between Zhao et al. (2005) and Krishnadasan et al. (2007), whose source population is the UCLA cohort, and Boice et al. (2006b) whose source population is the IEI cohort. A perspective of each study's characteristics may be obtained from Table B-6.

Table B-6. Characteristics of epidemiologic investigations of Rocketdyne workers

Study	Boice et al. (2006b)	Zhao et al. (2005)
Source population	41,351 administrative/scientific and nonadministrative male and female employees between 1949 and 1999 at Rocketdyne SSFL and two nearby facilities	~55,000 subjects of SSFL and two nearby facilities employed between 1950 and 1993
TCE subcohort	1,111 male test stand mechanics with potential TCE exposure	6,107 males working at SSFL before 1980 and identified as test stand personnel, of whom 2,689 males had exposure scores greater than no- to low-TCE exposure potential
Pay-type (hourly)	100% of TCE subcohort	11.3%
Job title with potential TCE exposure	Test stand mechanics identified with greatest potential for TCE exposure Other job titles with direct association with test stand work—instrument mechanics, inspectors, test stand engineers, and research engineers—identified with lower exposure potential to TCE and included in referent population	High potential exposure group included job titles as propulsion/test mechanics or technicians; Medium potential exposure group included propulsion/test inspector, test or research engineer, and instrumentation mechanic; Low-exposure potential included employees who, according to job title may have been present during engine test firings but without direct contact
Exposure metric	Qualitative, yes/no, and employment duration	Cumulative exposure score = $\sum$ (exposure score (0–3) × number of years in job)
Endpoint	Mortality as of 1999	Mortality as of 2001 and Incidence as of 2000
Statistical analysis	SMR Proportional hazards modeling with covariates for birth year, hire year, and potential exposure to hydrazine.	Proportional hazards modeling with covariates for time since first employment, SES, age at event, and exposure to all other carcinogens, including hydrazine
Observed number of deaths:		
Total cancer	121	600
Lung	51	No/low, 99
		Medium, 62
		High, 33
Kidney	7	No/low, 7
		Medium, 7
		High, 3
Bladder	5	No/low, 8
		Medium, 6
		High, 3
NHL/Leukemia	6	No/low, 27
		Medium, 27
		High, 6

A number of strengths and limitations underlie these studies. First, the Zhao et al. (2005) and Krishnadasan et al. (2007) analyses is of a larger population and of more cancer cases or deaths; 600 cancer deaths and 691 cancer cases in Zhao et al. (2005) compared to 121 cancer deaths in the TCE subcohort of Boice et al. (2006b), and for prostatic cancer among all Rocketdyne workers, 362 incident prostatic cancer cases in Krishnandasan et al. (2007) compared to 193 deaths in Boice et al. (2006b). Second, exposed populations appear appropriately selected in the three studies although questions exist regarding the referent population in Boice et al. (2006b) whose referent population included subjects with some direct association with test stand work but whose job title was other than test stand mechanic. As a result, it appears that these studies identify TCE exposure potential different for possibly similar job titles. For example, jobs as instrument mechanics, inspectors, test stand engineers, and research engineers are identified with medium potential exposure in Zhao et al. (2005). Boice et al. (2006b) on the other hand included these subjects in the referent population and assumed they had background exposure. TCE use at SSFL was also widespread and rocket engine cleaning occurred at other locations besides at test sites (Morgenstern et al., 1999), locations from which the referent population in Boice et al. (2006b) arose. If referents in Boice et al. (2006b) had more than background exposure, the bias introduced leads to an underestimation of risk. Third, Zhao et al. (2005) and Krishnadasan et al. (2007) studies include an examination of incidence, and are likely to have a smaller bias associated with disease misclassification than Boice et al. (2006b) who examines only mortality. Fourth, use of cumulative exposure score although still subject to biases is preferred to qualitative approach for exposure assessment. Last, all three studies adjusted for potentially confounding factors such as smoking, SES, and other carcinogenic exposures using different approaches either in the design of the study, such as Boice et al. (2006b) limitation to only hourly workers, or in the statistical analysis such as Zhao et al. (2005) and Krishnadansen et al. (2007). For this reason, the large difference in hourly workers between the UCLA cohort and Boice et al. (2006b) is not likely to greatly impact observations.

B.3.1.1.2. **Blair et al. (1998), Radican et al. (2008).**

B.3.1.1.2.1. **Radican et al. (2008)) abstract.**

> OBJECTIVE: To extend follow-up of 14,455 workers from 1990 to 2000, and evaluate mortality risk from exposure to trichloroethylene (TCE) and other chemicals. METHODS: Multivariable Cox models were used to estimate relative risk (RR) for exposed vs. unexposed workers based on previously developed exposure surrogates. RESULTS: Among TCE-exposed workers, there was no statistically significant increased risk of all-cause mortality (RR = 1.04) or death from all cancers (RR = 1.03). Exposure-response gradients for TCE were relatively flat and did not materially change since 1990. Statistically significant excesses were found for several chemical exposure subgroups and causes and

were generally consistent with the previous follow-up. CONCLUSIONS: Patterns of mortality have not changed substantially since 1990. Although positive associations with several cancers were observed, and are consistent with the published literature, interpretation is limited due to the small numbers of events for specific exposures.

B.3.1.1.2.2. **Blair et al. (1998) abstract.**

OBJECTIVES: To extend the follow up of a cohort of 14,457 aircraft maintenance workers to the end of 1990 to evaluate cancer risks from potential exposure to trichloroethylene and other chemicals. **METHODS:** The cohort comprised civilians employed for at least one year between 1952 and 1956, of whom 5727 had died by 31 December 1990. Analyses compared the mortality of the cohort with the general population of Utah and the mortality and cancer incidence of exposed workers with those unexposed to chemicals, while adjusting for age, sex, and calendar time. **RESULTS:** In the combined follow up period (1952–90), mortality from all causes and all cancer was close to expected (standardized mortality ratios (SMRs) 97 and 96, respectively). Significant excesses occurred for ischemic heart disease (SMR 108), asthma (SMR 160), and cancer of the bone (SMR 227), whereas significant deficits occurred for cerebrovascular disease (SMR 88), accidents (SMR 70), and cancer of the central nervous system (SMR 64). Workers exposed to trichloroethylene showed non-significant excesses for non-Hodgkin's lymphoma (relative risk (RR) 2.0), and cancers of the oesophagus (RR 5.6), colon (RR 1.4), primary liver (RR 1.7), breast (RR 1.8), cervix (RR 1.8), kidney (RR 1.6), and bone (RR 2.1). None of these cancers showed an exposure-response gradient and RRs among workers exposed to other chemicals but not trichloroethylene often had RRs as large as workers exposed to trichloroethylene. Workers exposed to solvents other than trichloroethylene had slightly increased mortality from asthma, non-Hodgkin's lymphoma, multiple myeloma, and breast cancer. **CONCLUSION:** These findings do not strongly support a causal link with trichloroethylene because the associations were not significant, not clearly dose-related, and inconsistent between men and women. Because findings from experimental investigations and other epidemiological studies on solvents other than trichloroethylene provide some biological plausibility, the suggested links between these chemicals and non-Hodgkin's lymphoma, multiple myeloma, and breast cancer found here deserve further attention. Although this extended follow up cannot rule out a connection between exposures to solvents and some diseases, it seems clear that these workers have not experienced a major increase in cancer mortality or cancer incidence.

B.3.1.1.2.3. **Study description and comment.**

This historical cohort study of 14,457 (9,400 male and 3,138 female) civilian personnel employed at least 1 year between 1942 and 1956 at Hill Air Force Base in Utah examines mortality to the end of 1982 (Spirtas et al., 1991) to the end of 1990 (Blair et al., 1998), or to the end of 2000 (Radican et al., 2008). About half of the cohort was identified with exposure to

TCE (6,153 white men and 1,051 white women). One-fourth of subjects were born before 1909 with an attained age of 43 years at cohort's identification date of 1952 and whose first exposure could have been as early as 1939, a cohort considered as a "survivor cohort."

As of December 2008, the end of follow-up in Radican et al. (2008), 8,580 deaths (3,628 in TCE subcohort) were identified, an increase of 2,853 deaths with the additional 8 years follow-up period compared to Blair et al. (1998) (5,727 total deaths, 2,813 among TCE subcohort subjects), with a larger proportion deaths among non-TCE exposed subjects (58%) as of December 2008 compared to the December 2000 (51%). Approximately 50% of TCE-exposed subjects and 60% of all cohort subjects had died, with mean age of 75 years for TCE-exposed subjects still alive and ≥45 years since the cohort's definition (1953 to 1955), a time period longer than that typically considered for an induction or latent window for detecting an adverse outcome like cancer. Blair et al. (1998) additionally examined cancer incidence among white TCE-exposed workers alive on 1-1-1973, a period of 31 years after the cohort's inception date, to the end of 1990. Incident cancer cases are likely under ascertained for this reason.

Statistical analyses in Spirtas et al. (1991) and Blair et al. (1998) focus on site-specific mortality for white subjects or subjects with unknown race who were assumed to as white since 97% of all subjects with known race were white. SMRs are presented with expected numbers of deaths based upon age-, race-, and year-specific mortality rates of the Utah population (Blair et al., 1998; Spirtas et al., 1991) or rate ratios for mortality or cancer incidence for the TCE subcohort from Poisson regression models, adjusting for date of birth, calendar year of death, and sex where appropriate, and an internal standard of mortality rates of the cohort's nonchemical exposed subjects (internal referents) (Blair et al., 1998). Blair et al. (1998), in addition to their presentation in the published papers of risk estimates associated with TCE exposure, also, presented risk estimates for subjects with an aggregated category of "any solvent exposure" (ever exposed) and for exposure to 14 solvents. To compare with risk ratios from Poisson regression models of Blair et al. (1998), Radican et al. (2008) adopted Cox proportional hazard models to reanalyze mortality observations of follow-up through 1990. For most site-specific cancers, Radican et al. (2008) did not observe large differences between the Cox hazard ratio and Poisson rate ratio of Blair et al. (1998), although difference between risk estimates from Cox proportional hazard and Poisson regression of ≥20% was observed for kidney cancer (increased risk estimate) and primary liver cancer (decreased risk estimate). Radican et al. (2008), furthermore, noted hazard ratios for all subjects were similar to results for white subjects only; therefore, their analyses of follow-up through 2000 included all subjects.

The original exposure assessment of Stewart et al. (1991) who conducted a detailed exposure assessment of TCE exposures at Hill Air Force Base was used by Radican et al. (2008), Blair et al. (1998), and Spirtas et al. (1991). Their study was limited linking subjects with exposures principally because solvent exposures were associated with work in "shops," but work

records listed only broad job titles and administrative units. As a result, exposures were probably substantially misclassified, particularly in "mixed solvent group." TCE was used principally for degreasing and hand cleaning in work areas during 1955–1968. TCE was the predominant solvent used in the few available vapor degreasers located in the electroplating (main hanger), propeller, and engine repair shops before the mid-1950 and, afterwards, as a cold state solvent, replacing Stoddard solvent. Solvents, notably TCE after 1955, were used primarily by aircraft mechanics with short but high exposures and sheet metal workers for spot clean aircraft surfaces. The investigators determined that 32% had "frequent" exposures to peak concentrations (one or two daily peaks of about 15 minutes to TCE at 200–600 ppm) during vapor degreasing. Work areas were located in very large buildings with few internal partitions, which aided dispersion of TCE. While TCE exposures were less controlled in the 1950s, by the end of 1960s, TCE exposure had been reduced significantly. Only a small number of subjects with "high" exposure had long-duration exposures, no more than 16%. Few workers were exposed only to TCE; most had mixed exposures to other chlorinated and nonchlorinated solvents. Person-years of exposure were computed from date of first exposure, which could have been as early as 1939, to the end of 1982.

Overall, Blair et al. (1998) and Radican et al. (2008) are studies with approximately half of the larger cohort identified as having some potential for TCE exposure (the TCE subcohort) and calculation of cancer risk estimates for TCE exposure, either risk ratios in Blair et al. (1998) or hazard ratios in Radican et al. (2008), using workers in the cohort without any chemical exposures as referent population, superior to SMRs of Spirtas et al. (1991) who first reported on mortality and TCE exposure. Use of an internal referent population of workers from the same company or plant, but lacking the exposure of interest, is considered to reduce bias associated with the healthy worker effect. For follow-up in Radican et al. (2008) who examined mortality 45 years after first exposure and likely at the tail of or beyond a window for cancer induction time, any influence on exposure on disease development or detection times would be diminished or less evident if exposures like TCE shortened induction time, e.g., if exposure shortened the natural course of disease development, which would become evident in an unexposed subjects with longer follow-up periods. The induction time of 35 years in Blair et al. (1998) may also fall outside a cancer induction window; however, it is more consistent with cancer induction times observed with other chemical carcinogens such as aromatic amines (Weistenhofer et al., 2008) and vinyl chloride (Du and Wang, 1998). A strong exposure assessment was performed, but precision in the exposure assignment was limited by vague personnel data. The cohort had a modest number of highly exposed (about 100 ppm) subjects, but overall most were exposed to low concentrations (about 10 ppm) of TCE.

Radican L, Blair A, Stewart P, Wartenberg D. (2008). Mortality of aircraft maintenance workers exposed to trichloroethylene and other hydrocarbons and chemicals: extended follow-up. J Occup Environ Med 50:1306–1319.

Blair A, Hartge P, Stewart PA, McAdams M, Lubin J. (1998). Mortality and cancer incidence of aircraft maintenance workers exposed to trichloroethylene and other organic solvents and chemicals: extended follow-up. Occup Environ Med 55:161–171.

Spirtas R, Stewart PA, Lee JS, Marano DE, Forbes CD, Grauman DJ, Pettigrew HM, Blair A, Hoover RN, Cohen JL. (1991). Retrospective cohort mortality study of workers at an aircraft maintenance facility. I. Epidemiological results. Br J Ind Med 48:515–530.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Abstract: "...to evaluate cancer risks from potential exposure to trichloroethylene and other chemicals."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	All civilians employed at Hill Air Force Base for ≥1 yr between 1-1-1952 and 12-31-1956; cohort of 14,457 workers identified form earnings records. TCE subcohort—7,204 white males and females (50%). External referents, all civilian cohort—Utah population rates, 1953–1990. Internal referents, TCE subcohort analysis of mortality (Blair et al., 1998); Radican et al. (2008) and incidence (Blair et al., 1998)—workers without chemical exposures.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality, all civilian cohort and TCE subcohort. Incidence, TCE subcohort.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Underlying and contributing causes of deaths as coded to ICDA 8.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Detailed records on setting and job activities, worker interviews; work done in large open shops; shops not recorded in personnel records, link of job with IH data was weak. Limited exposure IH measurements for TCE between 1960 and 1990. Plant JEM, rank order assignments by history; determined exposure duration during vapor degreasing tasks about 2,000 ppm-hr and hard degreasing about 20 ppm-hr. **Median exposure were about 10 ppm for rag and bucket (cold degreasing process); 100–200 ppm for vapor degreasing (Stewart et al., 1991). Cherrie et al. (2001) estimated long-term exposure as ~50 ppm with short-term excursion up to ~600 ppm. NRC (2006) concluded the cohort had a modest number of highly exposed (about 100 ppm) subjects, but overall most were exposed to low TCE concentrations (about 10 ppm).**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	97% of cohort traced successfully to 12-31-1982.
>50% cohort with full latency	Yes, all subjects followed minimum of 35 yrs (Blair et al., 1998) or 45 yrs (Radican et al., 2008).
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	TCE subcohort—2,813 deaths (39%), 528 cancer deaths, and 549 incident cancers (1973-1990) (Blair et al., 1998); 3,628 deaths (50%). 729 cancer deaths (Radican et al., 2008).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	SMR analysis evaluates age, sex, and calendar year (Spirtas et al., 1991). Date of hire, calendar year of death, and sex in Poisson regression analysis (Blair et al., 1998). Age, gender, and race (to compare with RR of Blair et al. (1998), or age and gender for follow-up to 2000 in Cox proportional hazard analysis (Radican et al., 2008).

Statistical methods	External analysis is restricted to Caucasian subjects—Life table analysis for mortality (Spirtas et al., 1991). Internal analysis restricted to Caucasian subjects or subject of unknown race assumed to be Caucasian and followed to 1990—Poisson regression (Blair et al., 1998) or Cox Proportional Hazard (Radican et al., 2008). Internal analysis—all subjects followed to 2000 (Radican et al., 2008).
Exposure-response analysis presented in published paper	Risk ratios from Poisson regression model and hazard ratios from Cox Proportional Hazard model for exposure rankings but no formal statistical trend test presented in papers.
Documentation of results	Adequate.

B.3.1.1.3. **Boice et al. (1999).**

B.3.1.1.3.1. **Author's abstract.**

OBJECTIVES: To evaluate the risk of cancer and other diseases among workers engaged in aircraft manufacturing and potentially exposed to compounds containing chromate, trichloroethylene (TCE), perchloroethylene (PCE), and mixed solvents. **METHODS:** A retrospective cohort mortality study was conducted of workers employed for at least 1 year at a large aircraft manufacturing facility in California on or after 1 January 1960. The mortality experience of these workers was determined by examination of national, state, and company records to the end of 1996. Standardized mortality ratios (SMRs) were evaluated comparing the observed numbers of deaths among workers with those expected in the general population adjusting for age, sex, race, and calendar year. The SMRs for 40 causes of death categories were computed for the total cohort and for subgroups defined by sex, race, and position in the factory, work duration, year of first employment, latency, and broad occupational groups. Factory job titles were classified as to likely use of chemicals, and internal Poisson regression analyses were used to compute mortality risk ratios for categories of years of exposure to chromate, TCE, PCE, and mixed solvents, with unexposed factory workers serving as referents. **RESULTS:** The study cohort comprised 77,965 workers who accrued nearly 1.9 million person-years of follow up (mean 24.2 years). Mortality follow-up, estimated as 99% complete, showed that 20,236 workers had died by 31 December 1996, with cause of death obtained for 98%. Workers experienced low overall mortality (all causes of death SMR 0.83) and low cancer mortality (SMR 0.90). No significant increases in risk were found for any of the 40 specific causes of death categories, whereas for several causes the numbers of deaths were significantly below expectation. Analyses by occupational group and specific job titles showed no remarkable mortality patterns. Factory workers estimated to have been routinely exposed to chromate were not at increased risk of total cancer (SMR 0.93) or of lung cancer (SMR 1.02). Workers routinely exposed to TCE, PCE, or a mixture of solvents also were not at increased risk of total cancer (SMRs 0.86, 1.07, and 0.89, respectively), and the numbers of deaths for specific cancer sites were close to expected values. Slight to moderately increased rates of non-Hodgkin's lymphoma were found among workers exposed to TCE or PCE, but none was significant. A significant increase in testicular cancer was found among those with exposure to mixed solvents, but the excess was based on only six deaths and could not be linked to any particular solvent or job activity. Internal cohort analyses showed no significant trends of increased risk for any cancer with increasing years of exposure to chromate or solvents.

The results from this large scale cohort study of workers followed up for over 3 decades provide no clear evidence that occupational exposures at the aircraft manufacturing factory resulted in increases in the risk of death from cancer or other diseases. Our findings support previous studies of aircraft workers in which cancer risks were generally at or below expected levels.

B.3.1.1.3.2. **Study description and comment.**

This study was conducted on an aircraft manufacturing worker cohort employed at Lockheed-Martin in Burbank, California with exposure assessment described by Marano et al. (2000). This large cohort study of 77,965 subject workers with at least 1 year employment on or after 1-1-1960, examined causes of mortality in the entire cohort, but also by broad job titles and for selected chemical exposures including TCE. Mortality was assessed as of 12-31-1996, with subjects lacking death certificates presumed alive at end of follow-up. Exposure assessment developed using a method of exposure assignment by job categories based on job histories (Kardex cards) and the judgment of long-term employees. Job histories were not available for every worker, and, if missing, auxiliary sources of job information were used to broadly classify workers into various job categories. Only subjects with job histories as recorded on Kardex cards are included in exposure duration analyses. TCE was used for vapor degreasing on routine basis prior to 1966 and, given the cohort beginning date of 1960, only a small percentage of the total cohort was identified as having potential TCE exposure. The investigators determined that 5,443 factory workers had potential TCE exposure. Of these subjects, 3% (2,267/77,965 subjects) had "routine" defined as use of TCE as part of daily job activities and an additional 3,176 subjects (4%) had potential "intermittent" based upon job title and judgment of nonroutine or nondaily TCE usage and were included in the mortality analysis. No information was provided on building and working conditions or the frequency of exposure-related tasks, and no atmospheric monitoring data were available on TCE, although some limited data were available after 1970 on other solvents such as perchloroethylene, which replaced TCE in 1966 in vapor degreasing, methylene chloride, and 1,1,1-trichloroethane. Without more information, it is not possible to determine the quality of some of the TCE assignments. This study had limited ability to detect exposure-related effects given its use of duration of exposure, a poor exposure metric given subjects may have differing exposure intensity with similar exposure duration (NRC, 2006). Lacking monitoring information, analyses examining the number of years of routine and intermittent TCE exposure are likely biased due to exposure misclassification related to inability to account for changes in process and chemical usage patterns over time. Stewart et al. (1991) show atmospheric TCE concentrations decreased over time. Similarly, an observation of inverse relationship between some site-specific causes of death and duration of exposure may be due to selection bias or to misallocation of person-years of follow-up (NYSDOH, 2006).

Boice JD, Marano DE, Fryzek JP, Sadler CJ, McLaughlin JK. (1999). Mortality among aircraft manufacturing workers. Occup Environ Med 56:581–597.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract: "To evaluate the risk of cancer and other diseases among workers engaged in aircraft manufacturing and potentially exposed to compounds containing chromate, trichloroethylene (TCE), perchloroethylene, and mixed solvents."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	All workers employed on or after 1-1-1960 for at least 1 yr at Lockheed Martin aircraft manufacturing factories in California. Control population: U.S. mortality rates or factory workers no exposed to any solvent (internal referents).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD code in use at the time of death.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Qualitative. Few exposure measurements existed prior to the late 1970s, a period after TCE had been discontinued at Lockheed-Martin aircraft manufacturing factories. Subjects are categorized as potentially TCE exposed received on a routine basis (2,075 subjects), daily job activity, or routine and intermittent basis (3,016 subjects), nonroutine or nondaily TCE usage, based on information on Service Record and Permanent Employment Record (Kardex) and other sources of job history information for subjects lacking Kardex cards.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	This study does not adopt methods to verify vital status of employees. All workers for which death certificate were not found are assumed to be alive until end of follow-up.
>50% cohort with full latency	Average follow-up of TCE cohort was 29 yrs.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,100 total deaths and 277 cancer deaths in TCE subcohort.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	SMR analysis—age, sex, and calendar-time. Poisson regression using internal referents—birth date, date first employed, date of finishing employment, race, and sex.
Statistical methods	SMR for routine TCE exposure subcohort. Poisson regression for routine and intermittent TCE exposure subcohort.
Exposure-response analysis presented in published paper	Duration of exposure for subjects with Kardex cards only— 2-sides test for linear trend.
Documentation of results	Adequate.

B.3.1.1.4. **Morgan et al. (1998).**

B.3.1.1.4.1. **Author's abstract.**

> We measured mortality rates in a cohort of 20,508 aerospace workers who were followed up over the period 1950-1993. A total of 4,733 workers had occupational exposure to trichloroethylene. In addition, trichloroethylene was present in some of the washing and drinking water used at the work site. We developed a job-exposure matrix to classify all jobs by trichloroethylene exposure levels into four categories ranging from "none" to "high" exposure. We calculated standardized mortality ratios for the entire cohort and the trichloroethylene exposed subcohort. In the standardized mortality ratio analyses, we observed a consistent elevation for nonmalignant respiratory disease, which we attribute primarily to the higher background rates of respiratory disease in this region. We also compared trichloroethylene-exposed workers with workers in the "low" and "none" exposure categories. Mortality rate ratios for nonmalignant respiratory disease were near or less than 1.00 for trichloroethylene exposure groups. We observed elevated rare ratios for ovarian cancer among those with peak exposure at medium and high levels] relative risk (RR) = 2.74; 95% confidence interval (CI) = 0.84-8.99] and among women with high cumulative exposure (RR = 7.09; 95% CI = 2.14-23.54). Among those with peak exposures at medium and high levels, we observed slightly elevated rate ratios for cancers of the kidney (RR = 1.89; 95% CI = 0.85-4.23), bladder (RR = 1.41; 95% CI = 0.52-3.81), and prostate (RR = 1.47; 95% CI = 0.85-2.55). Our findings do not indicate an association between trichloroethylene exposure and respiratory cancer, liver cancer, leukemia or lymphoma, or all cancers combined.

Erratum:

> One of the authors of the article entitled Mortality of aerospace workers exposed to trichloroethylene, by Robert W. Morgan, Michael A. Kelsh, Ke Zhao, and Shirley Heringer, published in Epidemiology (1998);9:424-431, informed us of some errors in one of the tables. In Table 5, the authors had inadvertently included both genders in counting person-years, rather than presenting gender-specific risk ratios for prostate and ovarian cancer. In addition, one subject, in the high trichloroethylene (TCE) exposure category, had been incorrectly classified with a diagnosis of ovarian cancer, instead of other female genital cancer. The authors report that correction of these errors did not change the overall conclusions of the study. The correct estimates of effect for prostate and ovarian cancer are presented in the Table below.

B.3.1.1.4.2. **Study description and comment.**

This study of a cohort of 20,508 aircraft manufacturing workers employed for at least 6 months between 1950 and 1985 at Hughes Aircraft in Arizona was followed through 1993 for mortality. Cause-specific SMRs are resented for the entire cohort and the TCE-subcohort using U.S. mortality rates from 1950 to 1992 as referents. Additionally, internal cohort analyses fitting Cox proportional hazards models are presented comparing risks for those with TCE exposure to

never-exposed subjects. Morgan et al. (2000, 1998) do not identify job titles of individuals in the never-exposed group; however, it is assumed these individuals were likely white-collar workers, administrative staff, or other blue-collar worker with chemical or solvents exposures other than TCE.

The company conducted a limited semiquantitative assessment of TCE exposure based on the judgment of long-term employees. Most TCE exposure occurred in vapor degreasing units between 1952 and 1977. No details were provided on the protocol for processing the jobs in the work histories into job classifications; no examples were provided. Additionally, no information is provided other chemical exposures that may also have been used in the different jobs. Of the 20,508 subjects, 4,733 were identified with TCE exposure. Exposure categories were assigned to job classifications: high = worked on degreasers (industrial hygiene reported exposures were >50 ppm); medium = worked near degreasers; and low = work location was away from degreasers but "occasional contact with (trichloroethylene)." There was also a "no exposure" category. No data were provided on the frequency of exposure-related tasks. Without more information, it is not possible to determine the quality of some of these assignments. Only the high category is an unambiguous setting. Depending on how the degreasers were operated, operator exposure to TCE might have been substantially >50 ppm. Furthermore, TCE intensity likely changed over time with changes in degreaser operations and exposure assignment based on job title only is able to correctly place subjects with a similar job title but held at different time periods. Furthermore, there are too many possible situations in which an exposure category of medium or low might be assigned to determine whether the ranking is useful. Therefore, the medium and low rankings are likely to be highly misclassified. Deficiencies in job rankings are further magnified in the cumulative exposure groupings. Internal analyses examine TCE exposed, defined as low and high cumulative exposure, compared to never-TCE exposed subjects. Low cumulative exposure group includes any workers with the equivalent of up to 5 years of exposure at jobs at low exposure or 1.4 years of medium exposure; all other workers were placed in the high cumulative exposure grouping. Ambiguity in low and medium job rankings and the lack of exposure data to define "medium" and "low" precludes meaningful analysis of cumulative exposure, specifically, and exposure-response, generally.

The development of exposure assignments in this study was insufficient to define exposures of the cohort and bias related to exposure misclassification is likely great. The inability to account for changes in TCE use and exposure potential over time introduces bias and may dampen observed risks. This study had limited ability to detect exposure-related effects and, overall, limited ability to provide insight on TCE exposure and cancer outcomes.

Morgan RW, Kelsh MA, Zhao K, Heringer S. (1998). Mortality of aerospace workers exposure to trichloroethylene. Epidemiol 9:424–431.

Morgan RW, Kelsh MA, Zhao K, Heringer S. (2000). Mortality of aerospace workers exposed to trichloroethylene. Erratum. Epidemiology 9:424–431.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	"measured mortality rates in a cohort of aerospace workers, comparing TCE workers with workers in low and none exposure categories."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	20,508 male and female workers are identified using company records and who were employed at plant for at least 6 months between 1-1-1950 and 12-31-1985. TCE subcohort—4,733 (23%) male and female subjects. External referents—U.S. population rates, 1950–1992. Internal referents—Analysis of peak exposure, low or no TCE exposure; analysis of cumulative exposure, never exposed to TCE. Internal referents are likely white-collar workers, administrative staff, and blue-collar workers with chemical exposure other than TCE. White-collar and administrative staff subjects are not representative of blue-collar workers due to SES and sex differences. Also, the never-TCE exposed blue-collar workers may potentially have other chlorinated solvents exposures, exposures that may be associated with a similar array of targets as TCE. These individuals may not be representative of a nonchemical exposed population as that used in Blair et al. (1998).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality
Changes in diagnostic coding systems for lymphoma, particularly NHL	No, ICD in use at time of death (ICD 7, 8, 9).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Semiquantitative. Limited IH measurements before 1975. Jobs ranked into high, medium, or low intensity exposure categories; categories are undefined as to TCE intensity. **Jobs with high intensity exposure rating involved work on degreaser machines with TCE exposure equivalent to 50 ppm**; assigned exposure score of 9. Job with medium rating were near (distance undefined in published paper) degreasing area and a score of 4. Jobs with low rating were away (undefined distance) from degreasing area and assigned score of 1. Cumulative exposure score = $\sum$ (duration exposure × score). Peak exposure defined by job with highest ranking score.

CATEGORY D: FOLLOW-UP (Cohort)	
More than 10% loss to follow-up	No, 27 subjects were excluded from analysis due to missing information.
>50% cohort with full latency	Average 22 yrs of follow-up for TCE subcohort.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	TCE subcohort—917 total deaths (19%) of subcohort, 270 cancer deaths.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, race, sex, and calendar year in SMR analysis. Internal analysis- age (for bladder, prostate, ovarian cancers), and age and sex (liver, kidney cancers).
Statistical methods	Life table analysis (SMR). Cox proportional hazards modeling (unexposed subjects as internal referents)—peak and two-levels of cumulative exposure (Morgan et al., 1998; EHS, 1997); any TCE exposure (EHS, 1997).
Exposure-response analysis presented in published paper	Qualitative presentation, only; no formal statistical test for linear trend.
Documentation of results	Adequate.

B.3.1.1.5. Costa et al. (1989).

B.3.1.1.5.1. Author's abstract.

> Mortality in a cohort of 8626 workers employed between 1954 and 1981 in an aircraft manufacturing factory in northern Italy was studied. Total follow up was 132,042 person-years, with 76% accumulated in the age range 15 to 54. Median duration of follow up from the date of first employment was 16 years. Vital status was ascertained for 98.5% of the cohort. Standardized mortality ratios were calculated based on Italian national mortality rates. Altogether 685 deaths occurred (SMR = 85). There was a significant excess of mortality for melanoma (6 cases, SMR = 561). Six deaths certified as due to pleural tumors occurred. No significant excess of mortality was found in specific jobs or work areas.

B.3.1.1.5.2. Study description and comment.

This study assesses mortality in a small cohort of 8,626 aircraft manufacturing workers employed between 1954 and the end of follow-up in June, 1981. A period of minimum employment duration before accumulating person-years was not a prerequisite for cohort definition. The cohort included employees identified as blue collar workers, technical staff, administrative clerks, and white-collar workers. Blue-collar workers comprised 7,105 of the 8,626 cohort subjects. Mortality was examined for all workers and included job title of blue collar workers, technical staff members, administrative clerks, and white-collar workers, not otherwise specified. No exposure assessment was used and the published paper does not identify chemical exposures. In fact, Costa et al. (1989) do not even mention TCE in the paper.

Overall, the lack of exposure assessment, the inability to identify TCE as an exposure to this cohort, and the inclusion of subjects who likely do not have potential TCE exposure are reasons why this study is not useful for determining whether TCE may cause increased risk of disease.

Costas G, Merletti F, Segnan N. (1989). A mortality study in a north Italian aircraft factory. Br J Ind Med 46:738–743.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The 1st paragraph of the paper identified this study was carried out to investigate an apparently high number of malignant tumors among employees that were brought to the attention of the local health authority by staff representative. This study was not designed to examine TCE exposure and cancer outcomes.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cohort is defined as all workers every employed between 1-1-1954 and 6-30-1981 (end of follow-up) at a north Italian aircraft manufacturing factory. Cohort include 8.626 subjects: 950 women (636 clerks, 314 blue-collar workers/technical staff) and 7,676 men (5,625 blue collar workers, 965 technical staff, 571 administrative clerks, and 515 white collar workers). External referent—Age, year (5-yr periods over 1955–1981)-sex and cause-specific death rates of Italian population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Causes and underlying causes of death coded to ICD rule in effect at the time of death and grouped into categories consistent with ICD 8th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Exposure is defined as employment in the factory. TCE is not mentioned in published paper and no exposure assessment was carried out by study investigators.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	Vital status ascertained for 98% of cohort; 2% could not be traced (1% unknown and 1% had emigrated).
>50% cohort with full latency	Average mean follow-up: males, 17 yrs; females, 13 yrs.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	642 total deaths, 168 cancer deaths.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and calendar year.
Statistical methods	SMR.
Exposure-response analysis presented in published paper	No.
Documentation of results	Adequate.

B.3.1.1.6. Garabrant et al. (1988).

B.3.1.1.6.1. Author's abstract.

A retrospective cohort mortality study was conducted among men and women employed for four or more years, between 1958 and 1982, at an aircraft manufacturing company in San Diego County. Specific causes of death under investigation included cancer of the brain and nervous system, malignant melanoma, and cancer of the testicle, which previous reports have suggested to be associated with work in aircraft manufacturing. Follow-up of the cohort of 14,067 subjects for a mean duration of 15.8 yr from the date of first employment resulted in successful tracing of 95% of the cohort and found 1,804 deaths through 1982. Standardized mortality ratios (SMRs) were calculated based on U. S. national mortality rates and separately based on San Diego County mortality rates. Mortality due to all causes was significantly low (SMR = 75), as was mortality due to all cancer (SMR = 84). There was no significant excess of cancer of the brain, malignant melanoma, cancer of the testicle, any other cancer site, or any other category of death. Additional analyses of cancer sites for which at least ten deaths were found and for which the SMR was at least 110 showed no increase in risk with increasing duration of work or in any specific calendar period. Although this study found no significant excesses in cause-specific mortality, excess risks cannot be ruled out for those diseases that have latency periods in excess of 20 to 30 yr, or for exposures that might be restricted to a small proportion of the cohort.

B.3.1.1.6.2. Study description and comment.

This study reported on the overall mortality of a cohort of workers in the aircraft manufacturing industry in southern California who had worked 1 day at the facility and had at least 4 years duration of employment. Fifty-four percent of cohort entered cohort at beginning date (1-1-1958). This is a survivor cohort. This study lacks exposure assessment for study subjects. The only exposure metric was years of work. Examination of jobs held by 70 study subjects, no details provided in paper on subject selection criteria, identified 37% as having possible TCE exposure, but no information was presented on how they were exposed, frequency or duration of exposure, or job titles associated with exposure. No information is provided on possible TCE exposure to the remaining ~14,000 subjects in this cohort. The exposure assignment in this study was insufficient to define exposures of the cohort and the frequency of exposures was likely low. Given the enormous misclassification on exposure, the effect of exposure would have to be very large to be detected as an overall risk for the population. Null findings are to be expected due to bias likely associated with a survivor cohort and to exposure misclassification. Therefore, this study provides little information on whether TCE is related to disease risk.

Garabrant DH, Held J, Langholz B, Bernstein L. (1988). Mortality of Aircraft Manufacturing Workers in Southern California. Am J Ind Med 13:683–693.

Langholz B, Goldstein L. (1996). Risk Set Sampling in Epidemiologic Cohort Studies. Stat Sci 11:35–53.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	"Our objects were to evaluate the oval mortality among the [aircraft manufacturing] workers and to test the hypotheses that brain tumors, malignant melanoma, and testicular neoplasms are associated with work in this industry." [Introduction] This study was not designed to evaluate any specific exposure, but rather employment in aircraft manufacturing industry.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	14,067 males and females working at least 4 yrs with a large aircraft manufacturing company and who had worked for at least 1 d at a factory in San Diego County, California. Person-year accrued from the anniversary date of an individual's 4th yr of service or from 1-1-1958 to end of follow-up 12-31-1982. External referents—age-, race-, sex-, calendar year-, and cause-specific mortality rates of U.S. population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD revision in effect at the date of death. Lymphomas in four groupings: lymphosarcoma and reticulosarcoma, HD, leukemia and aleukemia, and other.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD revision in effect at the date of death. Lymphomas in four groupings: lymphosarcoma and reticulosarcoma, HD, leukemia and aleukemia, and other.
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Exposure assessment is lacking for all subjects except 70 deaths (14 esophageal and 56 others) who were included in a nested case-control study. Of the 362 jobs held by these 70 subjects, 37% were identified as having potential for TCE exposure.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	4.7% with unknown vital status.
>50% cohort with full latency	Average 16-yr follow-up.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,804 deaths (12.8% of cohort), 453 cancer deaths.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, race, sex, and calendar year.
Statistical methods	SMR.
Exposure-response analysis presented in published paper	No.
Documentation of results	SMR analysis, adequate; published paper lacks documentation of nested case-control study of esophageal cancer.

B.3.1.2. Cancer Incidence Studies Using Biological Monitoring Databases

Finland and Denmark historically have maintained national databases of biological monitoring data obtained from workers in industries where toxic exposures are a concern. Legislation required that employers provide workers exposed to toxic hazards with regular health examinations, which must include biological monitoring to assess the uptake of toxic chemicals, including TCE. In Sweden, the only local producer of TCE operated a free exposure-surveillance program for its customers, measuring U-TCA. These programs used the linear relationship found for average inhaled TCE vs. U-TCA: TCE (mg/m^3) = 1.96; U-TCA (mg/L) = 0.7 for exposures <375 mg/m^3 (69.8 ppm) (Ikeda et al., 1972). This relationship shows considerable variability among individuals, which reflects variation in urinary output and activity of metabolic enzymes. Therefore, the estimated inhalation exposures are only approximate for individuals but can provide reasonable estimates of group exposures. There is evidence of nonlinear formation of U-TCA above about 400 mg/m^3 or 75 ppm of TCE. The half-life of U-TCA is about 100 hours. Therefore, the U-TCA value represents roughly the weekly average of exposure from all sources, including skin absorption. The Ikeda et al. (1972) relationship can be used to convert urinary values into approximate airborne concentration, which can lead to misclassification if tetrachloroethylene and 1,1,1-trichloroethane are also being used because they also produce U-TCA. In most cases, the Ikeda et al. relationship (1972) provides a rough upper boundary of exposure to TCE.

B.3.1.2.1. Hansen et al. (2001).

B.3.1.2.1.1. Author's abstract.

> Human evidence regarding the carcinogenicity of the animal carcinogen trichloroethylene (TCE) is limited. We evaluated cancer occurrence among 803 Danish workers exposed to TCE, using historical files of individual air and urinary measurements of TCE-exposure. The standardized incidence ratio (SIR) for cancer overall was close to unity for both men and women who were exposed to TCE. Men had significantly elevated SIRs for non-Hodgkin's lymphoma (SIR = 3.5; n = 8) and cancer of the esophagus (SIR = 4.2; n = 6). Among women, the SIR for cervical cancer was significantly increased (SIR = 3.8; n = 4). No clear dose-response relationship appeared for any of these cancers. We found no increased risk for kidney cancer. In summary, we found no overall increase in cancer risk among TCE-exposed workers in Denmark. For those cancer sites where excesses were noted, the small numbers of observed cases and the lack of dose-related effects hinder etiological conclusions.

B.3.1.2.1.2. Study description and comment.

This Danish study evaluated cancer incidence in a small cohort of individuals (n = 803) who had been monitored for TCE exposures in a national surveillance program between 1947 and 1989 for U-TCA or TCE in breath since 1974. In all, 2,397 samples were analyzed for U-

TCA of workers at 275 companies and 472 breathing zone samples of TCE from workers at 81 companies. Individual workers could not be identified for roughly one-third of the U-TCA measurements and 50% of breathing zone measurements; many of the individuals most likely had died prior to 1968, the start of the Central Population Registry from which workers were identified and follow-up for cancer incidence. A cohort of 658 males and 145 females were identified from the remaining 1,519 U-TCA and 245 air-TCE measurements. Only two of 803 cohort subjects had both urine and air measurements. Follow-up for cancer incidence ended as of 12-31-1996.

The retirement and measurement records contained general information about the type of employer and the subject's job. The subjects in this study came predominantly from the iron and metal industry with jobs such as metal-product cleaner. Each subject had 1–27 measurements of U-TCA measurements, an average of 2.2 per subject, going back to 1947. Using the linear relationship from Ikeda et al. (1972), the historic median exposures estimated from the U-TCA concentrations were low: 9 ppm for 1947–1964, 5 ppm for 1965–1973, 4 ppm for 1974–1979, and 0.7 ppm for 1980–1989. However, the distributions were highly skewed. Additionally, 5% of the cohort had urine or air samples below the limit of detection. Overall, median exposure in this cohort was 4 ppm and suggests that, in general, workers in a wide variety of industry and job groups and identified as "exposed" in this study had low TCE intensity exposures. Overall, the cohort in this study is small, drawn from a wide variety of industries, predominantly degreasing and metal cleaning, and had generally low exposures (most <20 ppm). The study has a lower power to examine TCE exposure and cancer for these reasons.

Hansen J, Raaschou-Nielsen O, Christensen JM, Johansen I, McLaughlin JK, Lipworth L, Blot WJ, Olsen JH. (2001). Cancer incidence among Danish workers exposed to trichloroethylene. J Occup Environ Med 43:133–139.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From introduction—A study of incidence was carried out to address shortcomings in earlier TCE studies related to the lack of direct exposure information and to assessment of mortality as opposed to incidence.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	803 subjects identified from biological monitoring of urine TCA from 1947 to 1989 (1,519 measurements) or breathing zone TCE since 1974 (245 measurements) and who were alive as of 1968, followed to 1996. External referents—cancer incidence rates of Danish population (age-, sex-, calendar years-, and site-specific).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD, 7th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Biological marker of TCE in urine or in breath used to assign TCE exposure to cohort subject. Historic median exposures estimated from the U-TCA were low: 9 ppm for 1947 to 1964, 5 ppm for 1965 to 1973, 4 ppm for 1974 to 1979, and 0.7 ppm for 1980 to 1989. **Overall, median TCE exposure to cohort was 4 ppm (arithmetic mean, 12 ppm).**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No.
>50% cohort with full latency	Unable to determine given insufficient information in paper; however, text notes follow-up for most subjects achieved a full latency.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	128 incident cancers among 804 cohort subjects (15%).

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and calendar year.
Statistical methods	SIR, Life table analysis.
Exposure-response analysis presented in published paper	Yes, as dichotomous variable for mean exposure (<4 ppm, 4+ ppm) and for cumulative exposure.
Documentation of results	Adequate.

B.3.1.2.2. **Anttila et al. (1995).**

B.3.1.2.2.1. **Author's abstract.**

> Epidemiologic studies and long-term carcinogenicity studies in experimental animals suggest that some halogenated hydrocarbons are carcinogenic. To investigate whether exposure to trichloroethylene, tetrachloroethylene, or 1,1,1-trichloroethane increases carcinogenic risk, a cohort of 2050 male and 1924 female workers monitored for occupational exposure to these agents was followed up for cancer incidence in 1967 to 1992. The overall cancer incidence within the cohort was similar to that of the Finnish population. There was an excess of cancers of the cervix uteri and lymphohematopoietic tissues, however. Excess of pancreatic cancer and non-Hodgkin lymphoma was seen after 10 years from the first personal measurement. Among those exposed to trichloroethylene, the overall cancer incidence was increased for a follow-up period of more than 20 years. There was an excess of cancers of the stomach, liver, prostate, and lymphohematopoietic tissues combined. Workers exposed to 1,1,1-trichloroethane had increased risk of multiple myeloma and cancer of the nervous system. The study provides support to the hypothesis that trichloroethylene and other halogenated hydrocarbons are carcinogenic for the liver and lymphohematopoietic tissues, especially for non-Hodgkin lymphoma. The study also documents excess of cancers of the stomach, pancreas, cervix uteri, prostate, and the nervous system among workers exposed to solvents.

B.3.1.2.2.2. **Study description and comment.**

This Finnish study evaluated cancer risk in a small cohort of individuals (2,050 males and 1,924 females) who had been monitored between 1965 and 1982 for exposures to TCE by measuring their U-TCA. The main source of exposure was identified as degreasing or cleaning metal surfaces. Some workplaces identified rubber work, gluing, and dry-cleaning. There was an average of 2.7 measurements per person. Using the Ikeda et al. (1972) conversion relationship, the exposure for TCE was approximately 7 ppm in 1965, which declined to approximately 2 ppm in 1982; the 75th percentiles for these dates were 14 and 7 ppm, respectively. The maximum values for males were approximately 380 ppm during 1965 to 1974 and approximately 96 ppm during 1974 to 1982. Females showed a similar pattern over time but had somewhat higher exposures than males before the 1970s. Median TCE exposure for females of 4 ppm compared to 3 ppm for males; maximum values were similar for both sexes. Duration of exposure was counted from the first measurement of U-TCA, which might underestimate the length of exposure. Without job histories, the length of exposure is uncertain. Another concern is the sampling strategy; it was not reported how the workers were chosen for monitoring. Therefore, it is not clear what biases might be present, especially the possibility of under-sampling highly exposed workers.

Overall, this TCE exposed cohort drawn from a wide variety of industries was twice the size of other Nordic biomonitoring studies (Hansen et al., 2001; Axelson et al., 1994) with urine

TCA measurements from a more recent period, 1965–1982, compared to other Nordic studies of Danish cohorts, 1947–1980s, or Swedish cohorts, 1955–1975 (Raaschou-Nielsen et al., 2002; Hansen et al., 2001; Axelson et al., 1994). Exposures to TCE were generally low, <14 ppm for the 75th percentile of all measurements, and median TCE exposures decreasing from 7 to 2 ppm over the 17-year period. The medians are similar to estimated exposures to Danish workers with biological markers of U-TCA (Hansen et al., 2001; Raaschou-Nielsen et al., 2001). The duration of exposure was uncertain.

Anttila A, Pukkala E, Sallmen M, Hernberg S, Hemminki K. (1995). Cancer incidence among Finnish workers exposed to halogenated hydrocarbons. J Occup Environ Med 37:797–806.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes, study aim was to assess cancer incidence among workers biologically monitored for exposure to TCE, PERC, and 1,1,1-trichloroethane.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	3, 976 subjects identified from biological monitoring of urine TCA between 1965 to 1982; PERC in blood, 1974 to 1983; and, 1,1,1-trichloroethane in blood, 1975 to 1983 (a total of 10.743 measurements). 109 of cohort subjects with TCE poisoning report between 1965 and 1976. Follow-up for mortality between 1965 and 1991 and for cancer between 1967 and 1992. TCE subcohort—3,089 (1,698 males, 1,391 females). External referents—age-, sex-, calendar year-, and site-specific cancer incidence rates of the Finnish population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality and cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD, 7th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Biological marker of TCE in urine used to assign TCE exposure for TCE subcohort. There were on average 2.5 U-TCA measurements per individual. 6% of cohort had measurements for two or all three solvents. The overall medians of U-TCA for females and males were 8.3 and 6.3 mg/L, respectively, and before 1970, 10–13 mg/L for females and 13–15 mg/L for males. **Using Ikeda et al. (1972) relationship for U-TCA and TCE concentration, median TCE exposures over the period of study were roughly <4–9 ppm (median, 4 ppm; arithmetic mean, 6 ppm).**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No.
>50% cohort with full latency	Yes, 18-yr mean follow-up period.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	208 cancers among 3,089 TCE-exposed subjects (7%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and calendar year.
Statistical methods	SMR and SIR, Life table analysis.
Exposure-response analysis presented in published paper	Yes, U-TCA as dichotomous variable (<6 ppm, 6+ ppm).
Documentation of results	Adequate for SIR analysis; details on SMR analysis of TCE subcohort are few.

B.3.1.2.3. **Axelson et al. (1994).**

B.3.1.2.3.1. **Author's abstract.**

There is limited evidence for mutagenicity and carcinogenicity of trichloroethylene (TRI) in experimental test systems. Whether TRI is a human carcinogen is unclear, however. This paper presents an update and extension of a previously reported cohort of workers exposed to TRI, in total 1670 persons. Among men (n = 1421), the overall standardized mortality ratio (SMR) and cancer morbidity ratio (SIR) were close to the expected, with SMR, 0.97; 95% confidence interval (CI), 0.86 to 1.10; and SIR, 0.96; 95% CI, 0.80 to 1.16, respectively. The cancer mortality was significantly lower than expected (SMR, 0.65; 95% CI, 0.47 to 0.89), whereas an increased mortality from circulatory disorders (cardiovascular, cerebrovascular) was of borderline significance (SMR, 1.17; 95% CI, 1.00 to 1.37). No significant increase of cancer of any specific site was observed, except for a doubled incidence of nonmelanocytic skin cancer without correlation with the exposure categories. In the small female subcohort (n = 249), a nonsignificant increase of cancer and circulatory deaths was observed (SMR, 1.53 and 2.02, respectively). For both genders, however, excess risks were largely confined to groups of workers with lower exposure levels or short duration of exposure or both. It is concluded that this study provides no evidence that TRI is a human carcinogen, i.e., when the exposure is as low as for this study population.

B.3.1.2.3.2. **Study description and comment.**

This Swedish study evaluated cancer risk in a small cohort of individuals (1,421 males and 249 females), who were monitored for U-TCA as part of a surveillance system by the TCE producer during 1955 to 1975. Both mortality between 1955 and 1986 and cancer morbidity between 1958 and 1987 are assessed in males only due to the small number of female subjects. Eighty-one percent of the male subjects had low exposures (<50 mg/L), corresponding to an airborne concentration of TCE of approximately 20 ppm. There was uncertainty about the beginning and end of exposure. Exposure was assumed to begin with the first urine sample and to end in 1979 (the reason for this date is unclear). Because the investigators did not have job histories, there is considerable uncertainty about the duration of exposure. No information is, additionally, presented to evaluate if a large proportion of the cohort had a full latency period for cancer development. Most subjects appear to have had short durations of exposure, but these might have been underestimated. Another concern is the sampling strategy. It was not reported how the workers were chosen for monitoring. Therefore, it is not clear what biases could be present in the data, especially the possibility of under sampling highly exposed workers.

Overall, this study had a small cohort drawn from a wide variety of industries, predominantly from industries involving degreasing and metal cleaning. Exposure to TCE was generally low (most <20 ppm). The duration of exposure was uncertain and bias related to under sampling of higher exposed workers is possible but cannot be evaluated.

Axelson O, Selden A, Andersson K, Hogstedt C. (1994). Updated and expanded Swedish cohort study on trichloroethylene and cancer risk. J Occup Environ 36:556–562.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes- "This paper present an update and extension of a previously reported cohort of workers exposure to TCE."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	1,670 subjects (1,421 males, 249 females) with records of biological monitoring of urine TCA from 1955 and 1975. Analysis restricted to 1,421 males. External referents—age-, sex-, calendar year-, and site-specific mortality or cancer incidence rates of Swedish population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence from 1958 to 1987 and all-cause mortality from 1955 to 1986.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD, 7th revision. ICD, 8th revision from 1975 onward for all lympho-hematopoietic system cancers.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Biological marker of TCE in urine used to assign TCE exposure to cohort subject. No extrapolation of U-TCA data to air-TCE concentration. **Roughly ¾ of cohort had U-TCA concentrations equivalent to <20 ppm TCE.**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No
>50% cohort with full latency	Insufficient to estimate for full cohort; however, 42% of person years in subjects with 2+ exposure years also had 10+ yrs of latency.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	229 deaths (16% of male subjects). 107 incident cancer cases.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and calendar year.
Statistical methods	SMR—age, sex, and calendar-year. SIR—analyses restricted to males—age and calendar-year.
Exposure-response analysis presented in published paper	Yes, by three categories of U-TCA concentration.
Documentation of results	Adequate.

B.3.1.3. Studies in the Taoyuan Region of Taiwan

B.3.1.3.1. Sung et al. (2008; 2007).

B.3.1.3.1.1. Sung et al.(2008) abstract.

There is limited evidence on the hypothesis that maternal occupational exposure near conception increases the risk of cancer in offspring. This study is to investigate whether women employed in an electronics factory increases childhood cancer among first live born singletons. We linked the databases of Birth Registration and Labor Insurance, and National Cancer Registry, which identified 40,647 female workers ever employed in this factory who gave 40,647 first live born singletons, and 47 of them developed cancers during 1979-2001. Mothers employed in this factory during their periconceptional periods (3 months before and after conception) were considered as exposed and compared with those not employed during the same periods. Poisson regression model was constructed to adjust for potential confounding by maternal age, education, sex, and year of birth. Based on 11 exposed cases, the rate ratio of all malignant neoplasms was increased to 2.26 [95% confidence interval (CI), 1.12-4.54] among children whose mothers worked in this factory during periconceptional periods. The RRs were associated with 6 years or less (RR=3.05; 95% CI, 1.20-7.74) and 7-9 years (RR=2.49; 95% CI, 1.26-4.94) of education compared with 10 years or more. An increased association was also found between childhood leukemia and exposed pregnancies (RR=3.83; 95% CI, 1.17-12.55). Our study suggests that maternal occupation with potential exposure to organic solvents during periconception might increase risks of childhood cancers, especially for leukemia.

B.3.1.3.1.2. Sung et al. (2007) abstract.

Background In 1994, a hazardous waste site, polluted by the dumping of solvents from a former electronics factory, was discovered in Taoyuan, Taiwan. This subsequently emerged as a serious case of contamination through chlorinated hydrocarbons with suspected occupational cancer. The objective of this study was to determine if there was any increased risk of breast cancer among female workers in a 23-year follow-up period. **Methods** A total of 63,982 female workers were retrospectively recruited from the database of the Bureau of Labor Insurance (BLI) covering the period 1973-1997; the data were then linked with data, up to 2001, from the National Cancer Registry at the Taiwanese Department of Health, from which standardized incidence ratios (SIRs) for different types of cancer were calculated as compared to the general population. **Results** There were a total of 286 cases of breast cancer, and after adjustment for calendar year and age, the SIR was close to 1. When stratified by the year 1974 (the year in which the regulations on solvent use were promulgated), the SIR of the cohort of workers who were first employed prior to 1974 increased to 1.38 (95% confidence interval, 1.11-1.70). No such trend was discernible for workers employed after 1974. When 10 years of employment was considered, there was a further increase in the SIR for breast cancer, to 1.62. Those workers with breast cancer who were first employed prior to 1974 were employed at a younger age and for a longer period. Previous qualitative studies of interviews with the

workers, corroborated by inspection records, showed a short-term high exposure to chlorinated alkanes and alkenes, particularly trichloroethylene before 1974. There were no similar findings on other types of cancer. **Conclusions** Female workers with exposure to trichloroethylene and/or mixture of solvents, first employed prior to 1974, may have an excess risk of breast cancer.

B.3.1.3.1.3. **Study description and comment.**

Sung et al. (2007) examined breast cancer incidence among females in a cohort of electronic workers with employment at one factory in Taoyuan, Taiwan between 1973 and 1992, date of factory closure, and followed to 2001. Some female subjects in Sung et al. (2007) overlap those in Chang et al. (2005; 2003) who included workers from the same factory whose employment dates were between 1978 and 1997, the closing date of the study a date of vital status ascertainment. A total of 64,000 females were identified with 63,982 in the analysis after the exclusion of 15 women with <1 full day of employment and three women with cancer diagnoses prior to the time of first employment; approximately 6,000 fewer female subjects compared to Chang et al. (2005) (70,735 females). Cancer incidence between 1979 and 2001 as identified using the National Cancer Registry which contained 80% of all cancer cases in Taiwan is examined using life table methods with exposure lag periods of 5–15 years, depending on the cancer site, and cancer rates from the larger Taiwanese population as referent.

Company employment records were lacking and the cohort was constructed using the Bureau of Labor Insurance database that contained computer records since 1978 and paper records for the period 1973–1978. Duration of employment was calculated from the beginning of coverage of labor insurance and is likely an underestimate. Labor insurance hospitalization data and a United Labor Association list of names were used to verify cohort completeness. While these sources may have been sufficient to identified current employees, their ability to identify former employees may be limited, particularly from the hospitalization data if the subject's current employer was listed.

This study assumes all employees in the factory were exposed to chlorinated organic solvent vapors and the primary exposure index was duration of employment at the plant. Most subjects had employment durations of <1 year (65%). Durations of exposure were likely underestimated as dates of commencement and termination of insurance coverage were incomplete, 7.5 and 6%, respectively. There is little to no information on chemical usage and exposure assignment to individual cohort subjects. As reported in Chang et al. (2005; 2003), records of the Department of Labor Inspection ad Bureau of International Trade, in addition, to recall of former industrial hygienists were used to identify chemicals used after 1975 in the plants. No information is available prior to this date.

Sung et al. (2008) presents an analysis of childhood cancer incidence (1979–2001) among first liveborn singleton births (1978 and 2001) of female subjects employed at the plant during a period 3 months before and after beginning of pregnancy, an estimate derived by Sung

et al. (2008) from the date of birth and estimated length of gestation plus 14 days. Sung et al. (2007) used Poisson regression methods and cancer incidence among first liveborn births of all other women in Taiwan in the same time to calculate RRs associated with leukemia risk among exposed offspring. Poisson models were adjusted for maternal age, maternal educational level, child's sex, and year of birth. A total of 8,506 first born singleton births among 63,982 female subjects were identified from the Taiwan Birth Registry database, and 11 cancers, including 6 leukemia cases and no brain/CNS cases identified from the National Cancer Registry database.

Overall, these studies do not provide substantial weight for determining whether TCE may cause increased risk of disease. The lack of TCE-assessment to individual cohort subjects; grouping cohort subjects with different exposure potential, both to different solvents and different intensities; and deficiencies in the record system used to construct the cohort introduce uncertainty.

Sung T-I, Chen P-C, Lee L J-H, Lin Y-P, Hsieh G-Y, Wang J-D. (2007). Increased standardized incidence ratio of breast cancer in female electronics workers. BMC Public Health 7:102. http://www.biomedcentral.com/content/pdf/1471-2458-7-102.pdf**.**

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract "This study is to investigate whether women employed in an electronics factory increases childhood cancer among first live born singletons." This study was not able to evaluate TCE exposures uniquely.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	63,982 females, some who were also subjects were also in cohort of Chang et al. (2005; 2003) with 70,735 females. Cohort initially established using labor insurance records (computer records after 1978 and paper records from 1973 and 1978) in the absence of company records. Cohort definition dates are not clearly identified. Cohort identified from records covering period 1973 and 1997 with vital status ascertained as of 2001. Factory closed in 1992. External referents: age-, calendar-, and sex-specific incidence rates of the Taiwanese general population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence as ascertained from National (Taiwan) Cancer Registry (80% of all cancers reported to Registry).
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-Oncology, a supplement to ICD-9.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	All employees assumed to be potentially exposed to chlorinated organic solvent vapors; study does not assign potential chemical exposures to individual subjects. No information on specific chemical exposures or intensity. Limited identification of solvents used in manufacturing process from the period after 1975 inferred from records of Department of Labor Inspection, Bureau of International Trade, and former industrial hygienists recall. No information on solvent usage was available before 1975. Exposure index defined as duration of exposure which was likely underestimated. 21% of cohort with ≥10 yrs duration of employment and 53% with <1 yr duration.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No information on loss to follow-up. Subject was assumed disease free at end of follow-up if lacking cancer diagnosis as recorded in the National Cancer Registry.
>50% cohort with full latency	No, 57% of cohort employed after November 21, 1978.

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,311 cancer cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age-, calendar-, and sex-specific incidence rates.
Statistical methods	SIR, analyses include a lag period of 5, 10, or 15 yrs since first employment (as indicated by labor insurance record).
Exposure-response analysis presented in published paper	Cancer incidence examined by duration of employment; however, employment durations were likely underestimates as dates of commencement and termination dates on of insurance coverage date were incomplete and misclassification bias is likely present.
Documentation of results	Inadequate—analyses that do not include a lag are not presented nor discussed in published paper or in supplemental documentation.

Sung T-I, Wang J-D, Chen P-C. (2008). Increased risk of cancer in the offspring of female electronics workers. Reprod Toxicol 25:115–119.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract "The study was designed to examine whether breast cancer risk in females was increased, as had been observed in Chang et al. (2005; 2003) in a cohort with earlier employment dates." This study was not able to evaluate TCE exposure.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	11 cancers among 8,506 first born singleton births between 1978 and 2001 in 63,982 female subjects of Sung et al. (2007). Cancers identified from National Cancer Registry and births identified from Taiwan Birth Registration database. External referents: cancer incidence among all other first birth singleton births among Taiwanese females over the same time period.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence as ascertained from National (Taiwan) Cancer Registry (80% of all cancers reported to Registry).
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-Oncology, a supplement to ICD-9, specific leukemia subtypes not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	All births were among subjects with employment at factory during a period 3 months before and after beginning of pregnancy. All mothers were assumed potentially exposed to chlorinated organic solvent vapors; specific solvents are not identified nor assigned to individual subjects. Limited identification of solvents used in manufacturing process from the period after 1975 inferred from records of Department of Labor Inspection, Bureau of International Trade, and former industrial hygienists recall. No information on solvent usage was available before 1975.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No information on loss to follow-up for females in Sung et al. (2007).
>50% cohort with full latency	66% of births would have been 16 yrs of age as of 2001, the date cancer incidence ascertainment ended.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	11 cancer cases among 8,506 first born singleton births.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Maternal age, maternal educational level, child's sex, and child's year of birth.
Statistical methods	Poisson regression using childhood cancer incidence among all other first live born children in Taiwan during same time period.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.1.3.2. Chang et al. (2005; 2003).

B.3.1.3.2.1. Chang et al. (2005) abstract.

A retrospective cohort morbidity study based on standardized incidence ratios (SIRs) was conducted to investigate the possible association between exposure to chlorinated organic solvents and various types of cancers in an electronic factory. The cohort of the exposure group was retrieved from the Bureau of Labor Insurance (BLI) computer database records dating for 1978 through December 31, 1997. Person-year accumulation began on the date of entry to the cohort, or January 1, 1979 (whichever came later), and ended on the closing date of the study (December 31, 1997), if alive without contracting any type of cancers, or the date of death, or the date of the cancer diagnosis. Vital status and cases of cancer of study subjects were determined from January 1, 1979 to December 31, 1997 by linking cohort data with the National Cancer Registry Database. The cancer incidence of the general population was used for comparison. After adjustment for age and calendar year, only SIR for breast cancer in the exposed female employees were significantly elevated when compared with the Taiwanese general population, based on the entire cohort without exclusion. The SIR of female breast cancer also showed a significant trend of period effect, but no significant dose-response relationship on duration of employment. Although the total cancer as well as the cancer for the trachea, bronchus[,] and lung for the entire female cohort was not significantly elevated, trend analysis by calendar-year interval suggested an upward trend. However, when duration of employment or latency was taken into consideration, no significantly elevated SIR was found for any type of cancer in either male or female exposed workers. In particular, the risk of female breast cancer was not indicated to be increased. No significant dose-response relationship on duration of employment and secular trend was found for the above-mentioned cancers. This study provides no evidence that exposure to chlorinated organic solvents at the electronics factory was associated with elevated human cancers.

B.3.1.3.2.2. Chang et al. (2003) abstract.

PURPOSE: A retrospective cohort mortality study based on standardized mortality ratios (SMRs) was conducted to investigate the possible association between exposure to chlorinated organic solvents and various types of cancer deaths. **METHODS**: Vital status and causes of death of study subjects were determined from January 1, 1985 to December 31, 1997, by linking cohort data with the National Mortality Database. Person-year accumulation began on the date of entry to the cohort, or January 1, 1985 (whichever came later), and ended on the closing date of the study (December 31, 1997), if alive; or the date of death. **RESULTS**: This retrospective cohort study examined cancer mortality among 86,868 workers at an electronics factory in the northern Taiwan. Using various durations of employment and latency and adjusting for age and calendar year, no significantly elevated SMR was found for any cancer in either male or female exposed workers when compared with the general Taiwanese population. In particular, the risk of female breast cancer was not found to be increased.

Although ovarian cancer suggested an upward trend when analyzed by length of employment, ovarian cancer risk for the entire female cohort was not elevated. **CONCLUSIONS**: It is concluded that this study provided no evidence that exposure to chlorinated organic solvents was associated with human cancer risk.

B.3.1.3.2.3. **Study description and comment.**

Both Chang et al. (2003) and Chang et al. (2005) studied a cohort of 86,868 subjects employed at an electronics factory between 1985 and 1997, and both administrative and nonadministrative (blue-collar) workers were included in the cohort. Cancer incidence between 1979 and 1997 was presented by Chang et al. (2005) and cancer mortality from 1985 to 1997 in Chang et al. (2003). The cohort was predominantly composed of females. The factory operated between 1968 and 1992, and the inclusion in the cohort of subjects after factory closure is questionable. Incidence was ascertained from the Taiwan National Cancer Registry, which contains 80% of all cancer cases in Taiwan (Parkin et al., 2002). The factory could be divided into three plants by manufacturing process: manufacture of television remote controls, manufacture of solid state and integrated circuit products, and manufacture of printed circuit boards. Furthermore, a factory waste disposal site was found to have contaminated the underground water supply of area communities with organic solvents; however, Chang et al. (2005) does not provide information on possible exposure to factory employees through ingestion. The analysis of communities adjacent to the factory is described in Lee et al. (2003).

Company employment records were lacking and the cohort was constructed using the Bureau of Labor Insurance database that contained computer records since 1978. Labor insurance hospitalization data and a United Labor Association list of names were used to verify cohort completeness. While these sources may have been sufficient to identified current employees, their ability to identify former employees may be limited, particularly from the hospitalization data if the subject's currently employer was listed.

All employees in the factory were assumed with potential exposure to chlorinated organic solvent vapors with duration of employment at the factory as the exposure surrogate. Subjects had varying exposure potentials and employment durations of <1 year (65% of cohort in Chang et al. (2005)). Durations of exposure were likely underestimated as dates of commencement and termination of insurance coverage were incomplete, 7.5 and 6%, respectively. Three plants comprised the factory and with different production processes. A wide variety of organic solvents were used in each process including dichloromethane, toluene, and methyl ethyl alcohol, used at all three plants, and perchloroethylene, propanol, and DCE, which were used at one of the three plants (Chang et al., (2005). Records of the Department of Labor Inspection and Bureau of International Trade, in addition, to recall of former industrial hygienists were used to identify chemicals used after 1975 in the plants. No information is available prior to this date. These sources documented the lack of TCE use between 1975 and 1991 and perchloroethylene was after 1981. No information was available on TCE and perchloroethylene usage during other

periods. Given the period of documented lack of TCE usage is before the cohort start date of 1978 and factory closure, there is great uncertainty of TCE exposure to cohort subjects.

Overall, both studies are not useful for determining whether TCE may cause increased risk of disease. The lack of TCE-assessment to individual cohort subjects and uncertainty of TCE usage in the factory; potential bias likely introduced through missing employment dates; and, examination of incidence using broad organ-level categories (i.e., lymphatic and hematopoietic tissue cancer together) decrease the sensitivity of this study for examining TCE and cancer. Furthermore, few cancers are expected, 1% of the cohort expected with cancer, and results in low statistical power from the cohort's young average age of 39 years.

Chang Y-M, Tai C-F, Yang S-C, Lin R, Sung F-C, Shin T-S, Liou S-H. (2005). Cancer Incidence among Workers Potentially Exposed to Chlorinated Solvents in An Electronics Factory. J Occup Health 47:171–180.

Chang Y-M, Tai C-F, Yang S-C, Chan C-J, S Shin T-S, Lin RS, Liou S-H. (2003). A cohort mortality study of workers exposed to chlorinated organic solvents in Taiwan. Ann Epidemiol 13:652–660.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The study was not designed to uniquely evaluate TCE exposure but rather chlorinated solvents exposures. From abstract: "... to investigate the possible association between chlorinated organic solvents and various types of cancer in an electronics factory." This study is quite limited to meet stated hypothesis by the inclusion of all factory employees in the cohort and lack of exposure assessment on individual study subjects to TCE, specifically, and to chlorinated solvents, generally.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	n = 86,868 in cohort. Cohort initially established using labor insurance records in the absence of company records. Cohort definition dates are not clearly identified. Cohort identified from labor insurance records covering period 1978 and 1997; yet, plant closed in 1992. All subjects followed through 1997. Paper states cohort was completely identified; however, former workers who were eligible for cohort membership may not have been identified if validation sources did not identify former employer. Duration of employment reconstructed from insurance records: ~40% of subjects had employment durations <3 months, 9% employed >5 yrs, 0.7% employed >10 yrs. External referents: Age-, calendar-, and sex-specific incidence rates of the Taiwanese general population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence as ascertained from National (Taiwan) Cancer Registry (80% of all cancers reported to Registry) (Chang et al., 2005). Mortality. ICD revision is not identified other than that used in 1981 (Chang et al., 2003).
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-Oncology, a supplement to ICD-9 (Chang et al., 2005). ICD, 9th revision was in effect in 1981, but paper does not identify to which ICD revision used to assign cause of death (Chang et al., 2003).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	All employees assumed to be potentially exposed to chlorinated organic solvent vapors. No information on specific chemical exposures or intensity. Limited identification of solvents used in manufacturing process from the period after 1975 inferred from records of Department of Labor Inspection, Bureau of International Trade, and former industrial hygienists recall. No information on solvent usage was available before 1975.

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No information on loss to follow-up. Subject was assumed disease free at end of follow-up if lacking cancer diagnosis as recorded in the National Cancer Registry.
>50% cohort with full latency	Average 16-yr follow-up (incidence) and 12 yrs (mortality).
Other	Subject's age determined by subtracting year of birth from 1997; however, insurance records did not contain DOB for 6% of subjects. Furthermore, commencement and termination dates were incomplete on insurance records, 7 and 6%, respectively.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,031 cancer cases. 1,357 total deaths (1.6% of cohort), 316 cancer deaths.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age-, calendar-, and sex-specific incidence rates (Chang et al., 2005) or age-, calendar-, and sex-specific mortality rates (Chang et al., 2003).
Statistical methods	SIR (Chang et al., 2005) and SMR (Chang et al., 2003).
Exposure-response analysis presented in published paper	Cancer incidence and mortality examined by duration of employment; however, employment durations were likely underestimates as dates of commencement and termination dates on of insurance coverage date were incomplete and calculated from date on insurance records. Misclassification bias is likely present.
Documentation of results	Adequate.

B.3.1.4. Studies of Other Cohorts

B.3.1.4.1. Clapp and Hoffman (2008).

B.3.1.4.1.1. Author's abstract.

BACKGROUND: In response to concerns expressed by workers at a public meeting, we analyzed the mortality experience of workers who were employed at the IBM plant in Endicott, New York and died between 1969 and 2001. An epidemiologic feasibility assessment indicated potential worker exposure to several known and suspected carcinogens at this plant. METHODS: We used the mortality and work history files produced under a court order and used in a previous mortality analysis. Using publicly available data for the state of New York as a standard of comparison, we conducted proportional cancer mortality (PCMR) analysis. RESULTS: The results showed significantly increased mortality due to melanoma (PCMR = 367; 95% CI: 119, 856) and lymphoma (PCMR = 220; 95% CI: 101, 419) in males and modestly increased mortality due to kidney cancer (PCMR = 165; 95% CI: 45, 421) and brain cancer (PCMR = 190; 95% CI: 52, 485) in males and breast cancer (PCMR = 126; 95% CI: 34, 321) in females. CONCLUSION: These results are similar to results from a previous IBM mortality study and support the need for a full cohort mortality analysis such as the one being planned by the National Institute for Occupational Safety and Health.

B.3.1.4.1.2. Study description and comment.

This proportional cancer mortality ratio study of deaths between 1969 and 2001 among employees at an IBM facility in Endicott, New York, who were included on the IBM Corporate Mortality File compared the observed number of site-specific cancer deaths are compared to the expected proportion, adjusted for age, using 10-year rather than 5-year grouping, and sex, of site-specific cancer deaths among New York residents during 1979 to 1998. Of the 360 deaths identified of Endicott employees, 115 deaths were due to cancer, 11 of these with unidentified site of cancer. Resultant PMRs estimates do not appear adjusted for race nor does the paper identify whether referent rates excluded deaths among New York City residents or are for New York deaths. The IBM Corporate Mortality File contained names of employees who had worker >5 years, who were actively employed or receiving retirement or disability benefits at time of death, or whose family had filed a claim with IBM for death benefits and Endicott plant employees were identified using worker employment data from the IBM Corporate Employee Resource Information System. Study investigators had previously obtained the IBM Corporate Mortality file through a court order and litigation.

The Endicott plant began operations in 1991 and manufactured a variety of products including calculating machines, typewriters, guns, printers, automated machines, and chip packaging. The most recent activities were the production of printed circuit boards. It was estimated from a National Institute of Occupational Safety and Health (NIOSH) feasibility study that a larger percentage of the plant's employee were potentially exposure to multiple chemicals,

including asbestos, benzene, cadmium, nickel compounds, vinyl chloride, tetrachloroethylene, TCE, PCBs, and o-toluidine. Chlorinated solvents were used at the plant until the 1980s. The study does not assign exposure potential to individual study subjects.

This study provides little information on cancer risk and TCE exposure given its lack of worker exposure history information and absence of exposure assignment to individual subjects. Other limitations in this study which reduces interpretation of the observations included incomplete identification of deaths, the analysis limited to only vested employees or to those receiving company death benefits, incomplete identification of all employees at the plant, the inherent limitation of the PMR method and instability of the effect measure particularly in light of bias resulting of excesses or deficits in deaths, and observed differences in demographic (race) between subjects and the referent (New York) population.

Clapp RW, Hoffman K. (2008). Cancer mortality in IBM Endicott plant workers, 1969–2001: an update on a NY production plant. Environ health 7:13.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract "…In response to concerns expressed by workers at a public meeting, we analyzed the mortality experience of workers who were employed at the IBM plant in Endicott, New York and died between 1969 and 2001."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Deaths among IBM workers identified in IBM Corporate Mortality File; workers with ≥5 yrs employment, who were actively employed or receiving retirement or disability benefits at time of death, or whose family had filed a claim with IBM for death benefits. Expected number of site-specific cancer deaths calculated from proportion of cancer deaths among New York residents. Paper does not identify if referent included all New York residents or those living upstate.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD 9.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	This study lacks exposure information. TCE and other chemicals were used at the factory and inclusion on the employee list served as a surrogate for TCE exposure of unspecified intensity and duration.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	Not able to evaluate given inability to identify complete cohort.
>50% cohort with full latency	Not able to evaluate given lack of work history records.
Other	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	360 deaths, 115 due to cancer, between 1969 and 2001.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and gender. No information was available on race and PMRs are unadjusted for race.
Statistical methods	PMR.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.1.4.2. ATSDR (2004a).

B.3.1.4.2.1. Author's abstract.

The View-Master stereoscopic slide viewer has been a popular children's toy since the 1950s. For nearly half a century, the sole U.S. manufacturing site for the View-Master product was a factory located on Hall Boulevard in Beaverton, Oregon. Throughout this period, an on-site supply well provided water for industrial purposes and for human consumption. In March 1998, chemical analysis of the View-Master factory supply well revealed the presence of the degreasing solvent trichloroethylene (TCE) at concentrations as high as 1,670 micrograms per liter (⌠g/L)—the U.S. Environmental Protection Agency maximum contaminant level is 5 ⌠g/L. Soon after the contamination was discovered, the View-Master supply well was shut down. Up to 25,000 people worked at the plant and may have been exposed to the TCE contamination. In September of 2001, the Oregon Department of Human Services (ODHS) entered into a cooperative agreement with the Agency for Toxic Substances and Disease Registry (ATSDR) to determine both the need for and the feasibility of an epidemiological study of the View-Master site. In this report, ODHS compiles the findings of the feasibility investigation of worker exposure to TCE at the View-Master factory.

On the basis of the levels of TCE found in the supply well, the past use of the well as a source of drinking water, and the potential for adverse health effects resulting from past exposure to TCE, ODHS determined that the site posed a public health hazard to people who worked at or visited the plant prior to the discovery of the contamination. Because the use of the View-Master supply well was discontinued when the contamination was discovered in March 1998, the View-Master supply well does not pose a current public health hazard. No other drinking water wells tap into the contaminated aquifer, and the long-term remediation efforts appear to be containing the contamination.

ATSDR and ODHS obtained a list of 13,700 former plant workers from the Mattel Corporation. In collaboration with ATSDR, ODHS conducted a preliminary analysis of mortality and identified excesses in the proportions of deaths due to kidney cancer and pancreatic cancer among the factory's former employees. Although this analysis was limited by the lack of information about the entire worker population and individual exposures to TCE, the preliminary findings underscore the need to fully investigate the impact of TCE exposure on the population of former View-Master workers.

The findings of this feasibility investigation are:

- TCE appears to have been the primary contaminant of the drinking water at the plant;
- Contamination was likely present for a long period of time (estimated to have been present in the groundwater since the mid-1960s);
- A large number were likely exposed to the contamination:
- The primary route of exposure (for the last 18 years the factory operated) was through contaminated drinking water;

- Levels of TCE contamination were 300 time the maximum contaminant levels; and
- A significant portion of the former workers of their next of kin can indeed be located and invited to participate in a public health evaluation of their exposures.

Therefore, ODHS recommends further investigation to include the following:

1. A fate and transport assessment to better establish when TCE reached the supply well, and to provide a historical understanding of the concentration of TCE in the well, and
2. Epidemiological studies among former workers to determine their exposure and whether they have experienced adverse health and reproductive outcomes associated with TCE exposure at the plant, to determine the mortality experience of the population, and to document the cancer incidence in this population.

B.3.1.4.2.2. **Study description and comment.**

This PMR study of deaths between 1995 and 2001 among 13,697 former employees at a View-Master toy factory in Beaverton, Oregon contains no exposure information on individual study subjects. The PMR analysis was conducted as a feasibility study for further epidemiologic investigations of these subjects by Oregon Department of Health on behalf of ATSDR, and findings have not been published in the peer-reviewed literature. A former plant owner provided a listing of former employees; however, employees were not identified using IRS records and the roster was known to be incomplete. Additionally, work history records were not available and not information was available on employment length or job title. The goal of the feasibility analysis was to evaluate ability to identify completeness of death identification using several sources.

Monitoring of a water supply well in March 1998 showed detectable concentrations of TCE, and this study assumes all subjects had exposure to TCE in drinking water. TCE had been used in large quantities for metal degreasing at the factory between 1952 and 1980; this activity mostly occurred in the paint shop located in one building. At the time metal degreasing ceased, company records suggested historical use of TCE was up to 200 gallons per month. Historical practices resulted in releases of hazardous substances at the factory site and former employees reported waste TCE from the degreased was transported to other sites on the premises, and discharged to the ground (ATSDR, 2004a). Additionally, chemical spills allegedly occurred in the paint shop and one report in 1964 of an inspection of the degreaser indicated atmospheric TCE concentrations above occupational limits. TCE was detected at concentrations between 1,220 and 1,670 µg/L in four water samples and the Oregon Department of Environmental Quality estimated the well had been contaminated for over 20 years. Other VOCs besides TCE

detected in the supply well water in March 1998 included cis-1,2-DCE at levels up to 33 µg/L and perchloroethylene at concentrations up to 56 µg/L. The 160-foot-deep supply well was on the property since original construction in 1950 and it supplied water for drinking, sanitation, fire fighting, and industrial use. Connection to municipal water supply occurred in 1956; however, although municipal water was directed to some parts of the plant, the supply well continued to serve the facility's needs, including most of the drinking and sanitary water (ATSDR, 2003b).

This study provides little information on cancer risk and TCE exposure given the absence of monitoring data beyond a single time period, absence of estimated TCE concentrations in drinking water, and exposure pathways other than ingestion. Other limitation in this study which reduces interpretation of the observations included incomplete identification of employees with the result of missing deaths likely, the inherent limitation of the PMR method and instability of the effect measure particularly in light of bias resulting of excesses or deficits in deaths, and observed differences in demographic (age and male/female ratio) between subjects and the referent (Oregon) population.

ATSDR (Agency for Toxic Substances and Disease Registry). (2004a). Feasibility investigation of worker exposure to trichloroethylene at the View-Master Factory in Beaverton, Oregon. Final Report. Submitted by Environmental and Occupational Epidemiology, Oregon Department of Human Services. December 2004.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The goal of this feasibility investigation for a cohort epidemiologic study of former employees at a plant manufacturing stereoscopic slide viewers examined the ability to identify former employees and ascertain vital status.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Name of ~13,000 former employee names were provided to ATSDR by the former plant owner. The current list of employees was known to be incomplete. The proportion of site-specific mortality among workers between 1989 and 2001 was compared to the proportion expected using all death in Oregon for a similar time period.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD 9 and ICD 10.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	This study lacks actual exposure information; work history records were not available. TCE was used at the factory and inclusion on the employee list served as a surrogate for TCE exposure of unspecified intensity and duration.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	Not able to evaluate given inability to identify complete cohort.
>50% cohort with full latency	Not able to evaluate given lack of work history records.
Other	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	616 deaths between 1989 and 2001.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and gender. No information was available on race and PMRs are unadjusted for race.
Statistical methods	PMR.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.1.4.3. Raaschou-Nielsen et al. (2003).

B.3.1.4.3.1. Author's abstract.

Trichloroethylene is an animal carcinogen with limited evidence of carcinogenicity in humans. Cancer incidence between 1968 and 1997 was evaluated in a cohort of 40,049 blue-collar workers in 347 Danish companies with documented trichloroethylene use. Standardized incidence ratios for total cancer were 1.1 (95% confidence interval (CI): 1.04, 1.12) in men and 1.2 (95% CI: 1.14, 1.33) in women. For non-Hodgkin's lymphoma and renal cell carcinoma, the overall standardized incidence ratios were 1.2 (95% CI: 1.0, 1.5) and 1.2 (95% CI: 0.9, 1.5), respectively; standardized incidence ratios increased with duration of employment, and elevated standardized incidence ratios were limited to workers first employed before 1980 for non-Hodgkin's lymphoma and before 1970 for renal cell carcinoma. The standardized incidence ratio for esophageal adenocarcinoma was 1.8 (95% CI: 1.2, 2.7); the standardized incidence ratio was higher in companies with the highest probability of trichloroethylene exposure. In a subcohort of 14,360 presumably highly exposed workers, the standardized incidence ratios for non-Hodgkin's lymphoma, renal cell carcinoma, and esophageal adenocarcinoma were 1.5 (95% CI: 1.2, 2.0), 1.4 (95% CI: 1.0, 1.8), and 1.7 (95% CI: 0.9, 2.9), respectively. The present results and those of previous studies suggest that occupational exposure to trichloroethylene at past higher levels may be associated with elevated risk for non-Hodgkin's lymphoma. Associations between trichloroethylene exposure and other cancers are less consistent.

B.3.1.4.3.2. Study description and comment.

Raaschous-Nielsen et al. (2003) examined cancer incidence among a cohort of workers drawn from 347 companies with documented TCE. Almost half of these companies were in the iron and metal industry. The cohort was identified using the Danish Supplementary Pension Fund, which includes type of industry of a company and a history of employees, for the years 1964 to 1997. Altogether, 152,726 workers were identified of whom 39,074 were white-collar and assumed not to have TCE exposure, 56,970 workers were of unknown status, and 56,578 blue-collar workers, of which 40,049 had been employed at the company for >3 months and are the basis of the analysis. The cohort was relatively young, 56% were 38 to 57 years old at end of follow-up, and 29% of subjects were older than 57 years of age. Cancer rates typically increase with increasing ages; thus, the lower age of this cohort likely limits the ability of this study to fully examine TCE and cancer, particularly cancers that may be associated with aging. Observed number of site-specific incident cancers are obtained from 4-1-1968 to the end of 1997 and compared to expected numbers of site-specific cancers based on incidence rates of the Danish population.

A separate exposure assessment was conducted using regulatory agency data from 1947 to 1989 (Raaschou-Nielsen et al., 2002). This assessment identified three factors as increasing potential for TCE exposure, duration of employment, year of first employment, and number of

employees, to increase the likelihood of cohort subjects as TCE exposed. The percentage of exposed workers was found to decrease as company size increased: 81% for <50 workers, 51% for 50–100 workers, 19% for 100–200 workers, and 10% for >200 workers. About 40% of the workers in the cohort were exposed (working in a room where TCE was used). Smaller companies had higher exposures. Median exposures to TCE were 40–60 ppm for the years before 1970, 10–20 ppm for 1970–1979, and approximately 4 ppm for 1980–1989. Additionally, an assessment of TCA concentrations in urine of Danish workers suggested a similar trend over time; mean concentrations of 58 mg/L for the period between 1960 and 1964 and 14 mg/L in sample taken between 1980 and 1985 (Raaschou-Nielsen et al., 2001).

Only a small fraction of the cohort was exposed to TCE. The highest exposures occurred before 1970 at period in which 21.2% of blue-collar workers had begun employment in a TCE-using company. The iron and metal industry doing degreasing and cleaning with TCE had the highest exposures, with a median concentration of 60 ppm and a range up to about 600 ppm. Overall, strengths of this study include its large numbers of subjects; however, the younger age of the cohort and the small fraction expected with TCE exposure limit the ability of the study to provide information on cancer risk and TCE exposure. For these reasons, positive associations observed in this study are noteworthy.

Raaschou-Nielsen O, Hansen J, McLaughlin JK, Kolstad H, Christensen JM, Tarone RE, Olsen JH. (2003). Cancer risk among workers at Danish companies using trichloroethylene: a cohort study. Am J Epidemiol 158:1182–1192.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study was designed to evaluate associations observed in Hansen et al. (2001) with TCE exposure and NHL, esophageal adenocarcinoma, cervical cancer, and liver-biliary tract cancer.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cohort of 40,049 blue-collar workers employed in 1968 or after with >3 months employment duration identified by linking 347 companies, who were considered as having a high likelihood for TCE exposure, with the Danish Supplementary Pension Fund to identify employees and with Danish Central Population Registry. External referents are age-, sex-, calendar year-, and site-specific cancer incidence rates of the Danish population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence between 4-1-1968 and 12-31-1997 as identified from records of Danish Cancer Registry.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD, 7th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Qualitative exposure assessment. A previous industrial hygiene survey of Danish companies identified several characteristics increase likelihood of TCE exposure-duration of employment, year of 1st employment, and number of employees in company (Raaschou-Nielsen et al., 2002). Exposure index defined as duration of employment. **Median exposures to TCE were 40–60 ppm for the years before 1970, 10–20 ppm for 1970–1979, and approximately 4 ppm for 1980–1989. Additionally, an assessment of TCA concentrations in urine of Danish workers suggested a similar trend over time; mean concentrations of 58 mg/L for the period between 1960 and 1964 and 14 mg/L in sample taken between 1980 and 1985 (Raaschou-Nielsen et al., 2001).**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	Danish Cancer Registry is considered to have a high degree of reporting and accurate cancer diagnoses.
>50% cohort with full latency	Yes, average follow-up was 18 yrs.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	3.244 cancers (8% of cohort had developed a cancer over the period from 1968 to 1997). Although of a large number of subjects, this cohort is of a young age, 29% of cohort was >57 yrs of age at end of follow-up.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and calendar year.
Statistical methods	SIR using life-table analysis.
Exposure-response analysis presented in published paper	Yes, duration of employment.
Documentation of results	Adequate.

B.3.1.4.4. Ritz (1999a).

B.3.1.4.4.1. Author's abstract.

Data provided by the Comprehensive Epidemiology Data Resource allowed us to study patterns of cancer mortality as experience by 3814 uranium-processing workers employed at the Fernald Feed Materials Production Center in Fernald, Ohio. Using risk-set analyses for cohorts, we estimated the effects of exposure to trichloroethylene, cutting fluids, and kerosene on cancer mortality. Our results suggest that workers who were exposed to trichloroethylene experienced an increase in mortality from cancers of the liver. Cutting-fluid exposure was found to be strongly associated with laryngeal cancers and, furthermore, with brain, hemato- and lymphopoietic system, bladder, and kidney cancer mortality. Kerosene exposure increased the rate of death from several digestive-tract cancers (esophageal, stomach, pancreatic, colon, and rectal cancers) and from prostate cancer. Effect estimates for these cancers increased with duration and level of exposure and were stronger when exposure was lagged.

B.3.1.4.4.2. Study description and comment.

This study of 3,814 white male uranium processing workers employed for at least 3 months between 1-1-1951 and 12-31-1972 at the Fernald Feed Materials Production Center in Fernald, Ohio, was of deaths as of 1-1-1990. Subjects were part of a larger cohort study of Fernald workers with potential uranium and products of uranium decay exposures that observed associations with lung cancer and lymphatic/hematopoietic cancer (Ritz, 1999b). Average length of follow-up time was 31.5 years. During this period, 1,045 deaths were observed with expected numbers of deaths based upon age- and calendar-specific U.S. white male mortality rates and age- and calendar-specific white male mortality rates from the NIOSH Computerized Occupational Referent Population System (CORPS) (Zahm, 1992). Internal analyses based upon risk-set sampling and Cox proportional hazards modeling compared workers with differing exposure intensity rankings (light and moderate) and a category for no- TCE exposure/<2 year duration TCE exposure.

Fernald produced uranium metal products for defense programs (Hornung et al., 2008). Subjects had potential exposures to uranium, mainly as insoluble compounds and varying from depleted to slight enriched, small amounts of thorium, an alpha particle emitter, respiratory irritants such as tributyl phosphate, ammonium hydroxide, sulfuric acid, and hydrogen fluoride, TCE, and cutting fluids (Ritz, 1999a, b). Exposure assessment for analysis of chemical exposures utilized a JEM to assign intensity of TCE, cutting fluids, and kerosene to individual jobs from the period 1952–1977. Industrial hygienists, a plant foreman, and an engineer during the late 1970s and early 1980s determined the likelihood of exposure to TCE, cutting fluids, and kerosene for each job title and plant area. Based on work records, the workforce appeared stable and 54% were employed ≥5 years and had held only one job title during employment. Both intensity or exposure level and duration of exposure in years were used to rank subjects into four

categories of no exposure (level 0), light exposure (level 1), moderate exposure (level 2), and heavy exposure (level 3). Seventy eight percent of the cohort was identified with some potential for TCE exposure, 2,792 subjects were identified with low TCE exposure (94%), 179 with moderate exposure (6%), and no subjects were identified with heavy TCE exposure. TCE exposure was highly correlated with other chemical exposures and with alpha radiation (Hornung et al., 2008; Ritz, 1999a, b). Fernald subjects had higher exposures to radiation compared to those of radiation-exposed Rocketdyne workers (Ritz et al., 2000; Ritz, 1999b; Ritz et al., 1999). Atmospheric monitoring information is lacking on TCE exposure conditions as is information on changes in TCE usage over time. The cohort was identified from company rosters and personnel records and it is not known whether these were sources for a subject's job title information. Analysis of TCE exposure carried out using conditional logistic regression adjusting for pay status, time since first hired, external and internal radiation dose, and previous chemical exposure. Relative risks for TCE exposure are also presented with a lag time period of 15 years.

Overall, strengths of this study are the long follow-up time and a large percentage of the cohort who had died by the end of follow-up. TCE exposure intensity is low in this cohort, 94% of TCE exposed subjects were identified with "light" exposure intensity, and all subjects had potential for radiation exposure, which was highly correlated with chemical exposures. No information is presented on the definition of "light" exposure and monitoring data are lacking. Only 179 subjects were identified with TCE exposure above "light" and the number of cancer deaths not presented. The published paper reported limited information on site-specific cancer and TCE exposure; risk estimates are reported for lymphatic and hematopoietic cancers, esophageal and stomach cancer, liver cancer, prostate cancer and brain cancer. Risk estimates for bladder and kidney cancer and TCE exposure are found in NRC (2006). Few deaths were observed with moderate TCE exposure and exposure durations of >2 years: one death due to lymphatic and hematopoietic cancer, no deaths due to kidney or bladder cancer (as noted in NRC (2006)), and two liver cancer deaths among these subjects. Low statistical power reflecting few cases with moderate TCE exposure and multicollinearity of chemical and radiation exposures greatly limits the support that this study provides in an overall weight-of-evidence analysis.

Ritz B. (1999a). Cancer mortality among workers exposed to chemicals during uranium processing. J Occup Environ Med 41:556–566.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The hypothesis in this study was to examine the influence of chemical exposures in the work environment of the Fernald Feed Materials Production Center (FFMPC) in Fernald, Ohio, on cancer mortality with a focus on the effects of TCE, cutting fluids, and a combination of kerosene exposure with carbon (graphite) and other solvents.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	3,814 white male subjects identified from company rosters and personnel records, hired between 1951 and 1972 and who were employed continuously for 3 months and monitored for radiation. 2,971 subjects identified as exposed to TCE at "light" and "moderate" exposures. Subjects were identified in a previous study of cancer mortality and radiation exposure and most subjects had radiation exposures above 10+ mSV (Ritz, 1999b). External analysis: U.S. white male mortality rates and NIOSH-Computerized Occupational Referent Population System mortality rates. Internal analysis: cohort subjects according to level and duration of chemical exposure.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality. Vital status searched through Social Security Administration records, before 1979, and National Death Index for the period 1979–1989.
Changes in diagnostic coding systems for lymphoma, particularly NHL	External analysis: ICDA, 8th revision. Internal analysis: aggregation of several subsite causes of deaths into larger categories based on ICD, 9th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Semiquantitative approach and development of JEM. JEM developed by expert assessment by plant employees to classify jobs into four levels of chemical exposures for the period 1952 to 1977. Intensity using the four-level scale and duration of exposure to TCE, cutting fluids and kerosene were assigned to individual cohort subjects using JEM. 73% of cohort identified as TCE exposed (2,971 male with TCE exposure in cohort of 3,814 subjects). Only 4% of TCE-exposed subjects with exposure identified as "moderate" and no subjects with "high" exposure. High correlation between TCE and other chemical exposure and radiation exposure.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	All workers without death certificate assumed alive at end of follow-up.
>50% cohort with full latency	Average follow-up time, 31.5 yrs.
Other	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,045 deaths (27% of cohort), 328 due to cancer. No information on number of all-cancer deaths among TCE exposed subjects, although reported numbers for specific sites reported by Ritz (1999a) or NRC (2006): >2-yr exposure duration, hemato- and lymphopoietic cancer (n = 18 with light exposure, 1 with moderate exposure), esophageal and stomach cancer (n = 15 with light exposure, 0 with moderate exposure), liver cancers (n = 3 with light exposure, 1 with moderate exposure), kidney and bladder cancers, (n = 7 with light exposure, 0 with moderate exposure) prostate cancers (n = 10 with light exposure, 1 with moderate exposure), and brain cancers (n = 6 with light exposure, 1 with moderate exposure).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	External analysis: age- and calendar-specific mortality rates for white males. Internal analysis: pay status, time since first hired, and cumulative time-dependent external- and internal-radiation doses (continuous); indirect assessment of smoking through examination of smoking distribution by chemical exposure.
Statistical methods	SMR (external analysis) and RR (internal analysis).
Exposure-response analysis presented in published paper	Yes, RR presented for exposure to TCE (level 1 and level 2, separately) by duration of exposure.
Documentation of results	Adequate.

B.3.1.4.5. **Henschler et al. (1995).**

B.3.1.4.5.1. **Author's abstract.**

> A retrospective cohort study was carried out in a cardboard factory in Germany to investigate the association between exposure to trichloroethene (TRI) and renal cell cancer. The study group consisted of 169 men who had been exposed to TRI for at least 1 year between 1956 and 1975. The average observation period was 34 years. By the closing day of the study (December 31, 1992) 50 members of the cohort had died, 16 from malignant neoplasms. In 2 out of these 16 cases, kidney cancer was the cause of death, which leads to a standard mortality ratio of 3.28 compared with the local population. Five workers had been diagnosed with kidney cancer: four with renal cell cancers and one with an urothelial cancer of the renal pelvis. The standardized incidence ratio compared with the data of the Danish cancer registry was 7.97 (95% CI: 2.59-18.59). After the end of the observation period, two additional kidney tumors (one renal cell and one urothelial cancer) were diagnosed in the study group. The control group consisted of 190 unexposed workers in the same plant. By the closing day of the study 52 members of this cohort had died, 16 from malignant neoplasms, but none from kidney cancer. No case of kidney cancer was diagnosed in the control group. The direct comparison of the incidence on renal cell cancer shows a statistically significant increased risk in the cohort of exposed workers. Hence, in all types of analysis the incidence of kidney cancer is statistically elevated among workers exposed to TRI. Our data suggest that exposure to high concentrations of TRI over prolonged periods of time may cause renal tumors in humans. A causal relationship is supported by the identity of tumors produced in rats and a valid mechanistic explanation on the molecular level.

B.3.1.4.5.2. **Study description and comment.**

This was a cohort study of workers in a cardboard factory in the area of Arnsberg, Germany. TCE was used in this area until 1975 for degreasing and solvent needs. Plant records indicated that 2,800–23,000 L/year was used. Small amounts of tetrachloroethylene and 1,1,1-trichloroethane were used occasionally, but in much smaller quantities than TCE. TCE was used in three main areas: cardboard machine, locksmith's area, and electrical workshop. Cleaning the felts and sieves and cleaning machine parts of grease were done regularly every 2 weeks, in a job that required 4–5 hours, plus whatever additional cleaning was needed. TCE was available in open barrels and rags soaked in it were used for cleaning. The machines ran hot (80–120°C) and the cardboard machine rooms were poorly ventilated and warm (about 50°C), which would strongly enhance evaporation. This would lead to very high concentrations of airborne TCE. Cherrie et al. (2001) estimated that the machine cleaning exposures to TCE were >2,000 ppm. Workers reported frequent strong odors and a sweet taste in their mouths. The odor threshold for TCE is listed as 100 ppm (ATSDR, 1997c). Workers often left the work area for short breaks "to get fresh air and to recover from drowsiness and headaches." Based on reports of anesthetic effects, it is likely that concentrations of TCE exceeded 200 ppm (Stopps and McLaughlin,

1967). Those reports, the work setting description, and the large volume of TCE used are all consistent with very high concentrations of airborne TCE. The workers in the locksmith's area and the electrical workshop also had continuous exposures to TCE associated with degreasing activities; parts were cleaned in cold dip baths and left on tables to dry. TCE was regularly used to clean floors, work clothes, and hands of grease, in addition to the intense exposures during specific cleaning exercises, which would produce a background concentration of TCE in the facility. Cherrie et al. (2001) estimated the long-term exposure to TCE was approximately 100 ppm.

The subjects in this study clearly had substantial peak exposures to TCE that exceeded 2,000 ppm and probably sustained long-term exposures >100 ppm, which are not confounded by concurrent exposures to other chlorinated organic solvents.

Henschler D, Vamvakas S, Lammert M, Dekant W, Kraus B, Thomas B, Ulm K. (1995). Increased incidence of renal cell tumors in a cohort of cardboard workers exposed to trichloroethene. Arch Toxicol 69:291–299.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract "…retrospective cohort study was carried out in a cardboard factory I Germany to investigate the association between exposure to trichloroethene and renal cell cancer."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Employee records were used to identify 183 males employed in a cardboard factory for at least 1 yr between 1956 and 1975 and with presumed TCE exposure and a control group of 190 male workers at same factory during the same period of time but in jobs not involving possible TCE exposure. Mortality rates from German population residing near factory used as referent in mortality analysis. Renal cancer incidence rates from Danish Cancer Registry used to calculate expected number of incident cancer. The age-standardized rate in the late 1990s among men in Denmark was 10.6 per 100,000 and in Germany, it was 1.2 per 100,000 (Ferlay et al., 2004). If these differences in rates apply when the study was carried out, this would imply that the expect number of deaths would have been inflated by about 14% (and the rate ratio underestimated by that amount).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality and renal cell cancer incidence.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-9 for deaths. Hospital pathology records were used to verify diagnosis of RCC.
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Walkthrough survey and interviews with long-term employees were used to identify work areas and jobs with potential TCE exposure. The workers in the locksmith's area and the electrical workshop also had continuous exposures to TCE associated with degreasing activities; parts were cleaned in cold dip baths and left on tables to dry. **Cherrie et al. (2001) estimated that the machine cleaning exposures to TCE were >2,000 ppm with average long-term exposure as 10–225 ppm. Estimated average chronic exposure to TCE was ~100 ppm to subjects using TCE in cold degreasing processes.**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	14 exposed subjects (8%) were excluded from life-table analysis and no information is presented in paper on loss-to-follow-up among control subjects.
>50% cohort with full latency	Median follow-up period was over 30 yrs for both exposed and control subjects.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	

Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	50 total deaths (30%) and 15 cancer death among exposed subjects. 52 deaths (27%) and 15 cancer deaths among control subjects.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and calendar-year.
Statistical methods	SMR and SIR. Analysis excludes person-years of subjects excluded from exposed population with the number of person-years underestimated and an underestimate of the expected numbers of deaths and incident renal carcinoma cases.
Exposure-response analysis presented in published paper	No.
Documentation of results	Adequate.

B.3.1.4.6. **Greenland et al. (1994).**

B.3.1.4.6.1. **Author's abstract.**

To address earlier reports of excess cancer mortality associated with employment at a large transformer manufacturing plant each plant operation was rated for seven exposures: Pyranol (a mixture of polychlorinated biphenyls and trichlorobenzene), trichloroethylene, benzene, mixed solvents, asbestos, synthetic resins, and machining fluids. Site-specific cancer deaths among active or retired employees were cases; controls were selected from deaths (primarily cardiovascular deaths) presumed to be unassociated with any of the study exposures. Using job records, we then computed person-years of exposure for each subject. All subjects were white males. The only unequivocal association was that of resin systems with lung cancer (odds ratio = 2.2 at 16.6 years of exposure, P = 0.0001, in a multiple logistic regression including asbestos, age, year of death, and year of hire). Certain other odds ratios appeared larger, but no other association was so robust and remained as distinct after considering the multiplicity of comparisons. Study power was very limited for most associations, and several biases may have affected our results. Nevertheless, further investigation of synthetic resin systems of the type used in the study plant appears warranted.

B.3.1.4.6.2. **Study description and comment.**

This nested case-control study at General Electric's Pittsfield, Massachusetts, plant was of deaths reported to the GE pension fund among employees vested in the pension fund. The cohort from which cases and controls were identified was defined as plant employees who worked at the facility before 1984; whose date of deaths was between 1969, the date pension records became available, and 1984; and existence of a job history record. The size of the underlying employee cohort was unknown because work history records did not exist for a large fraction of former employees, especially in the earlier years of deaths. All deaths were identified from records maintained by GE's pension office; other record sources such as the Social Security Administration and National Death Index were not utilized. Requirements for eligibility or "vestment" for a pension varied over time, but for most of the study period, required 10–15 years employment with the company. The analysis was restricted to white males because of few deaths among females and nonwhite males. A total of 1,911 deaths were identified from pension records and cases and controls, with 90 deaths excluded as possible cases and controls due to several reasons. Cases were identified as site-specific deaths and controls were selected from the remaining noncancer deaths due to circulatory disease, respiratory disease, injury, and other causes. No information was available on the number of controls selected per case. Controls were not matched to cases, were slightly older than cases, and were from earlier birth cohorts, which have a lower job history availability or greater frequency of missing exposure ratings in work history records (Salvan, 1990). Statistical analysis of the data included covariates for age and year of death.

The company's job history record served as the source for exposure rating. The JEM linked possible exposures to over 1,000 job title from 50 separate departments and 100 buildings. A categorical ranking was developed for exposure to seven exposures (Pyranol, TCE, benzene, other solvents, asbestos, resin systems, machining fluids) from 1901 to 1984 based upon on-site interviews with 18 long-term employees and knowledge of one of the study investigators who was an industrial hygienist. Two categories were used for potential TCE exposure: Level 1, duration of indirect exposure (TCE in workplace but does not work directly with TCE) and Level 2, duration of direct work with TCE, with the continuous exposure scores rescaled to the 97th percentile of controls (Salvan, 1990). Statistical analyses in Greenland et al. (1994) collapsed these two categories into a dichotomous ranking of no exposure or any exposure. In many instances, exposure levels were inaccurately estimated and some exposures were highly correlated (Salvan, 1990). Although of low correlation, TCE exposure was statistically significantly correlated with exposure to other solvents (r = 0.11), benzene (r = 0.22) and machining fluids (r = 0.28) (Salvan, 1990). Industrial hygiene monitoring data were not available before 1978 and limited production and purchase records did not extend far back in time (Salvan, 1990). TCE was used as a degreaser since the 1930s and discontinued between 1966 and 1975, depending on department. In all, fewer than 10% of jobs were identified as have TCE exposure potential, primarily through indirect exposure and not directly working with TCE. In fact, few subjects were identified with as working directly with TCE (Salvan, 1990). It is not surprising that exposure score distributions were highly skewed towards zero (Salvan, 1990). No details were provided on the protocol for processing the jobs in the work histories into job classifications.

Job history information was missing for roughly 35% of the cases and controls, particularly from subjects with earlier years of death. The highest percentage of missing information among cases was for leukemia deaths (43% of deaths) and the lowest percentage for rectal deaths (11%). Moreover, work history records did not exist for a large fraction of former employees, especially in the earlier years of death. Bias resulting from exposure misclassification is likely high due to the lack of industrial monitoring to support rankings and the inability of the JEM to account for changes in TCE exposure concentrations over time.

This study had a number of weaknesses with the likely result of dampening observed risks. Deaths were underestimated given nonpensioned employees are not included in the analysis; possible differences in exposure potential between pensioned and nonpensioned workers may introduce bias, particularly if a subject leaves work as a consequence of a precondition related to exposure, and would dampen observed associations (Robins, 1987). Misclassification bias related to exposure is highly likely given missing job history records for over one-third of deaths, mostly among deaths from the earlier study period, a period when TCE was used. Salvan (1990) noted "exposure measurements should be regarded as heavily nondifferentially misclassified relative to the true exposure does" and exposure associations with

outcomes will be underestimated. For TCE specifically, the development of exposure assignments in this study was insensitivity to define TCE exposures of the cohort-industrial hygiene data were not available for the time period of TCE use, exposure rates applied to a job-building-operation time matrix and may not reflect individual variation, and exposure ratings obtained by employee interview are subject to subjective assessment and measurement error. NRC (2006) also noted a low likelihood of exposure potential to subjects in this nested case-control study. Last, the lymphoma category includes Hodgkin lymphoma, in addition to traditional NHL forms such as reticulosarcoma and lymphosarcoma. Overall, the sensitivity of this study for evaluating cancer and TCE exposure is quite limited. The inability of this study to detect associations for two known human carcinogens, benzene and leukemia and asbestos and lung cancer, provides ancillary support for the study's low sensitivity and statistical power.

Greenland S, Salvan A, Wegman DH, Hallock MF, Smith TH. (1994). A case-control study of cancer mortality at the transformer-assembly facility. Int Arch Occup Environ Health 66:49–54.

Greenland S. (1992). A semi-Bayes approach to the analysis of correlated multiple associations with an application to an occupational cancer-mortality study. Stat Med 11:219–230.

Salvan A. (1990). Occupational exposure and cancer mortality at an electrical manufacturing plant: A case-control study. Ph.D. Dissertation, University of California, Los Angeles.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The study was carried out to reevaluate an earlier observation from a PMR study of GE employment and excess leukemia and colorectal cancer risks.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Selection of cases and controls is not adequate because only deaths among pensioned workers were included in the analysis. Also, the size of the underlying cohort was not known and potential for selection bias is likely given cases and controls are drawn from a select population. Cases were identified from deaths among white males employed before 1984, who had died between 1969 and 1984, and for whom a job history record was available. Controls selected from noncancer deaths due to cardiovascular disease, circulatory disease, respiratory disease, injury, or other causes. Controls are not matched to cases on covariates such as age, or date of hire. In total, 2,653 subjects were identified as meeting criteria for inclusion in subject, either as a case or as a control. Job history records were available for 1,714 (512 cases, 1,202 controls) of these subjects (65%).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD, 8th revision. Lymphomas, Codes 200–202 and includes Hodgkin lymphoma.
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Dichotomous ranking, not exposed/exposed, for indirect and direct exposure potential. Most subjects identified with indirect TCE exposure. The company's job history record served as the source for exposure rating. The JEM linked possible exposures to over 1,000 job title from 50 separate departments and 100 buildings. Potential TCE exposure assigned to 10% of all job titles. The seven exposures were highly correlated. NRC (2006) noted a low likelihood of TCE exposure potential to subjects in this nested case-control study.
CATEGORY D: FOLLOW-UP (COHORT)	

More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	Record study.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	220 of 732 cases and 1,202 or 1,921 possible controls had job history records; job history records are missing for 35% of all possible cases and controls. Any potential TCE exposure prevalence among cases: Laryngeal, pharyngeal cancer, 38% Liver and biliary passages, 22% Pancreas, 45% Lung, 33% Bladder, 30% Kidney, 33% Lymphoma, 27% Leukemias, 36% Brain, 31% Control exposure prevalence, 34%.
Control for potential confounders in statistical analysis	Age and year of death. Other unidentified covariates are included if risk estimate is altered by >20%.
Statistical methods	Logistic regression with: (1) dichotomous exposure (Greenland et al., 1994); (2) epoch analysis (Salvan, 1990); and (3) empirical Bayes models (Greenland, 1992).
Exposure-response analysis presented in published paper	No.
Documentation of results	Adequate.

B.3.1.4.7. Sinks et al. (1992).

B.3.1.4.7.1. Author's abstract.

> A physician's alert prompted us to investigate workers' can cancer risk at a paperboard printing manufacturer. We conducted a retrospective cohort mortality study of all 2,050 persons who had worked at the facility for more than 1 day, calculated standardized incidence ratios (SIRs) for bladder and renal cell cancer, and conducted a nested case-control study for renal cell cancer. Standardized mortality ratios (SMRs) from all causes [SMR = 1.0, 95% confidence interval (CI) = 0.9 – 1.2] and all cancers (SMR = 0.6, 95% CI = 0.3 – 1.0) were not greater than expected. One bladder cancer and one renal cell cancer were included in the mortality analysis. Six incident renal cell cancers were observed, however, compared with less than two renal cell cancers expected (SIR = 3.7, 95% CI = 1.4 – 8.1). Based on a nested case-control analysis, the risk of renal cell carcinoma was associated with overall length of employment but was not limited to any single department or work process. Although pigments containing congeners of dichlorobenzidine and o-toluidine had been used at the plant, environmental sampling could not confirm any current exposure. Several limitations and a potential selection bias limit the inferences that can be drawn.

B.3.1.4.7.2. Study description and comment.

Sinks et al. (1992) is the published report of analyses examining morbidity and mortality among employees at a James River Corporation plant in Newnan, Georgia. This plant manufactured paperboard (cardboard) packaging. The study was carried out as a NIOSH, Health Hazard Evaluation to investigate a possible cluster of urinary tract cancers and work in the plant's Finishing Department (NIOSH, 1992)\. A cohort of 2,050 white and nonwhite, male and female, subjects were identified from company personnel and death records, considered complete since 1-1-1957, and were follows for site-specific mortality and cancer morbidity to 6-30-1988. Records of an additionally 36 subjects were missing hire dates or birth dates, indicated employment duration of <1 day, and or employment outside the study period and these subjects were excluded from the analysis. This study suffers from missing information. A large percentage of personnel records did not identify a subject's race and these subjects were considered as white in statistical analyses. Additionally, vital status was unknown for approximately 10% of the cohort. Life-table analyses are based upon U.S. population age-, race-, sex-, calendar- and cause-specific mortality rates. Expected numbers of incident bladder and kidney cancers for white males were derived using white male age-specific bladder and renal cell incidence rates from the Atlanta-SEER registry for the years 1973–1977.

A nested case-control analysis of the incident renal carcinoma cases was also undertaken. This analysis is based on 6 RCC cases and 48 controls (1:8 matching) who were selected by risk set sampling of all employees born within 5 years of the case, the same sex as the case, and having attained the age at which the case was diagnosed or died if date of diagnosis was not known. A diagnosis of renal carcinoma was confirmed for four of the six cases through

pathologic examination. Both the nested case-control analysis and the life-table analyses of morbidity included a renal carcinoma case from the original cluster.

Exposures are poorly defined in this study assessing renal cancer among paper board printing workers. TCE was mentioned in material-safety data sheets for one or more materials used by the process but no information was provided regarding TCE usage and use by job title. It was not possible to assess the degree of contact with TCE or the printing inks which were identified as containing benzidine. Furthermore, the lack of monitoring data precludes evaluation of possible exposure intensity. This study is limited for assessing risks associated with exposures to TCE due to the large percentage of missing information and due to its exposure assessment approach.

Sinks T, Lushniak B, Haussler BJ, Sniezek J, Deng J-F, Roper P, Dill P, Coates R. (1992). Renal cell cancer among paperboard printing workers. Epidemiol 3:483–489.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The purpose of the cohort and nested case-control investigations was to determine whether an excess of bladder or renal cell cancer had occurred among workers in a paperboard packaging plant and, if so, to determine whether it was associated with any specific exposure or work-related process.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cohort analysis: 2,050 males and females employed at the plant between 1-1-1957 and 6-30-1988. External referents for mortality analysis were age-, sex-, race-, and calendar- cause specific mortality rates of the U.S. population. External referents for morbidity analysis were age-specific bladder and renal-cell cancer rate for white males from the Atlanta-SEER registry for the years 1973–1977. Nested case-control analysis: Cases were all subjects with renal cell cancer; eight non-RCC controls chosen from a risk set of all employees matched to case on date of birth (within 5 yrs), sex and attained age of cancer diagnosis or death, if diagnosis date unknown.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD revision in effect at the time of death; incident cases of RCC diagnoses confirmed with pathology reports for four of six cases.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Exposure in cohort analysis defined broadly at level of the plant and, in case-control study, department worked as identified on company's personnel.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	Yes, 10% of cohort with unknown vital status (n = 204). P-Y for these workers were censored at the date of last follow-up.
>50% cohort with full latency	18-yr average follow-up.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Department assignment based on company personnel records.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	141 total deaths (7% of cohort had died by end of follow-up), 16 cancer deaths.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Mortality analysis: Age, race, sex, and calendar year. Morbidity analysis limited to white males: age. Nested case-control analysis: Risk set sampling matching controls to cases on date of birth (within 5 yrs), sex, and attained age at diagnosis.
Statistical methods	SIR. Conditional logistic regression used for nested case-control analysis.
Exposure-response analysis presented in published paper	No.
Documentation of results	Adequate.

B.3.1.4.8. Blair et al. (1989).

B.3.1.4.8.1. Author's abstract.

> Work history records and fitness reports were obtained for 1767 marine inspectors of the U.S. Coast Guard between 1942 and 1970 and for a comparison group of 1914 officers who had never been marine inspectors. Potential exposure to chemicals was assessed by one of the authors (RP), who is knowledgeable about marine inspection duties. Marine inspectors and noninspectors had a deficit in overall mortality compared to that expected from the general U.S. population (standardized mortality ratios [SMRs = 79 and 63, respectively]). Deficits occurred for most major causes of death, including infectious and parasitic diseases, digestive and urinary systems, and accidents. Marine inspectors had excesses of cirrhosis of the liver (SMR = 136) and motor vehicle accidents (SMR = 107, and cancers of the lymphatic and hematopoietic system (SMR = 157, whereas noninspectors had deficits for these causes of death. Comparison of mortality rates directly adjusted to the age distribution of the inspectors and noninspectors combined also demonstrated that mortality for these causes of death was greater among inspectors than noninspectors (directly adjusted ratio ratios of 190, 145, and 198) for cirrhosis of the liver, motor vehicle accidents, and lymphatic and hematopoietic system cancer, respectively. The SMRs rose with increasing probability of exposure to chemicals for motor vehicle accidents, cirrhosis of the liver, liver cancer, and leukemia, which suggests that contact with chemicals during inspection of merchant vessels may be involved in the development of these diseases among marine inspectors.

B.3.1.4.8.2. Study description and comment.

This cohort of 1,767 U.S. Coast Guard male officers and enlisted personnel performing marine inspection duties between 1942 and 1970 and 1,914 noninspectors matched to inspectors for registry, rank, and year that rank was achieved examined mortality as of January 1, 1980. Standardized mortality ratios compared the observed number of site-specific deaths among marine inspectors (n = 483, 27%) to that expected of the total U.S. white male population and to standardized mortality ratios of noninspectors (n = 369, 19%). The cohort was predominantly white (91%), race was unknown for the remaining 8% of subjects, considered in the statistical analysis as white, with a large percentage (69%) of the marine inspectors having >20-year employment duration. The minimum latent period was 10 years, calculated from the end date of cohort identification to the date of vital status ascertainment.

This study lacks exposure information on potential exposures of marine inspectors, who enter cargo tanks, void spaces, cofferdams, and pump rooms during inspections. TCE is identified in the paper as a possible exposure along with nine other agents. One authors acquainted with Coast Guard processes estimated the level of exposure to general chemical exposures during a marine inspection. A four-point rating scales was developed: nonexposed, person generally held administrative position; low exposed, assigned to staff with duties that occasionally required vessel inspections; moderate exposed, assigned to inspection duties that

did not regularly include hull structures, and regular inspection of hull structures in geographic areas where chemicals were not major items of cargo; and high exposed, assigned to subjects who performed hull inspections at ports were vessels transported chemicals. A cumulative exposure score was calculated by summing the product of the four-point rating scale and the duration in each job.

Overall, the exposure assessment in this study is insufficient for examining TCE exposure and cancer mortality. Furthermore, the few site-specific deaths among marine inspectors greatly limits statistical power.

Blair A, Haas T, Prosser R, Morrissette M, Blackman, Grauman D, van Dusen P, Morgan F. (1989). Mortality among United States Coast Guard marine Inspectors. Arch Environ Health 44:150–156.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The purpose of the cohort study was to examine mortality patterns among Coast Guard marine inspectors. This study was not designed to examine specific exposures, including TCE.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	1,767 U.S. Coast Guard male officers and enlisted personnel performing marine inspections between 1942 and 1970 and 1,914 noninspectors matched to inspectors on registry, rank, and year that rank was achieved. External referents: age-specific mortality rates of the U.S. white male population and noninspectors.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICDA, 8th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	TCE identified in paper as 1 of 10 potential exposures; however, no exposure assessment to TCE to individual subjects. Exposure in cohort analysis defined broadly at level of the plant and, in case-control study, department worked as identified on company's personnel. A cumulative exposure surrogate developed from duration in each job and a four-point rating scale: nonexposed, person generally held administrative position; low exposed, assigned to staff with duties that occasionally required vessel inspections; moderate exposed, assigned to inspection duties that did not regularly include hull structures, and regular inspection of hull structures in geographic areas where chemicals were not major items of cargo; and high exposed, assigned to subjects who performed hull inspections at ports were vessels transported chemicals.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No
>50% cohort with full latency	Not reported; minimum latent period was 10 yrs.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	483 deaths among marine inspectors (27% of cohort), 103 cancer deaths.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Mortality analysis: Age, race, sex, and calendar year. Directly adjusted rate ratios compared cause-specific SMR of marine inspectors to that of noninspectors.
Statistical methods	SMR and RR.
Exposure-response analysis presented in published paper	Yes, using a ranked cumulative exposure surrogate.
Documentation of results	Adequate.

B.3.1.4.9. **Shannon et al. (1988).**

B.3.1.4.9.1. **Author's abstract.**

> A historical prospective study of cancer in lamp manufacturing workers in one plant was conducted. All men and women who worked for a total of at least 6 months and were employed at some time between 1960 and 1975 were included. Work histories were abstracted and subjects were divided according to whether they had worked in the coiling and wire drawing area (CWD). Cancer morbidity from 1964 to 1982 was ascertained via the provincial registry, and was compared with the site-specific incidence in Ontario, adjusting for age, sex and calendar period. Of particular interest were primary breast and gynecological cancers in women.
> The cancers of a priori concern were significantly increased in women in CWD, but not elsewhere in the plant. The excess was greatest in those with more than 5 yr exposure (in CWD) and more than 15 yr since first working in CWD, with eight cases of breast and gynecological cancers observed in this category compared with 2.67 expected. Only three cancers occurred in men in CWD. Environmental measurements had not been made in the past and little information was available on substances used in the 1940s and 1950s, the period when the women with the highest excess began employment. It is known that methylene chloride and trichloroethylene have been used, but not enough is known about the dates and patterns.

B.3.1.4.9.2. **Study description and comments.**

This cohort of 1,770 workers (1,044 females, 826 males) employed >6 months and working between 1960 and 1975 at a General Electric plant in Ontario, Canada, in the lamp manufacturing department identified cancer incidence cases from a regional cancer registry from 1964, the first date of high quality information, to 1982. Office workers were included in the study population. The study was carried out in response to previous reports of excess breast and gynecological cancer in women employed in the CWD area. SIRs compared the observed number of site-specific incident cancers to that expected of the Ontario population and supplied by the regional cancer registry. SIR estimates were calculated for all lamp department workers, and for two subgroups defined by job title, workers in the coil and wire-drawing area (CWD), and workers in all other areas. The cohort was successfully traced, with low rates of lost to follow-up (6% among CWD workers, 7% of all other workers). A total of 98 incident cancer cases were identified (58 in females, 40 in males) and over half of the incident cancers in females (n = 31) due to breast and gynecological cancers. The number of incident cancers is likely underestimated given the 4-year period between cohort identification and the first date of high quality information in the cancer registry. Additionally, cancer cases among workers who moved from the province would not be found in the registry, leading to underascertainment of cases. This is likely a small number given follow-up tracing identified 2% of workers had left the province.

This study lacks exposure information on individual study subjects. Exposures in CWD were of concern given previous reports. The study lacks exposure monitoring data and potential exposures in CWD area were identified using purchase records. A number of chemicals were identified including methylene chloride from 1959 onward and TCE, which records suggested may have been used beforehand.

Overall, the exposure assessment in this study is insufficient for examining TCE exposure and cancer mortality. The inclusion of office workers, who likely have low potential exposure, would introduce a downward bias. Furthermore, the few site-specific deaths among CWD and all other workers greatly limits statistical power.

Shannon HS, Haines T, Bernholz C, Julian JA, Verma DK, Jamieson E, Walsh C. (1988). Cancer morbidity in lamp manufacturing workers. Am J Ind Med 14:281–290.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study was undertaken in response to previous report of apparent excess breast and gynecological cancers in women employed in the coil and wire drawing area of a lamp manufacturing plant.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cohort analysis: 1,770 workers (1,044 females, 826 males) in the lamp manufacturing department of a GE plant in Ontario Province, Canada. External referents: Age-, sex-, and race-specific site-specific cancer incidence rates for Ontario Province population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Not reported.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	This study does not assign TCE exposure to individual subjects. Job title and work in the CWD area used to assign exposure potential and chemical usage in CWD identified from purchase records. Methylene chloride used from 1959 onward, with one report from 1955 indicating TCE used as degreasing solvent.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No, follow-up was incomplete for 6% of CWD workers and 7% for all other workers.
>50% cohort with full latency	Not reported
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	98 incident cancer cases

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, race, sex, and calendar year.
Statistical methods	SIR.
Exposure-response analysis presented in published paper	No.
Documentation of results	Adequate.

B.3.1.4.10. **Shindell and Ulrich (1985).**

B.3.1.4.10.1. **Author's abstract.**

> A prospective study was conducted of 2,646 employees who worked three months or more during the period January, 1957, through July, 1983, in a manufacturing plant that used trichloroethylene as a degreasing agent throughout the study period. Ninety-eight percent of the study cohort were traced; they accounted for 16,388 person-years of employment and 38,052 person-years of follow-up. Mortality experience was found to be generally more favorable than that of the comparable segment of the U.S. population over the same period of time. For the white male cohort there were fewer deaths than expected from heart disease, cancer, and trauma (standard mortality rate for all causes = 0.79, p less than .01). Reports by current and former employees of health problems requiring medical treatment showed that there were only one third as many persons with heart disease or hypertension as were reported in a comparable reference population studied over the past five years.

B.3.1.4.10.2. **Study description and comment.**

This study of 2, 546 current and former office and production employees at a manufacturing plant in northern Illinois compares broad groupings of cause-specific mortality between 1957 and 1983 to expected number of deaths based on U.S. population mortality rates for the period. The published paper lacks an assessment of TCE exposure other than noting TCE was used as a degreasing agent at the plant. No information is presented on quantity used, job titles with potential exposure, or likely exposure concentrations. Not all study subjects had the same potential for exposure and the inclusion of office workers who had a very low exposure potential decreased the study's detection sensitivity. Deaths were identified from company records or from direct or indirect contact with former employees or next-of-kin for subjects not known to the company to be deceased instead of using national-based registries such as Social Security listings or National Death Index for identifying vital status. There were few deaths in this cohort, a total of 141 among male and female subjects; vital status could not be ascertained for 52 subjects. The few numbers of cancer deaths (21 total) precluded examination of cause-specific cancer mortality. Overall, this study provides no information on TCE and cancer; it lacked exposure assessment to TCE and the few cancer deaths observed greatly limited its detection sensitivity.

Shindell S, Ulrich S. (1985). A cohort study of employees of a manufacturing plant using trichloroethylene. J Occup Med 27:577–579.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study was designed to assess mortality patterns of office and production employees at an Illinois manufacturing plant.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	2,646 males and female workers employed from 1-1-1957 to 7-31-1983. Mortality rates of U.S. population used as referent. The paper lacks information on source for identifying cohort subjects and if company records were complete.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Not identified.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	The paper does not identify TCE usage other than as a degreaser. Conditions of exposure and jobs potentially exposure are not identified in paper. This study lacks an assessment of TCE exposure.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	2%.
>50% cohort with full latency	No information provided in paper.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	This study does not use standard approaches to verify deaths and vital status. Deaths are self-reported in response to contact by employer representative. 141 deaths (6%) were reported to employer, 9 deaths lacked a death certificate.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Sex and race.
Statistical methods	SMR.
Exposure-response analysis presented in published paper	No.
Documentation of results	The paper lacks discussion of process used to contact former employees to verify vital status and methods used to identify subjects.

B.3.1.4.11. Wilcosky et al. (1984).

B.3.1.4.11.1. Author's abstract.

> Some evidence suggests that solvent exposures to rubber industry workers may be associated with excess cancer mortality, but most studies of rubber workers lack information about specific chemical exposure. In one large rubber and tire-manufacturing plant, however, historical documents allowed a classification of jobs based on potential exposures to all solvents that were authorized for use in the plant. A case-control analysis of a 6,678 member cohort compared the solvent exposure histories of a 20% age-stratified random sample of the cohort with those of cohort members who died during 1964-1973 for stomach cancer, respiratory system cancer, prostate cancer, lymphosarcoma, or lymphatic leukemia. Of these cancers, only lymphosarcoma and lymphatic leukemia showed significant positive associations with any other potential solvents exposures. Lymphatic leukemia was especially strongly related to carbon tetrachloride (OR = 1.3, p< .0001) and carbon disulfide (OR = 8.9, p = .0003). Lymphosarcoma showed similar, but weaker, association with these two solvents. Benzene, a suspected carcinogen, was not significantly associated with any of the cancers.

B.3.1.4.11.2. Study description and comment.

Exposure was assessed in this nested case-control study of four site-specific cancers among rubber workers at a plant in Akron, Ohio through use of a JEM originally used to examine benzene specifically, but had the ability to assess 24 other solvents, including TCE, or solvent classes. Exposure was inferred using information on production operations and product specifications that indicated whether solvents were authorized for use during tire production, and by process area and calendar year. A subject's work history record was linked to the JEM to assign exposure potential to TCE. Overall, a low prevalence of TCE exposure, ranging from 9 to 20% for specific cancers was observed among cases.

The JEM was developed originally to assign exposure to benzene and other aromatic solvents in a nested case-control study of lymphocytic leukemia (Arp et al., 1983). Details of exposure potential to TCE are not described by either Arp et al. (1983) or Wilcosky et al. (1984). No data were provided on the frequency of exposure-related tasks. Without more information, it is not possible to determine the quality of some of the assignments. Similarly, the lack of industrial hygiene monitoring data precluded validation of the JEM.

Cases of respiratory, stomach and prostate cancers; lymphosarcoma and reticulum cell sarcoma; and lymphatic leukemia were identified from a previous study, which had observed associations with these site-specific cancers among a cohort of rubber workers employed at a large tire manufacturing plant in Akron, Ohio. Statistical power is low in this study, particularly for evaluation of lymphatic cancer for which there were 9 cases of lymphosarcoma and 10 cases of lymphatic leukemia. Controls were chosen from a 20% age-stratified random sample of the cohort. The published paper does not identify if subjects with other diseases associated with

solvents or TCE were excluded as controls. If no exclusion criteria were adopted, a bias may have been introduced which would dampen observed associations towards the null.

The few details provided in the paper on exposure assessment and JEM developments, few details of control selection, the low prevalence of TCE exposure and the few lymphatic cancer cases greatly limit the ability of this study for assessing risks associated with exposures to TCE.

Wilcosky TC, Checkoway H, Marshall EG, Tyroler HA. (1984). Cancer mortality and solvent exposure in the rubber industry. Am Ind Hyg Assoc J 45:809–811.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study was identified as "exploratory" to examine several site-specific cancer and specific solvents, primarily benzene.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Underlying population at risk was a cohort of 6,678 male workers employed in the rubber industry in 1964. Cases are deaths due to respiratory, stomach and prostate cancers; lymphosarcoma; and lymphatic leukemia observed in the cohort analysis—30 deaths due to stomach cancer, 333 deaths from prostate cancer, 9 deaths from lymphosarcoma, and 10 deaths from lymphatic leukemia. Controls were a 20% age-stratified random sample of the cohort (exclusion criteria not identified in paper).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICDA, 8th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Procedure to assign TCE and other solvent exposures based upon JEM developed originally to assess benzene and other solvent exposures (Arp et al., 1983). The JEM was linked to a detailed work history as identified from a subject's personnel record to assign TCE exposure potential. Details of JEM for TCE not well-described in Wilcosky et al. (1984). Multiple solvent exposures likely (McMichael et al., 1976).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Record study with exposure assignment using JEM and personnel records.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	N/A

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	TCE exposure prevalence: Stomach cancer, five exposed cases (17% exposure prevalence) Prostate cancer, three exposed cases (9% exposure prevalence) Lymphosarcoma, three exposed cases (33% exposure prevalence) Lymphatic leukemia, two exposed cases (20% exposure prevalence). No information presented in paper on exposure prevalence among control subjects.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age.
Statistical methods	Not described in published paper.
Exposure-response analysis presented in published paper	No.
Documentation of results	Methods and analyses not fully described in published paper.

B.3.2. Case-Control Studies

B.3.2.1. Bladder Cancer Case-Control Studies

B.3.2.1.1. Pesch et al. (2000a)

B.3.2.1.1.1. Author's abstract.

BACKGROUND: This multicentre population-based case-control study was conducted to estimate the urothelial cancer risk for occupational exposure to aromatic amines, polycyclic aromatic hydrocarbons (PAH), and chlorinated hydrocarbons besides other suspected risk factors. METHODS: In a population-based multicentre study, 1035 incident urothelial cancer cases and 4298 controls matched for region, sex, and age were interviewed between 1991 and 1995 for their occupational history and lifestyle habits. Exposure to the agents under study was self-assessed as well as expert-rated with two job-exposure matrices and a job task-exposure matrix. Conditional logistic regression was used to calculate smoking adjusted odds ratios (OR) and to control for study centre and age. RESULTS: Urothelial cancer risk following exposure to aromatic amines was only slightly elevated. Among males, substantial exposures to PAH as well as to chlorinated solvents and their corresponding occupational settings were associated with significantly elevated risks after adjustment for smoking (PAH exposure, assessed with a job-exposure matrix: OR = 1.6, 95% CI: 1.1-2.3, exposure to chlorinated solvents, assessed with a job task-exposure matrix: OR = 1.8, 95% CI: 1.2-2.6). Metal degreasing showed an elevated urothelial cancer risk among males (OR = 2.3, 95% CI: 1.4-3.8). In females also, exposure to chlorinated solvents indicated a urothelial cancer risk. Because of small numbers the risk evaluation for females should be treated with caution. CONCLUSIONS: Occupational exposure to aromatic amines could not be shown to be as strong a risk factor for urothelial carcinomas as in the past. A possible explanation for this finding is the reduction in exposure over the last 50 years. Our results strengthen the evidence that PAH may have a carcinogenic potential for the urothelium. Furthermore, our results indicate a urothelial cancer risk for the use of chlorinated solvents.

B.3.2.1.1.2. Study description and comment.

This multicenter study of urothelial (bladder, ureter, and renal pelvis) and RCC in Germany included the five regions (West Berlin, Bremen, Leverkusen, Halle, Jena), identified two case series from participating hospitals, 1,035 urothelial cancer cases and 935 RCC cases with a single population control series matched to cases by region, sex, and age (1:2 matching ratio to urothelial cancer cases and 1:4 matching ratio to RCC cases). Findings in Pesch et al. (2000a) are from analyses of urothelial cancer analysis and Pesch et al. (2000b) from analyses of RCC. In all, 1,035 (704 males, 331 females) urothelial carcinoma cases were interviewed face-to-face using with a structured questionnaire in the hospital within 6 months of first diagnosis and 4,298 randomly selected population controls were interviewed at home. Logistic regression models were fit separately to for males and females conditional on age (nine 5-year groupings), study region, and smoking, to examine occupational chemical exposures and urothelial carcinoma.

Two general JEMs, British and German, were used to assign exposures based on subjects' job histories reported in an interview. This approach was the same as that described for the RCC analysis of Pesch et al. (2000b). Researchers also asked about job tasks associated with exposure, such as metal degreasing and cleaning, and use of specific agents (organic solvents chlorinated solvents, including specific questions about carbon tetrachloride, TCE, and tetrachloroethylene) to evaluate TCE potential using a JTEM. A category of "any use of a solvent" mixes the large number with infrequent slight contact with the few noted earlier who have high intensity and prolonged contact. Analyses examining TCE exposure using either the JEM of JTEM assigned a cumulative TCE exposure index of none to low, medium high and substantial, defined as the product of exposure probability x intensity x duration with the following cutpoints: none to low, $<30^{th}$ percentile of cumulative exposure scores; medium, 30^{th}–$<60^{th}$ percentile; high, 60^{th}–$<90^{th}$ percentile; and, substantial, $\geq 90^{th}$ percentile. The use of the German JEM identified approximately twice as many cases with any potential TCE exposure (44%) compared to the JTEM (22%) and, in both cases, few cases identified with substantial exposure, 7% by JEM and 5% by JTEM. Pesch et al. (2000a) noted "exposure indices derived from an expert rating of job tasks can have a higher agent-specificity than indices derived from job titles." For this reason, the JTEM approach with consideration of job tasks is considered a more robust exposure metric for examining TCE exposure and urothelial carcinoma due to likely reduced potential for exposure misclassification compared to TCE assignment using only job history and title.

While this case-control study includes a region in the North Rhine-Westphalia region where the Arnsberg area is also located, several other regions are included as well, where the source of the TCE and chlorinated solvent exposures are expected as much less well defined. Few cases were identified as having substantial exposure to TCE and, as a result, most subjects identified as exposed to TCE probably had minimal contact, averaging concentrations of about 10 ppm or less (NRC, 2006).

Pesch B, Haerting H, Ranft U, Klimpel A, Oelschlagel B, Schill W, and the MURC Study Group. 2000a. Occupational risk factors for urothelial carcinoma: agent-specific results from a case-control study in Germany. Int J Epidemiol 29:238–247.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes, this case-control study was conducted to estimate urothelial carcinoma risk for exposure to occupational-related agents; chlorinated solvents including TCE were identified as exposures of a priori interest.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	1,035 urothelial (bladder, ureter, renal pelvis) carcinoma cases were identified from hospitals in a five-region area in Germany between 1991 and 1995. Cases were confirmed histologically. 4,298 population controls identified from local residency registries in the five-region area were frequency matched to cases by region, sex and age comprised the control series for both the urothelial carcinoma cases and the RCC cases, published as Pesch et al. (2000a). Participation rate: cases, 84%; controls, 71%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	No information in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	A trained interviewer interviewed subjects using a structured questionnaire which covered occupational history and job title for all jobs held >1 yr, medical history, and personal information. Two general JEMs, British and German, were used to assign exposures based on subjects' job histories reported in an interview. Researchers also asked about job tasks associated with exposure, such as metal degreasing and cleaning, and use of specific agents (organic solvents chlorinated solvents, including specific questions about carbon tetrachloride, TCE, and tetrachloroethylene) and chemical-specific exposure were assigned using a JTEM. Exposure index for each subject is the sum over all jobs of duration × probability × intensity. A four category grouping was used in statistical analyses defined by exposure index distribution of controls: no-low; medium, 30th percentile; high, 60th percentile; substantial, 90th percentile.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Interviewers carried out face-to-face interview with all cases and controls. All cases were interviewed in the hospital within 6 months of initial diagnosis. All controls had home interviews.
Blinded interviewers	No, by nature of interview location.

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No, all cases and controls were alive at time of interview.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	JEM: 460 cases with TCE exposure index of medium or higher (44% exposure prevalence among cases), 71 cases with substantial exposure (7% exposure prevalence). JTEM: 157 cases with TCE exposure index of medium or higher (22% exposure prevalence among cases), and 36 males assigned substantial exposure (5% exposure prevalence). No information is presented in paper on control exposure prevalence.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, study center, and smoking.
Statistical methods	Conditional logistic regression.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes.

B.3.2.1.2. Siemiatycki et al. (1994), Siemiatycki (1991).

B.3.2.1.2.1. Author's abstract.

A population-based case-control study of the associations between various cancers and occupational exposures was carried out in Montreal, Quebec, Canada. Between 1979 and 1986, 484 persons with pathologically confirmed cases of bladder cancer and 1,879 controls with cancers at other sites were interviewed, as was a series of 533 population controls. The job histories of these subjects were evaluated by a team of chemist/hygienists for evidence of exposure to a list of 294 workplace chemicals, and information on relevant non-occupational confounders was obtained. On the basis of results of preliminary analyses and literature review, 19 occupations, 11 industries, and 23 substances were selected for in-depth multivariate analysis. Logistic regression analyses were carried out to estimate the odds ratio between each of these occupational circumstances and bladder cancer. There was weak evidence that the following substances may be risk factors for bladder cancer: natural gas combustion products, aromatic amines, cadmium compounds, photographic products, acrylic fibers, polyethylene, titanium dioxide, and chlorine. Among the substances evaluated which showed no evidence of an association were benzo(a)pyrene, leather dust, and formaldehyde. Several occupations and industries were associated with bladder cancer, including motor vehicle drivers and textile dyers.

B.3.2.1.2.2. Study description and comment.

Siemiatycki et al. (1994) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and bladder cancer conducted in Montreal, Quebec (Canada) and part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 617 newly diagnosed cases of primary bladder cancer, confirmed on the basis of histology reports, between 1979 and 1985; 484 of these participated in the study interview (78% participation). One control group (n = 1,295) consisted of patients with other forms of cancer (excluding lung and kidney cancer) recruited through the same study procedures and time period as the bladder cancer cases. A population-based control group (n = 533, 72% response), frequency matched by age strata, was drawn using electoral lists and random digit dialing. Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents. The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Siemiatycki et al.

(1994) presents observations of analyses examining job title, occupation, and some chemical-specific exposures, but not TCE. Observations on TCE are found in the original report of Siemiatycki (1991). Any exposure to TCE was 2% among cases (n = 8) but <1% for substantial TCE exposure (n = 5); "substantial" is defined as ≥10 years of exposure for the period up to 5 years before diagnosis. Logistic regression models adjusted for age, ethnicity, SES, smoking, coffee consumption, and status of respondent (Siemiatycki et al., 1994) or Mantel-Henszel χ^2 stratified on age, family income, cigarette smoking, coffee, and respondent status (Siemiatycki, 1991). Odds ratios for TCE exposure are presented in Siemiatycki (1991) with 90% CIs.

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of bladder cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The JEM, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Siemiatycki J, Dewar R, Nadon L, Gérin M. (1994). Occupational risk factors for bladder cancer: results from a case-control study in Montreal, Quebec, Canada. Am J Epidemiol 140:1061–1080.

Siemiatycki J. (1991). Risk Factors for Cancer in the Workplace. Baca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	617 bladder cancer cases were identified among male Montreal residents between 1979 and 1985 of which 484 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of all other cancer controls excluding lung and kidney cancer cases. Participation rate: cases, 78%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-O, 188 (malignant neoplasm of bladder).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of a priori interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 300 exposures, including TCE. For each exposure, a three-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1–5%; medium, >5–30%; and high, >30%).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.
CATEGORY G: SAMPLE SIZE	

Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	484 cases (78% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 2% cases; Substantial TCE exposure (exposure for ≥10 yrs and up to 5 yrs before disease onset), <1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, income, index for cigarette smoking, coffee, and respondent status (Siemiatycki, 1991). Age, ethnicity, SES, smoking, coffee consumption, and status of respondent (Siemiatycki et al., 1994).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Siemiatycki et al., 1994).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.2. CNS Cancers Case-Control Studies

B.3.2.2.1. De Roos et al. (2001).

B.3.2.2.1.1. Author's abstract.

To evaluate the effects of parental occupational chemical exposures on incidence of neuroblastoma in offspring, the authors conducted a multicenter case-control study, using detailed exposure information that allowed examination of specific chemicals. Cases were 538 children aged 19 years who were newly diagnosed with confirmed neuroblastoma in 1992–1994 and were registered at any of 139 participating hospitals in the United States and Canada. One age-matched control for each of 504 cases was selected through random digit dialing. Self-reported exposures were reviewed by an industrial hygienist, and improbable exposures were reclassified. Effect estimates were calculated using unconditional logistic regression, adjusting for child's age and maternal demographic factors. Maternal exposures to most chemicals were not associated with neuroblastoma. Paternal exposures to hydrocarbons such as diesel fuel (odds ratio (OR) = 1.5; 95% confidence interval (CI): 0.8, 2.6), lacquer thinner (OR = 3.5; 95% CI: 1.6, 7.8), and turpentine (OR = 10.4; 95% CI: 2.4, 44.8) were associated with an increased incidence of neuroblastoma, as were exposures to wood dust (OR = 1.5; 95% CI: 0.8, 2.8) and solders (OR = 2.6; 95% CI: 0.9, 7.1). The detailed exposure information available in this study has provided additional clues about the role of parental occupation as a risk factor for neuroblastoma.

B.3.2.2.1.2. Study description and comment.

De Roos et al. (2001), a large multicenter case-control study of neuroblastoma in offspring and part of the pediatric collaborative clinical trial groups, the Children's Cancer Group and the pediatric Oncology Group, examined parental and maternal chemical exposures, focusing on solvent exposures, expanding the exposure assessment approach of Olshan et al. (1999) who examined parental occupational title among cases and controls. Neuroblastoma in patients under the age of 19 years was identified at 1 of 139 participating hospitals in the United States and Canada from 1992 to 1996. One population control per case s was using a telephone random digit dialing procedure and matched to the case on date of birth (+6 months for cases 3 years old or younger and +1 year for cases older than 3 years of age). A total of 741 cases and 708 controls were identified with direct interviews by telephone obtained from 538 case mothers (73% participation), 405 case fathers, 504 control mothers (71% participation), and 304 control fathers. Mothers served as proxy respondents for paternal information for 67 cases (12%) and 141 controls (28%).

A strength of the study was its use of industrial hygienist review of self-reported occupational exposure to increase specificity, reduce the number of false-positive information from self-reported exposures, and to minimize exposure misclassification bias. A parent was coded as having been exposed to individual chemicals or chemical group (halogenated hydrocarbons, paints, metals, etc.) if the industrial hygiene review determined probable exposure

in any job. Individual chemicals in the halogenated hydrocarbons grouping included carbon tetrachloride, chloroform, Freon, methylene chloride, perchloroethylene and TCE. Typical of population case-control studies, reported TCE exposure was uncommon among cases and controls. Only 6 case and 8 control mothers were identified by industrial hygiene review of occupational information to have probable exposure to halogenated hydrocarbons. The few numbers prevented examination of specific chemical exposure. Of the 538 cases and 504 controls, paternal exposure to TCE was self-reported for 22 cases (5%) and 12 controls (4%) were identified with paternal TCE exposure with fewer fathers with probable TCE exposure confirmed from industrial hygiene expert review, 9 cases (2%) and 7 controls (2%).

Overall, this study has a low sensitivity and statistical power for evaluating parental TCE exposure and neuroblastoma in offspring due to the low exposure prevalence to TCE. Although study investigators took effort to reduce false positive reporting, exposure misclassification bias may still be possible from false negative reporting of occupational information. As discussed by study authors, job duty information reported by parents was best used to infer exposure to chemical categories but was not detailed sufficiently to infer specific exposures. The study's reported risk estimates for TCE exposure are imprecise and do not provide support for or against an association.

De Roos AJ, Olshan AF, Teschke K, Poole Ch, Savitz DA, Blatt J, Bondy ML, Pollock BH. (2001). Parental occupational exposure to chemicals and incidence of neuroblastoma in offspring. Am J Epidemiol 154:106–114.

Olshan AF, De Roos AJ, Teschke K, Neglin JP, Stram DO, Pollock BH, Castleberry RP. (1999). Neuroblastoma and parental occupation. Cancer Causes Control 10:539–549.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This multicenter population case-control study examined parental chemical-specific occupational exposures using detailed exposure information.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	538 cases of neuroblastoma in children <19 yrs of age and diagnosed between 1992 and 1994 at any of 139 U.S. or Canadian hospitals participating in the Children's Cancer Group and Pediatric Oncology Group studies. 504 population controls were selected through random digit dialing and matched (1:1) with cases on date of birth. Controls could not be located for 34 cases. 538 of 741 potentially eligible cases (73% participation rate). 504 of 681 potentially eligible controls (74% participation rate).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Self-reported exposure to any of 65 chemicals, compounds, or broad categories was obtained from structured questionnaire. An industrial hygienist confirmed each respondent's self-reported chemical exposure responses. Exposures were not assigned using JEM. TCE exposure examined in analysis as separate exposure and as one of several chemicals in the broader category of "halogenated hydrocarbons."
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Telephone interview with mother and father of each case and control.
Blinded interviewers	Not identified in paper.
CATEGORY F: PROXY RESPONDENTS	

>10% proxy respondents	No proxy information on maternal exposure; direct interview with mother was obtained for 537 cases and 503 controls. Analysis of paternal chemical exposures did not include information on paternal exposure from proxy interviews.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Self-reported TCE exposure: 22 cases (5% exposure prevalence) and 12 controls (4% exposure prevalence). IH-reviewed TCE exposure: 9 cases (2% exposure prevalence) and 7 controls (2% exposure prevalence).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Analyses of maternal and paternal occupational exposure each adjusted for child's age, maternal race, maternal age, and maternal education.
Statistical methods	Separate analyses are conducted for maternal and paternal exposure using logistic regression methods.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes, results are well documented.

B.3.2.2.2. **Heineman et al. (1994).**

B.3.2.2.2.1. **Author's abstract.**

Chlorinated aliphatic hydrocarbons (CAHs) were evaluated as potential risk factors for astrocytic brain tumors. Job-exposure matrices for six individual CAHs and for the general class of organic solvents were applied to data from a case-control study of brain cancer among white men. The matrices indicated whether the CAHs were likely to have been used in each industry and occupation by decade (1920–1980), and provided estimates of probably and intensity of exposure for "exposed" industries and occupations. Cumulative exposure indices were calculated for each subject.

Associations of astrocytic brain cancer were observed with likely exposure to carbon tetrachloride, methylene chloride, tetrachloroethylene, and trichloroethylene, but were strongest for methylene chloride. Exposure to chloroform or methyl chloroform showed little indication of an association with brain cancer. Risk of astrocytic brain tumors increase with probability and average intensity of exposure, and with duration of employment in jobs considered exposed to methylene chloride, but not with a cumulative exposure score. These trends could not be explained by exposures to the other solvents.

B.3.2.2.2.2. **Study description and comment.**

Heineman et al. (1994) studied the association between astrocytic brain cancer (ICD-9 codes 191, 192, 225, and 239.7) and occupational exposure to chlorinated aliphatic hydrocarbons. Cases were identified using death certificates from southern Louisiana, northern New Jersey, and the Philadelphia area. This analysis was limited to white males who died between 1978 and 1981. Controls were randomly selected from the death certificates of white males who died of causes other than brain tumors, cerebrovascular disease, epilepsy, suicide, and homicide. The controls were frequency matched to cases by age, year of death, and study area.

Next-of-kin were successfully located for interview for 654 cases and 612 controls, which represents 88 and 83% of the identified cases and controls, respectively. Interviews were completed for 483 cases (74%) and 386 controls (63%). There were 300 cases of astrocytic brain cancer (including astrocytoma, glioblastoma, mixed glioma with astrocytic cells). The ascertainment of type of cancer was based on review of hospital records, which included pathology reports for 229 cases and computerized tomography reports for 71 cases. After excluding 66 controls with a possible association between occupational exposure to chlorinated aliphatic hydrocarbons and cause of death (some types of cancer, cirrhosis of the liver), the final analytic sample consisted of 300 cases and 320 controls.

In the next-of-kin interviews, the work history included information about each job held since the case (or control) was 15 years old (job title, description of tasks, name and location of company, kinds of products, employment dates, and hours worked per week). Occupation and industry were coded based on four-digit Standard Industrial Classification and Standard Occupational Classification (Department of Commerce) codes. The investigators developed

matrices linked to jobs with likely exposure to six chlorinated aliphatic hydrocarbons (carbon tetrachloride, chloroform, methyl chloroform, methylene dichloride, tetrachloroethylene, and TCE), and to organic solvents (Gómez et al., 1994). This assessment was done blinded to case-control status. Exposure was defined as the probability of exposure to a substance (the highest probability score for that substance among all jobs), duration of employment in the exposed occupation and industry, specific exposure intensity categories, average intensity score (the three-level semiquantitative exposure concentration assigned to each job multiplied by duration of employment in the job, summed across all jobs), and cumulative exposure score (weighted sum of years in all exposed jobs with weights based on the square of exposure intensity [1, 2, 3] assigned to each job). Secular trends in the use of specific chemicals were considered in the assignment of exposure potential. Exposures were lagged 10 or 20 years to account for latency. Thus, this exposure assessment procedure was quite detailed.

The strengths of this case-control study include a large sample size, detailed work histories including information not just about usual or most recent industry and occupation, but also about tasks and products for all jobs held since age 15, and comprehensive exposure assessment and analysis along several different dimensions of exposure. The major limitation was the lack of direct exposure information and potential inaccuracy of the description of work histories that was obtained from next-of-kin interviews. The authors acknowledge this limitation in the report, and in response to a letter by Norman (1968) criticizing the methodology and interpretation of the study with respect to the observed association with methylene chloride, Heineman et al. (1994) noted that while the lack of direct exposure information must be interpreted cautiously, it does not invalidate the results. Differential recall bias between cases and controls was unlikely because work histories came from next-of-kin for both groups and, the industrial hygienists made their judgments blinded to disease status. Nondifferential misclassification is possible due to underreporting of job information by next of kin and would, on average, attenuate true associations.

Heineman EF, Cocco P, Gomez MR, Dosemeci M, Stewart PA, Hayes RB, Zahm SH, Thomas TL, Blair A. (1994). Occupational exposure to chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. Am J Ind Med 26:155–169.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes, study further examines six specific solvents including TCE in a previous study of brain cancer which reported association with electrical equipment production and repair.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Brain cancer deaths among white males in southern Louisiana, northern New Jersey, and Philadelphia, Pennsylvania, were identified using death certificates (n = 741). Controls were randomly selected (source not identified in paper) among other cause-specific deaths among white male residents of these areas and matched to cases by age, year of death and study area (n = 741). Participation rate, 483 of 741 (65% of cases with brain cancer); 386 of 741 controls (52%). Of the 483, 300 deaths were due to astrocytic brain cancer.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD, 9th revision, Codes 191, 192, 225, 239.7.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	The job-exposure-matrix of Gomez et al. (1994) was used to assign potential exposure to six solvents including TCE.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Interview with next-of-kin but paper does not identify whether telephone or face-to-face.
Blinded interviewers	Interviewer was blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Proxy information was obtained from 100% of cases and controls.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	TCE exposure prevalence: 128 cases (43%) and 125 controls (39%).

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Stratified analysis controlled for age, year of death and study area; employment in electronics-related occupations was included in addition in logistic regression analyses.
Statistical methods	Stratified analysis using 2 × 2 tables and logistic regression.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes.

B.3.2.3. Colon and Rectal Cancers Case-Control Studies

B.3.2.3.1. Goldberg et al. (2001), Siemiatycki (1991).

B.3.2.3.1.1. Author's abstract.

BACKGROUND: We conducted a population-based case-control study in Montreal, Canada, to explore associations between hundreds of occupational circumstances and several cancer sites, including colon. METHODS: We interviewed 497 male patients with a pathologically confirmed diagnosis of colon cancer, 1514 controls with cancers at other sites, and 533 population-based controls. Detailed job histories and relevant potential confounding variables were obtained, and the job histories were translated by a team of chemists and industrial hygienists into a history of occupational exposures. RESULTS: We found that there was reasonable evidence of associations for men employed in nine industry groups (adjusted odds ranging from 1.1 to 1.6 per a 10-year increase in duration of employment), and in 12 job groups (OR varying from 1.1 to 1.7). In addition, we found evidence of increased risks by increasing level of exposures to 21 occupational agents, including polystyrene (OR for "substantial" exposure (OR(subst) = 10.7), polyurethanes (OR(subst) = 8.4), coke dust (OR(subst) = 5.6), mineral oils (OR(subst) = 3.3), polyacrylates (OR(subst) = 2.8), cellulose nitrate (OR(subst) = 2.6), alkyds (OR(subst) = 2.5), inorganic insulation dust (OR(subst) = 2.3), plastic dusts (OR(subst) = 2.3), asbestos (OR(subst) = 2.1), mineral wool fibers (OR(subst) = 2.1), glass fibers (OR(subst) = 2.0), iron oxides (OR(subst) = 1.9), aliphatic ketones (OR(subst) = 1.9), benzene (OR(subst) = 1.9), xylene (OR(subst) = 1.9), inorganic acid solutions (OR(subst) = 1.8), waxes, polishes (OR(subst) = 1.8), mononuclear aromatic hydrocarbons (OR(subst) = 1.6), toluene (OR(subst) = 1.6), and diesel engine emissions (OR(subst) = 1.5). Not all of these effects are independent because some exposures occurred contemporaneously with others or because they referred to a group of substances. CONCLUSIONS: We have uncovered a number of occupational associations with colon cancer. For most of these agents, there are no published data to support or refute our observations. As there are few accepted risk factors for colon cancer, we suggest that new occupational and toxicologic studies be undertaken focusing on the more prevalent substances reported herein.

B.3.2.3.1.2. Study description and comment.

Goldberg et al. (2001), and Siemiatycki (1991) reported data from a case-control study of occupational exposures and colon cancer conducted in Montreal, Quebec (Canada) and part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 607 newly diagnosed cases of primary colon cancer (ICD9, 153), confirmed on the basis of histology reports, between 1979 and 1985; 497 of these participated in the study interview (81.9% participation). One control group (n = 1,514) consisted of patients with other forms of cancer (excluding cancers of the lung, peritoneum, esophagus, stomach, small intestine, rectum, liver and intrahepatic bile ducts, gallbladder and extrahepatic bile ducts and pancreas) recruited through the same study procedures and time period as the colon cancer cases. A population-based control group (n = 533, 72% response), frequency matched by age strata, was

drawn using electoral lists and random digit dialing. Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents. The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Goldberg et al. (2001) presents observations of analyses examining industries, occupation, and some chemical-specific exposures, but not TCE. Observations on TCE are found in the original report of Siemiatycki (1991). Any exposure to TCE was 2% among cases (n = 12) and 1% for substantial TCE exposure (n = 7); "substantial" is defined as ≥10 years of exposure for the period up to 5 years before diagnosis.

Logistic regression models adjusted for a number of nonoccupational variables including age, ethnicity, birthplace, education, income, parent's occupation, smoking, alcohol consumption, tea consumption, respondent status, heating source and cooking source in childhood home, consumption of nonpublic water supply, and BMI (Goldberg et al., 2001) or Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, coffee, ethnic origin, and beer consumption (Siemiatycki, 1991). ORs for TCE exposure are presented in Siemiatycki (1991) with 90% CIs.

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of colon cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The JEM, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Goldberg MS, Parent M-E, Siemiatycki J, Desy M, Nadon L, Richardson L, Lakhani R, Lateille B, Valois M-F. (2001). A case-control study of the relationship between the risk of colon cancer in men and exposure to occupational agents. Am J Ind Med 39:5310–546.

Siemiatycki J. (1991). Risk Factors for Cancer in the Workplace. Baca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	607 colon cancer cases were identified among male Montreal residents between 1979 and 1985 of which 497 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of all other cancer controls excluding lung peritoneum and other digestive cancers. Participation rate: cases, 81.9%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-9, 153 (malignant neoplasm of colon).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of a priori interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a three-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1–5%; medium, >5–30%; and high, >30%).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	

>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	497 cases (81.9% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 2% cases; substantial TCE exposure (exposure for ≥10 yrs and up to 5 yrs before disease onset), 1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, ethnicity, birthplace, education, income, parent's occupation, smoking, alcohol consumption, tea consumption, respondent status, heating source and cooking source in childhood home, consumption of nonpublic water supply, and BMI (Goldberg et al., 2001). Age, family income, cigarette smoking, coffee, ethnic origin, and beer consumption (Siemiatycki, 1991).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Goldberg et al., 2001).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.3.2. Dumas et al.(2000), Siemiatycki (1991).

B.3.2.3.2.1. Author's abstract.

> In 1979, a hypothesis-generating, population-based case-control study was undertaken in Montreal, Canada, to explore the association between occupational exposure to 294 substances, 130 occupations and industries, and various cancers. Interviews were carried out with 3,630 histologically confirmed cancer cases, of whom 257 had rectal cancer, and with 533 population controls, to obtain detailed job history and data on potential confounders. The job history of each subject was evaluated by a team of chemists and hygienists and translated into occupational exposures. Logistic regression analyses adjusted for age, education, cigarette smoking, beer consumption, body mass index, and respondent status were performed using population controls and cancer controls, e.g., 1,295 subjects with cancers at sites other than the rectum, lung, colon, rectosigmoid junction, small intestine, and peritoneum. We present here the results based on cancer controls. The following substances showed some association with rectal cancer: rubber dust, rubber pyrolysis products, cotton dust, wool fibers, rayon fibers, a group of solvents (carbon tetrachloride, methylene chloride, trichloroethylene, acetone, aliphatic ketones, aliphatic esters, toluene, styrene), polychloroprene, glass fibers, formaldehyde, extenders, and ionizing radiation. The independent effect of many of these substances could not be disentangled as many were highly correlated with each other.

B.3.2.3.2.2. Study description and comment.

Dumas et al. (2000) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and rectal cancer conducted in Montreal, Quebec (Canada) and part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 304 newly diagnosed cases of primary rectal cancers, confirmed on the basis of histology reports, between 1979 and 1985; 257 of these participated in the study interview (84.5% response). One control group (n = 1,295) consisted of patients with other forms of cancer (excluding lung cancer and other intestinal cancers) recruited through the same study procedures and time period as the rectal cancer cases. A population-based control group (n = 533), frequency-matched by age strata, was drawn using electoral lists and random digit dialing (72% response). The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews. The percentage of proxy respondents was 15.2% for cases, 19.7% for other cancer controls, and 12.6% for the population controls.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Any exposure to

TCE was 5% among cases (n = 12) and 1% for substantial TCE exposure (n = 3); "substantial" is defined as ≥10 years of exposure for the period up to 5 years before diagnosis.

Logistic regression models adjusted for age, education, respondent status, cigarette smoking, beer consumption and BMI (Dumas et al., 2000) or Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, coffee, ethnic origin, and beer consumption (Siemiatycki, 1991). Dumas et al. (2000) presents observations of analyses examining industries, occupation, and some chemical-specific exposures, including TCE. Observations on TCE from Mantel-Haenszel analyses are found in the original report of Siemiatycki (1991). ORs for TCE exposure are presented in Siemiatycki (1991) with 90% CIs and 95% CIs in Dumas et al. (2000).

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of rectal cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The JEM, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Dumas S, Parent M-E, Siemiatycki J, Brisson J. (2000). Rectal cancer and occupational risk factors: a hypothesis-generating, exposure-based case-control study. Int J Cancer 87:874–879.

Siemitycki J. (1991). Risk Factors for Cancer in the Workplace. Boca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	304 rectal cancer cases were identified among male Montreal residents between 1979 and 1985 of which 294 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of all other cancer controls excluding lung and other intestinal cancer cases. Participation rate: cases, 84.5%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-O, 154 (malignant neoplasm of rectum, rectosigmoid junction and anus).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of a priori interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a three-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1–5%; medium, >5–30%; and high, >30%).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face to face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.
CATEGORY G: SAMPLE SIZE	

Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	294 cases (78% response), 533 population controls (72% response). Exposure prevalence: Any TCE exposure, 5% cases; substantial TCE exposure (exposure for ≥10 yrs and up to 5 yrs before disease onset), 1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, education, respondent status, cigarette smoking, beer consumption and BMI (Dumas et al., 2000). Age, family income, cigarette smoking, coffee, ethnic origin, and beer consumption (Siemiatycki, 1991).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Dumas et al., 2000).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.3.3. **Fredriksson et al. (1989).**

B.3.2.3.3.1. **Author's abstract.**

> A case-control study on colon cancer was conducted encompassing 329 cases and 658 controls. Occupations and various exposures were assessed by questionnaires. A decreased risk was found in persons with physically active occupations. This effect was most pronounced in colon descendens and sigmoideum with an odds ratio (OR) of 0.49 whereas no reduced risk was found for right-sided colon cancer. Regarding specific jobs, reduced ORs were found for agricultural, forestry, and saw mill workers and increased OR for railway employees. High-grade exposure to asbestos or to organic solvents gave a two-fold increased risk. Regarding exposure to trichloroethylene in general, a slightly increased risk was found whereas such exposure among dry cleaners gave a 7-fold increase of the risk.

B.3.2.3.3.2. **Study description and comment.**

Fredriksson et al. (1989) reported data from a population case-control study of occupational and nonoccupational exposures and rectal cancer conducted in Ureå, Sweden. The investigators identified 329 diagnosed cases of rectal cancers (ICD 8, 153), between 1980 and 1983, confirmed on the basis of histology reports and alive at the time of data collect between 1984 and 1986; 302 (165 males and 165 females) of these participated in the study interview (92% response). A population-based control group (n = 658), matched by a 1:2 ratio to cases on age sex and county residence, was drawn using the Swedish National Population Register list; 623 (306 males and 317 females) returned mailed questionnaires and participated in the study (95% response).

The occupational assessment consisted of a detailed description of each job held during the working lifetime, including details on specific occupations and exposures. Occupation information was provided directly from each case and control given the study's eligibility requirement of being alive at the time of data collection. A team of experts independently classified three exposures of interest (asbestos, organic solvents, and impregnating agents) into two categories, low grade exposure and high grade exposure and other chemical-specific exposures, including TCE, as either "exposed" or "unexposed." Fredriksson et al. (1989) do not define these categories nor do they provide information on exposure potential, frequency of exposure, or concentration of exposure. No information is provided whether experts were blinded as to disease status.

Statistical analysis examining occupation and agent-specific exposures was carried out using Mantel-Haenszel χ^2 stratified on age, sex, and an index of physical activity. Odds ratios associated with specific chemical exposure are presented with their 95% CIs.

The strengths of this study were its specific information about job duties for all jobs held and a definitive diagnosis of rectal cancer. However, the study's assignment of exposure

potential from information using mailed questionnaires is considered inferior to information obtained directly from trained interviewers and expert assessment because of greater uncertainty and misclassification (Fritschi et al., 1996). The degree of potential exposure misclassification bias in this population case-control study of colon cancer is not known. Furthermore, exposure prevalence to TCE appears low, as judged by the wide CI around the OR. This study is considered as having decreased sensitivity for examining colon cancer and TCE given the apparent lower exposure prevalence and likely exposure misclassification bias associated with mailed questionnaire information.

Fredriksson M, Bengtsson N-O, Hardell L, Axelson O. (1989). Colon cancer, physical activity, and occupational exposure. A case-control study. Cancer 63:1838–1842.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Abstract—to evaluate occupational and nonoccupational exposures as risk factors for colon cancer.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	302 (165 males and 165 females) cases participated in study out of 329 eligible cases reported to the Swedish Cancer Registry between 1980 and 1983, among resident of Umeå, Sweden, alive at time of data collection 1984 and 1986, and with histological-confirmed diagnosis of colon cancer. 623 (306 males and 317 females) identified from Swedish Population Registry and matched for age, sex, and county of residence. Participation rate: cases, 92%; population controls, 95%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-8, 153 (malignant neoplasm of large intestine, except rectum).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Self-reported information on occupational exposure as obtained from a mailed questionnaire to study participants. Questionnaire sought information on complete working history, other exposures, and dietary habits. Procedure for assigning chemical exposures from job title information not described in paper.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Mailed questionnaire.
Blinded interviewers	No information in published paper.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No proxy respondents, all cases and controls alive at time of data collection.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	302 cases (92% response), 623 population controls (95% response). Exposure prevalence not calculated, published paper lacks number of TCE exposed cases and controls.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Yes, age, sex, and index of physical activity.
Statistical methods	Mantel-Haenszel.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.4. Esophageal Cancer Case-Control Studies

B.3.2.4.1. Parent et al. (2000a), Siemiatycki (1991).

B.3.2.4.1.1. Parent et al. (2000b) abstract.

OBJECTIVES: To describe the relation between oesophageal cancer and many occupational circumstances with data from a population based case-control study. METHODS: Cases were 99 histologically confirmed incident cases of cancer of the oesophagus, 63 of which were squamous cell carcinomas. Various control groups were available; for the present analysis a group was used that comprised 533 population controls and 533 patients with other types of cancer. Detailed job histories were elicited from all subjects and were translated by a team of chemists and hygienists for evidence of exposure to 294 occupational agents. Based on preliminary results and a review of literature, a set of 35 occupational agents and 19 occupations and industry titles were selected for this analysis. Logistic regression analyses were adjusted for age, birthplace, education, respondent (self or proxy), smoking, alcohol, and beta-carotene intake. RESULTS: Sulphuric acid and carbon black showed the strongest evidence of an association with oesophageal cancer, particularly squamous cell carcinoma. Other substances showed excess risks, but the evidence was more equivocal-namely chrysotile asbestos, alumina, mineral spirits, toluene, synthetic adhesives, other paints and varnishes, iron compounds, and mild steel dust. There was considerable overlap in occupational exposure patterns and results for some of these substances may be mutually confounded. None of the occupations or industry titles showed a clear excess risk; the strongest hints were for warehouse workers, food services workers, and workers from the miscellaneous food industry. CONCLUSIONS: The data provide some support for an association between oesophageal cancer and a handful of occupational exposures, particularly sulphuric acid and carbon black. Many of the associations found have never been examined before and warrant further investigation.

B.3.2.4.1.2. Study description and comment.

Parent et al. (2000b) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and esophageal cancer conducted in Montreal, Quebec (Canada) and part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 129 newly diagnosed cases of primary esophageal cancers, confirmed on the basis of histology reports, between 1979 and 1985; 99 of these participated in the study interview (76.7% response). One control group consisted of patients with other forms of cancer recruited through the same study procedures and time period as the esophageal cancer cases. A population-based control group (n = 533), frequency-matched by age strata, was drawn using electoral lists and random digit dialing (72% response). Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents.

The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and

any additional information that could furnish clues about exposure from the interviews. A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Any exposure to TCE was 1% among cases (n = 1) and 1% for substantial TCE exposure (n = 1); "substantial" is defined as ≥10 years of exposure for the period up to 5 years before diagnosis.

Logistic regression models adjusted for age, education, respondent status, birthplace, cigarette smoking, beer consumption spirits consumption and beta-carotene intake (Parent et al., 2000a) or Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, coffee, and an index for alcohol consumption (Siemiatycki, 1991). Parent et al. (2000b) presents observations of analyses examining industries, occupation, and some chemical-specific exposures, including solvents, but not TCE. Observations on TCE from Mantel-Haenszel analyses are found in the original report of Siemiatycki (1991). Odds ratios for TCE exposure are presented in Siemiatycki (1991) with 90% CIs and 95% CIs in Parent et al. (2000b).

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of esophageal cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The JEM, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Parent M-E, Siemiatycki J, Fritschi L. (2000b). Workplace exposures and oesophageal cancer. Occup Environ Med 57:325–334.

Siemitycki J. (1991). Risk Factors for Cancer in the Workplace. Boca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	129 esophageal cancer cases were identified among male Montreal residents between 1979 and 1985 of which 99 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of all other cancer controls. Participation rate: cases, 76.7%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-O, 150 (malignant neoplasm of esophagus).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of a priori interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a three-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1–5%; medium, >5–30%; and high, >30%).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	99 cases (76.7% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 1% cases; substantial TCE exposure (exposure for ≥10 yrs and up to 5 yrs before disease onset), 1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, education, respondent status, birthplace, cigarette smoking, beer consumption spirits consumption, and beta-carotene intake (Parent et al., 2000b). Age, family income, cigarette smoking, and index for alcohol consumption (Siemiatycki, 1991).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Parent et al., 2000b).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.5. Liver Cancer Case-Control Studies

B.3.2.5.1. Lee et al. (2003).

B.3.2.5.1.1. Author's abstract.

Aims: To investigate the association between cancer mortality risk and exposure to chlorinated hydrocarbons in groundwater of a downstream community near a contaminated site. Methods: Death certificates inclusive for the years 1966–97 were collected from two villages in the vicinity of an electronics factory operated between 1970 and 1992. These two villages were classified into the downstream (exposed) village and the upstream (unexposed) according to groundwater flow direction. Exposure classification was validated by the contaminant levels in 49 residential wells measured with gas chromatography/mass spectrometry. Mortality odds ratios (MORs) for cancer were calculated with cardiovascular-cerebrovascular diseases as the reference diseases. Multiple logistic regressions were performed to estimate the effects of exposure and period after adjustment for age. Results: Increased MORs were observed among males for all cancer, and liver cancer for the periods after 10 years of latency, namely, 1980–89, and 1990–97. Adjusted MOR for male liver cancer was 2.57 (95% confidence interval 1.21 to 5.46) with a significant linear trend for the period effect. Conclusion: The results suggest a link between exposure to chlorinated hydrocarbons and male liver cancer risk. However, the conclusion is limited by lack of individual information on groundwater exposure and potential confounding factors.

B.3.2.5.1.2. Study description and comment.

Exposure potential to chlorinated hydrocarbons was assigned in this community case-control study of liver cancer in males >30 years of age using residency as coded on death certificates obtained from local household registration offices. No information is available to assess the completeness of death reporting to the local registration office. Of the 1,333 deaths between 1966 and 1997 in two villages surrounding a hazardous waste site, an electronics factory operating between 1970 and 1992 in Taoyuan, Taiwan,[3] 266 cancer deaths were identified; 53 liver cancer deaths, 39 stomach cancer deaths, 26 colorectal deaths, and 41 lung cancer deaths. Controls were identified from 344 deaths due to cardiovascular and cerebrovascular diseases, without arrhythmia; 286 were included in the statistical analysis. Residents from a village north and northeast of the plant were considered exposed and residents living south considered unexposed to chlorinated hydrocarbons. Statistical analyses are limited to Mantel-Haenszel χ^2 approaches stratified by sex and age and, for male cases and controls, logistic regression with age as a covariate. Socioeconomic characteristics were similar between residents of the two villages (Wang, 2004). The study does not include control for potential confounding from hepatitis virus; high rates of hepatitis B and C are endemic to Taiwan and northern Taiwan, the location of this study, has a high prevalence of hepatitis C virus infection

[3]The factory's workers were subjects in the cohort studies of Chang et al. (2003, 2005) and Sung et al. (2007, 2008).

(Lee et al., 2003). Confounding would be introduced if the prevalence of hepatitis C differed between the two villages.

Exposure assessment is quite limited and misclassification bias likely high using residence address as recorded on the death certificate as a surrogate for consumption of contaminated drinking water. The paper not only lacks information on intensity and duration of hydrocarbon exposures to individual cases and controls, but no information is available on an estimate of the amount of TCE ingested. Information on residence length, population mobility, and chemical usage at the plant are lacking. Similarly, well water monitoring is sparse, based on seven chlorinated hydrocarbons monitored over a 7-month period between 1999 and 2000 in 69 groundwater samples from 44 wells to the north and northeast, or downstream from the factory, and in 5 groundwater samples from 2 wells to the south or upstream from the factory. Monitoring from other time periods is lacking with no information available to judge if current monitoring are representative of past concentrations. Median concentrations (µg/L or ppb) and ranges (µg/L or ppb) for these seven chemicals are identified in the table below. Highest concentration of contaminants was from wells closest to the factory boundary with concentrations detected at or close to maximum contaminant levels in wells located 0.5 mile (1,000 meters) away. A municipal system supplied water to upstream village residents (start date not identified); however, wells served as source for water to the north or downstream village residents. The exposure assessment does not consider potential occupational exposure.

Chemical	Downstream		Upstream	
	Median	Range	Median	Range
TCE	28	ND–1,791	0.1	0.1–0.1
Perchloroethylene	3	ND–5,228	0.05	ND–0.1
cis-1,2-DCE	3	ND–1,376	ND	ND
1,1-Dichloroethane	2	ND–228	0.05	ND–0.1
1,1-DCE	1	ND–1,240	ND	ND
Vinyl chloride	0.003	ND–72	ND	ND

ND = not detected

Lee L J-H, Chung C-W, Ma Y-C, Wang G-S, Chen P-C, Hwang Y-H, Wang J-D. (2003). Increased mortality odds ratio of male liver cancer in a community contaminated by chlorinated hydrocarbons in groundwater. Occup Environ Med 60:364–369.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Study hypothesis of investigating cancer mortality risk and exposure to chlorinated hydrocarbons in groundwater.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Deaths in 1966–1997 identified from local housing registration offices among residents in two villages were the source for case and control series. The two villages were north (contaminated community) and south (unexposed) of an electronics factory declared as a hazardous waste site. No information if all death among residents were reported to registration office. Cases: 53 liver cancer deaths in males and females, 51 included in statistical analysis (96%); stomach cancer deaths (n = 39), colon and rectum deaths (n = 26), and lung cancer deaths (n = 41). Paper does not present numbers of stomach, colo-rectal, and lung cancer deaths used in statistical analyses. Controls: 344 cardiovascular-cerebrovascular CV-CB disease deaths, 286 CV-CB deaths without arrhythmia included in statistical analysis (83%).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-9.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Exposure potential to chlorinated hydrocarbons in drinking water was inferred from residence address on deaths certificate.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	NA, Record based information.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	NA

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Liver cancer case exposure prevalence [downstream village resident], 53% (n = 24 males, n = 4 females). Control exposure prevalence [upstream village resident], 30% (n = 44 males, n = 41 females).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Sex and age (categorical). No control for potential confounding due to hepatitis virus (for liver cancer) or smoking (for lung cancer analyses).
Statistical methods	Mantel-Haenszel χ^2. Multiple logistic regressions (males deaths only).
Exposure-response analysis presented in published paper	No, MORs presented by time period.
Documentation of results	Inadequate, the paper does not discuss mobility patterns of residents, percentage of population who may have moved from area, or completeness of death ascertainment using certificates obtained from local housing registration offices.

MOR = mortality odds ratio

B.3.2.6. Lymphoma Case-Control Studies

B.3.2.6.1. Gold et al. (2011), Purdue et al. (2011)

B.3.2.6.1.1. Gold et al. (2011) abstract.

Objectives Few studies have examined whether exposure to chlorinated solvents is associated with multiple myeloma. We evaluated associations between multiple myeloma and occupational exposure to six chlorinated solvents: 1,1,1-trichloroethane, trichloroethylene (TCE), methylene chloride (DCM), perchloroethylene, carbon tetrachloride and chloroform. **Methods** In-person interviews obtained occupational histories and information on jobs with likely solvent exposure. We assigned exposure metrics of probability, frequency, intensity and confidence using job-exposure matrices modified by job-specific questionnaire information. We used logistic regression to estimate ORs and 95% CIs for associations between multiple myeloma and ever exposure to each, and any, chlorinated solvent and analysed whether associations varied by duration and cumulative exposure. We also considered all occupations that were given the lowest confidence scores as unexposed and repeated all analyses. **Results** Risk of multiple myeloma was elevated for subjects ever exposed to 1,1,1-trichloroethane (OR (95% CI): 1.8 (1.1 to 2.9)). Ever exposure to TCE or DCM also entailed elevated, but not statistically significant, risks of multiple myeloma; these became statistically significant when occupations with low confidence scores were considered unexposed (TCE: 1.7 (1.0 to 2.7); DCM: 2.0 (1.2 to 3.2)). Increasing cumulative exposure to perchloroethylene was also associated with increasing multiple myeloma risk. We observed non-significantly increased multiple myeloma risks with exposure to chloroform; however, few subjects were exposed. **Conclusions** Evidence from this relatively large case-control study suggests that exposures to certain chlorinated solvents may be associated with increased incidence of multiple myeloma; however, the study is limited by relatively low participation (52%) among controls.

B.3.2.6.1.2. Purdue et al. (2011) abstract.

BACKGROUND: Previous epidemiologic findings suggest an association between exposure to trichloroethylene (TCE), a chlorinated solvent primarily used for vapor degreasing of metal parts, and non-Hodgkin lymphoma (NHL). OBJECTIVES: We investigated the association between occupational TCE exposure and NHL within a population-based case-control study using detailed exposure assessment methods. METHODS: Cases (n = 1,189; 76% participation rate) and controls (n = 982; 52% participation rate) provided information on their occupational histories and, for selected occupations, on possible workplace exposure to TCE using job-specific interview modules. An industrial hygienist assessed potential TCE exposure based on this information and a review of the TCE industrial hygiene literature. We computed odds ratios (ORs) and 95% confidence intervals (CIs) relating NHL and different metrics of estimated TCE exposure, categorized using tertiles among exposed controls, with unexposed subjects as the reference group. RESULTS: We observed associations with NHL for the highest tertiles of estimated average weekly exposure (23 exposed cases; OR = 2.5; 95% CI, 1.1–6.1) and cumulative exposure (24 exposed cases; OR =

2.3; 95% CI, 1.0-5.0) to TCE. Tests for trend with these metrics surpassed or approached statistical significance (p-value for trend = 0.02 and 0.08, respectively); however, we did not observe dose–response relationships across the exposure levels. Overall, neither duration nor intensity of exposure was associated with NHL, although we observed an association with the lowest tertile of exposure duration (OR = 2.1; 95% CI, 1.0-4.7). CONCLUSIONS: Our findings offer additional support for an association between high levels of exposure to TCE and increased risk of NHL. However, we cannot rule out the possibility of confounding from other chlorinated solvents used for vapor degreasing and note that our exposure assessment methods have not been validated.

B.3.2.6.1.3. **Gold et al. (2011) study description and comment.**

The population case-control study of multiple myeloma in men and women who were residents of two SEER reporting sites, the Seattle-Puget Sound, Washington region and the Detroit, Michigan metropolitan area, evaluated occupational risk factors in relation to the risk of multiple myeloma (MM). Detailed exposure information obtained from job-specific questionnaires allowed evaluation of association between 1, 1, 1-trichloroethane, TCE, dichloromethane, perchloroethylene carbon tetrachloride, and chloroform. Histologically-confirmed incident cases of MM (ICD-O-2/3, Codes 9731, 9732) in men and women without a previous diagnosis of MM, NHL or HIV, between 35 and 74 years of age, and diagnosed between 2000 and 2002 were eligible as cases, with population controls having Seattle-Puget Sound, Washington or Detroit, Michigan metropolitan area addresses identified from random digit dialing if <65 years of age, or by random selection from Medicare or Medicaid files for controls 65–74 years of age. Controls for this study were the same as those participating in the population-based case-control study of NHL carried out at the same time in these SEER areas, in addition, to two other SEER areas. A greater proportion of controls than cases were from Seattle-Puget Sound area. Face-to-face interviews were completed for 181 cases (71% participation rate) and 418 (52% participation rate).

In-person interviews were conducted using a computer-assisted interview program with modules focused specifically on solvent exposures for jobs held >2 years in 20 occupations. Proxy interviews were not permitted but were allowed to aid in recalling occupational details. All jobs were coded according to the Standard Occupational Classification system. For each of the six solvents, exposure metrics of probability, frequency, intensity, and confidence were assigned by modifying JEMs based on the subjects' answers to the questionnaire's sections on work history and job module. The JEMs were developed for each decade for specific industries, occupational and tasks by an industrial hygienist after reviewing published paper and reports on chlorinated solvents (e.g., 2007 for TCE). The assignment of exposure probability defined as the theoretical percentage of workers reporting the same information that would have been likely to have had exposure to the solvent is one strength of the study. For all jobs with probability scores of at least 1 (≥1% of subjects were likely to have had exposure), frequency and intensity scores

were also assigned, with values of 1, 2, 3, or 4 for each variable. Additionally, depending on the information source for assigning the probability, frequency, and intensity score, whether from literature review or self-reported, a confidence level was assigned on a scale of 1–4. Exposure surrogates developed for each of the six solvents were ever exposed and cumulative exposure, defined as the sum over all jobs of the product of intensity, exposure duration, and frequency. Of the 180 cases, 66 (37%) were identified as having been ever exposed to TCE (confidence scores of 1 or higher) with 24 of the TCE exposed cases (13% of all cases) assigned to the highest cumulative exposure group. Moreover, roughly one-third of the TCE-exposed cases were identified as having a low confidence level score (no information was available on probability, frequency or intensity or contradictory information exists in the literature), suggesting a greater potential for exposure misclassification bias in TCE assignment.

Association between MM and individual occupational solvents exposure was assessed using unconditional logistic regression to estimate ORs and 95% CIs. Jobs with probability score of ≥2 (≥10% subjects in that job were likely to have had TCE exposure) were defined as ever exposed to TCE. A lag period of 10 years, e.g., summing TCE exposures up to a period 10 years before disease diagnosis, was also examined in analyses of cumulative exposure. All statistical models included covariates for sex, age (three categories), race (four categories), education (three categories), and SEER site. Each of the continuous exposure metrics was categorized into four groups according to quartiles of the control exposure distribution. For TCE, cumulative exposure scores were 2,218 ppm-year (median) (range, 1–50,000 ppm-year). Test of trend were conducted using a linear term for the median duration and cumulative scores among controls in each category. Gold et al. (2011) further reported findings from sensitivity analyses considering all cases and controls with confidence scores of 1 as unexposed to address potential misclassification bias resulting from the identification of unexposed individuals as exposed. In studies with low exposure prevalences like Gold et al. (2011) this misclassification bias would diminish observed associations between TCE and multiple myeloma (Stewart and Correa-Villaseor, 1991).

B.3.2.6.1.4. **Purdue et al. (2011) study description and comment.**

This population case-control study of NHL in four SEER reporting areas was designed to investigate the association between NHL and occupational factors and focused on TCE exposures with a detailed exposure assessment method. Histologically-confirmed incident cases of NHL in men and women between 20 and 74 years of age, diagnosed between 1998 and 2000, and without know HIV infection were identified from four SEER reporting areas—the State of Iowa, the Seattle, Washington and Detroit, Michigan metropolitan areas, and Los Angeles County, California—with populations controls having addresses in the four SEER reporting areas identified from random digit dialing for men and women <65 years of age, or by random

selection from Medicare files, for men and women 65–74 years of age. NHLs were classified using according to the ICD-O-2 (converted to ICD-O-3, Codes 967-972): B-cell lymphomas, including small B-cell lymphoma, large diffuse B-cell lymphoma, follicular, or precursor lymphoblastic leukemia, and T-cell lymphoma, including anaplastic T-cell, N/K, and lymphoblastic leukemia. Subjects with CLL were ineligible; however, 28 recruited cases of small lymphocytic lymphoma were later identified by pathology review to be cases of CLL and were retained because the two diagnoses comprise the same disease. Face-to-face interviews were completed for 1,321 NHL cases (76% participation rate) and 1,057 controls (52% participation rate). Of these, 132 cases and 75 controls that were never employed or had unknown occupation were excluded, leaving 1,189 cases and 982 controls for the analysis.

Subjects provided information on residential and occupation history from a mailed calendar, with an in-person interview and home visit using a computer-assisted interview program with modules on solvent exposure, added 1 year after the study's start date. Of the computer-assisted personal interviews, 682 cases and 640 controls included the solvent-focused modules. The occupational history gathered information on each job held by the subject for ≥1 year since the age of 16. For selected occupations, 1 of 32 job- or industry-specific modules was administered based on information collected in the occupational histories. The information collected in the modules included the average frequency of various solvent-related tasks, the average length of time it took to perform given solvent-related tasks, sensory descriptions, dermal exposure, work practices, engineering controls, and personal protective equipment use. Information was also sought from subjects who reported jobs that could involve degreasing on the usual number of hours per instance spent degreasing, the identity of the chemical used for degreasing, the percentage of time each chemical was used, whether the degreasing solvent was heated or at room temperature, and the manner in which parts were cleaned.

The 23 exposure matrices developed by the industrial hygienist using information from the literature review, including Bakke et al. (2007), the subject's occupational history, and the information collected in the job modules, an expert industrial hygienist assessed levels of probability, frequency, and intensity of TCE exposure for each job. The assignment of exposure probability defined as the theoretical percentage of workers reporting the same information that would have been likely to have had exposure to the solvent is one strength of this study. For all jobs with probability scores of at least 1 (≥1% of subjects were likely to have had exposure), frequency and intensity scores were also assigned on a scale of 1–4 for frequency and 1–5 for intensity. The intensity score also reflected dermal exposure. The job-specified estimates of frequency and intensity for each subject were integrated to develop several metrics of TCE exposure. A subject was identified as "unexposed" if all jobs had been assigned an exposure probability of 0%, "possibly exposed" if one or more jobs had been assigned an exposure probability of <50% (probability scores of 1, 2, or 3, and "probably exposed" if at least one job had been assigned an exposure probability of ≥50% (probability scores of 4 or 5). For subjects

defined as probably exposed, the following additional exposure metrics were calculated: exposure duration; cumulative exposure, defined as the sum, across all jobs with exposure probability scores of 4 or 5, of the product of intensity midpoint, the frequency midpoint, and the duration in weeks; average week exposure, defined as the cumulative exposure divided by exposure duration; and average exposure intensity defined as the duration-weighted average intensity level across all jobs with probability scores of 4 or 5. Of the 1,189 cases, 545 (46%) were assigned an exposure level of "possible" and 45 cases (4%) an exposure level of "probable." Among subjects with probable confidence TCE exposure, the median cumulative exposure score was 150 ppm-year [range, 1–≥234,000 ppm-year].

Association between NHL and TCE exposure metrics was assessed using unconditional logistic regression to estimate ORs and 95% CIs. Other than the ever/never analysis, all analyses include subjects with probable TCE exposure, those with probability scores of 4 or 5. The observed exposure prevalence among subjects assigned possible exposure, defined as holding a job with a confidence score of 1, 2, or 3, suggested poor specificity and was inconsistent with the narrow set of occupational applications for TCE from the literature review. The higher likelihood for possible exposure misclassification bias and the importance of high specificity exposure assessment, further analysis of this measure was judged as unlikely to be informative. All statistical analyses included covariates for age (three categories), sex, race (four categories), education (three categories) and SEER area. The exposure metrics were categorized using tertiles among probably exposed controls as cut-points. In addition, ORs and 95% CIs were reported for exposure defined as the difference between the second and third tertiles among exposed controls. Test of trend were performed by modeling exposure the exposure metrics as continuous variables. Last, the association between TCE exposure and specific histologically-defined NHL subtypes (diffuse large B-cell, follicular lymphoma, and small lymphocytic lymphoma/CLL, were reported using polytomous regression to explore possible heterogeneity.

Gold LS, Stewart PA, Milliken K, Purdue M, Severson R, Seixas N, Blair A, Hartge P, Davis S, Dr Roos AJ. (2011). The relationship between multiple myeloma and occupational exposure to six chlorinated solvents. Occup Environ Med 68:391-399. doi:10.1136/oem.2009.054809].

Purdue MP, Bakke B, Stewart P, De Roos AJ, Schenk M, Lynch CF, Bernstein L, Morton LM, Cerhan JR, Severson RK, Cozen W, Davis S, Rothman N, Martge P, Colt JS. (2011). A case-control study of occupational exposure to trichloroethylene and non-Hodgkin lymphoma. Environ Health Perspect 119:232–238 doi:10.1289/ehp.1002106 [Online 2 November 2010]

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Study hypotheses of investigating association between TCE exposure and NHL using detailed exposure assessment methods (Purdue et al., 2011) and evaluating associations between multiple myeloma (Gold et al., 2011) and occupational exposure to six chlorinated solvents: 1,1,1-trichloro-ethane, methylene chloride, perchloroethylene, carbon tetrachloride, and chloroform.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cases: 1,321 (2,248 eligible) histologically-confirmed NHL cases in males and females, 20–74 yrs of age, 1998–2000, and residents of four SEER reporting areas—Iowa, Los Angeles County, California, Seattle, Washington metropolitan area and Detroit, Michigan metropolitan area (Purdue et al., 2011); 181 (255 eligible) histologically-confirmed multiple myeloma cases in males and females, 35–74 yrs of age, 2000–2002, and residents of two SEER reporting areas—Seattle-Puget Sound, Washington area and Detroit, Michigan metropolitan area (Gold et al., 2011) Controls: 1,057 (2,409 eligible) controls identified from random digit dialing (<65 yrs old) or Medicare file (65–75 yrs old) who were residents in the four SEER areas (Purdue et al., 2011); 481 (1,133 eligible) controls identified from Purdue et al. (2011) who were 35–74 yrs of age, no previous diagnosis of HIV, MM, plasmacytoma, or NHL, spoke English, and residents of Seattle-Puget Sound, Washington area and Detroit, Michigan metropolitan area (Gold et al.).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	NHL and multiple myeloma incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-0-2 [Codes 967-972, NHL; 9731-9732, MM].
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Literature review, exposure matrices occupational histories and information collected in the job module supported assignment by expert industrial hygienist of probability, frequency, and intensity of TCE for each job held ≥12 months (Purdue et al., 2011) or ≥2 yrs (Gold et al., 2011).

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	In-person interview using questionnaire or computer-assisted personal interview (682 of 1,321 cases and 640 of 1,057 controls in Purdue et al. (2011) with modules for jobs of interest.
Blinded interviewers	Interviewer not blinded. Exposure assessment assigned blinded.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No proxy interviews.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,321 cases (76% participation rate); 1,051 controls (52% participation rate) (Purdue et al., 2011). Of these, 132 cases and 75 controls that were never employed or had unknown occupation were excluded, leaving 1,189 cases and 982 controls for the analysis. 181 cases (71% participation rate); 1,113 controls (52% participation rate) (Gold et al., 2011). Exposure prevalence, ever exposed to TCE (≥50% of subjects in job probably exposed), 27 (2.8%) NHL cases; 0.7% of cases in highest cumulative exposure category and 2.3% in highest average exposure intensity category (Purdue et al., 2011); ever exposed to TCE (>10% of subjects in job with probable exposure) (Gold et al., 2011).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, SEER center, race and education (Gold et al.; Purdue et al., 2011).
Statistical methods	Unconditional logistic regression.
Exposure-response analysis presented in published paper	Test for trend performed by modeling the exposure metrics as continuous variable (Purdue et al., 2011) or using median duration and cumulative scores among controls for each exposure category.
Documentation of results	Yes, study was well documented with supplemental material on publisher's webpage (Purdue et al., 2011).

B.3.2.6.2. Cocco et al. (2010).

B.3.2.6.2.1. Author's abstract.

BACKGROUND: Several studies have suggested an association between occupational exposure to solvents and lymphoma risk. However, findings are inconsistent and the role of specific chemicals is not known. Objective To investigate the role of occupational exposure to organic solvents in the aetiology of B-cell non-Hodgkin's lymphoma (B-NHL) and its major subtypes, as well as Hodgkin's lymphoma and T-cell lymphoma. METHODS: 2348 lymphoma cases and 2462 controls participated in a case-control study in six European countries. A subset of cases were reviewed by a panel of pathologists to ensure diagnostic consistency. Exposure to solvents was assessed by industrial hygienists and occupational experts based on a detailed occupational questionnaire. RESULTS: Risk of follicular lymphoma significantly increased with three independent metrics of exposure to benzene, toluene and xylene (BTX) (combined p=4 x 10(-7)) and to styrene (p=1 x 10(-5)), and chronic lymphocytic leukaemia (CLL) risk increased with exposure to solvents overall (p=4 x 10(-6)), BTX (p=5 x 10(-5)), gasoline (p=8 x 10(-5)) and other solvents (p=2 x 10(-6)). Risk of B-NHL for ever exposure to solvents was not elevated (OR=1.1, 95% CI 1.0 to 1.3), and that for CLL and follicular lymphoma was 1.3 (95% CI 1.1 to 1.6) and 1.3 (95% CI 1.0 to 1.7), respectively. Exposure to benzene accounted, at least partially, for the association observed with CLL risk. Hodgkin's lymphoma and T-cell lymphoma did not show an association with solvent exposure. CONCLUSION: This analysis of a large European dataset confirms a role of occupational exposure to solvents in the aetiology of B-NHL, and particularly, CLL. It is suggested that benzene is most likely to be implicated, but we cannot exclude the possibility of a role for other solvents in relation to other lymphoma subtypes, such as follicular lymphoma. No association with risk of T-cell lymphoma and Hodgkin's lymphoma was shown.

B.3.2.6.3. Study description and comment.

This population case control study of NHL in the Czech Republic, France, Germany, Italy, Ireland, and Spain was designed to examine possible personal and occupational risk factors for lymphoma subtypes as defined using the WHO classification (the Epilymph study). Observations in German subjects are reported separately in Seidler et al. (2007) (see B.3.2.6.6). The publication of Cocco et al. (2010) examined solvents and adopted expert assessment to assign exposure potential to organic solvents, specifically, chlorinated aliphatic hydrocarbons, benzene, toluene, xylene, gasoline, mineral spirits, styrene, and TCE. Cases of lymphoma in adults, >17 years of age, and diagnosed in 22 centers in 1998 and 2004 with population controls selected by sampling from the general population, and matched to cases on sex, age, and residence area, in Germany and Italy, or matched hospital controls limited to diagnoses other than cancer, infectious diseases, and immunodeficient diseases in the Czech Republic, France, Ireland, and Spain. The lymphoma diagnosis was classified according to the 2001 WHO classification of lymphoma, and slides of about 20% of cases from each center were reviewed

centrally by a panel of pathologists and reclassified when necessary. Lymphoma cases included in this study were B-cell lymphomas, including B-cell subtypes, T-cell lymphomas, and Hodgkin lymphoma. Informed consent was obtained for 2,348 lymphoma cases (88%) and 2,462 controls (81% hospital controls, 52% population controls) who participated in the study. Most cases were B-cell lymphomas (n = 1,869) with fewer T-cell (n = 133) and Hodgkin (n = 339) lymphoma.

Trained interviewers administered a structured questionnaire through in-person interviews with cases and controls to collect information on sociodemographic factors, lifestyle, health history, and complete work history for all full-time jobs held for ≥1 year. Special questionnaire modules for specific occupations gathered additional details on jobs and exposure of a priori interest. Industrial hygienists in each center reviewed the general and specific questionnaires and assessed exposure to 43 agents, including organic solvents according to confidence, intensity, and frequency of exposure. The paper does not report if proxy or next-of-kin provided information if the case or control was deceased. Confidence represented the degree of certainty that the worker had been exposed to the agent and was based both on probability of exposure and on the proportion of workers exposed in a give job, <40% (possible exposure), 40–90%, (probable exposure), and >90% (certain/definite exposure). Intensity of exposure was defined as a rank-ordered variable, unexposed (0), low (1), medium (2), high (3), with agent-specific cut-off points defined based on current threshold limit values, likely half the threshold limit value (TLV) (low), 51–150% (medium), and >150% (high) (Kiran et al., 2010). Exposure frequency expressed the proportion of work time involving contact with the agent: unexposed (coded as 0), 1–5% of the work time (coded as 1), >5–30% of the work time (coded as 2), and >30% of the work time (coded as 3). Exposure potential to TCE for cases and controls was based surrogates for overall exposure and cumulative exposure score. The cumulative exposure score was the sum over a subjects work history of the product of duration and frequency/3 to the power of intensity and results in a log distribution of exposure scores. Exposure prevalence to TCE is low in this study; Cocco et al. (2010) identifies 71 cases of B-cell lymphoma (4% exposure prevalence) and 117 controls (5% exposure prevalence) with high confidence overall TCE exposure and of these exposed subjects, 29 cases (2%) and 37 (2%) with a high-confidence, high-cumulative exposure score.

Association between B-cell lymphoma and B-cell lymphoma subtypes and individual occupational solvent exposures was assessed using unconditional logistic regression, which adjusted for age, sex, education, and center. Alcohol and smoking were not included as a potential confounder as previous analysis of the Epilymph data showed no association (Besson et al., 2006). Statistical analyses are limited to subjects whose jobs TCE exposure was assessed with high degree of confidence, defined as >90%of worker exposed in a given job. Lymphoma subtypes examined included diffuse large B-cell lymphoma, follicular lymphoma, CLL, and multiple myeloma. There were few cases of T-cell lymphomas with high confidence TCE exposure; six cases with overall exposure, two of which with high confidence high cumulative

score. Two-tailed 95% CIs of the OR were calculated with the Wald statistics and trend test defining cumulative exposure score as a continuous variable using Wald's test for trend. As common to epidemiological studies, the many statistical analyses and comparisons in Cocco et al. (2010) increases the potential for false positive errors and Cocco et al. (2010) used Bonferroni correction of individual CIs and trend tests as an attempt to reduce this type of bias.

This study adopted a detailed exposure assessment, current classification system for lymphomas, and was of a large number of cases and controls, although exposure prevalence to TCE was <5%, typical of population case-control studies. This study defines the cumulative exposure score using a log scale, in addition, to using a rank-order value for intensity instead of a midpoint of an range of exposure concentrations. Other cohort and case-control studies of TCE and NHL, e.g., Purdue et al. (2011), define their cumulative exposure score as a product of intensity, frequency, and duration. Each approach will produce a slightly different rank ordering (personal communication). In the cumulative exposure formula of Cocco et al. (2010), exposure duration contributes the greatest weight in light of the formula's treatment of 1/3 the value of frequency (Cocco et al., 2010). The direction of bias in estimated trends of disease risk by cumulative exposure depends on the variation of duration, with large variation in durations between exposure exposures leading to downward bias. Cocco et al. (2010), also, reported ORs and CIs for high confidence TCE exposure, assigned to a job title when over 90% of workers were exposed. In comparison, both Purdue et al. (2011) and Gold et al. (2011) defined probable exposure if at least one job has been assigned an exposure probability of ≥50%. Any differences in reported findings between Cocco et al. (2010) and the other NHL studies of Miligi et al. (2006), Wang et al. (2009), and Purdue et al. (2011) may be due to these differences.

Cocco P, Mannetje A, Fadda D, Melis M, Becker N, Sanjosé S, Foretova L, Marekova J, Staines A, Kleefeld S, Maynadié M, Nieters A, Brennan P, Boffetta P. (2010). Occupational exposure to solvents and risk of lymphoma subtypes: results from the Epilymph case-control study. Occup Environ Med 67:341–347.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study evaluated occupational exposure to organic solvents as risk factors of NHL in a population-based, case-control study of men and women in six European countries.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	2,348 hospital cases of NHL diagnosed between 1998 and 2004 among men and women, >17 yrs of age, and residents of Czech Republic, France, Germany, Ireland, Italy, and Spain; 2,462 population and hospital controls, identified from census lists in Germany and Italy or small hospitals as the cases, in all other countries, and matched to cases on age, sex, and study center.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Lymphoma incidence – B-cell lymphoma (CLL, follicular, and diffuse large B-cell), T-cell lymphoma, Hodgkin lymphoma, and multiple myeloma. Postransplant lymphoproliferative disorder or monoclonal gammopathies of undetermined significance were excluded as cases.
Changes in diagnostic coding systems for lymphoma, particularly NHL	WHO classification system
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	All jobs held for >1 yr assigned to standardized occupation (5-digit code). Industrial hygienists at each center assigned exposure to 43 agents, including TCE and other solvents (benzene, toluene, xylene, chlorinated aliphatic hydrocarbons, and gasoline) to subjects according to confidence (possible, probable, certain), intensity (unexposed, low, medium, high), and frequency. Exposure surrogates for overall exposure and cumulative exposure (low, medium, high).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Face-to-face interview with questionnaire for information about medical history, lifestyle factors, lifetime occupational history (all jobs held >1 yr) and supplemental modules for specific occupations to gather additional details on jobs and exposures of a priori interest.
Blinded interviewers	Unblinded interviews. Blinded exposure assessment.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Not reported in published paper.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	2,348 cases (88% participation rate) and 2,462 controls (81% participation rate, hospital controls, 52% participation rate, population controls). Exposure prevalence, subjects with high confidence overall TCE exposure, 71 (4%) all B-cell lymphoma, 6 (7%) T-cell lymphoma, and 48 (6%) NHL (B-cell diffuse and follicular subtypes and T-cell); subjects with high confidence high cumulative TCE exposure, 29 (2%) all B-cell lymphomas, 2 (2%) T-cell lymphoma, 14 (2%) NHL (B-cell diffuse and follicular subtypes and T-cell).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, education, and center.
Statistical methods	Unconditional logistic regression.
Exposure-response analysis presented in published paper	Yes, using cumulative exposure defined as low, medium, high.
Documentation of results	Yes.

B.3.2.6.4. Wang et al. (2009).

B.3.2.6.4.1. Author's abstract.

> A population-based case-control study involving 601 incident cases of non-Hodgkin lymphoma (NHL) and 717 controls was conducted in 1996-2000 among Connecticut women to examine associations with exposure to organic solvents. A job-exposure matrix was used to assess occupational exposures. Increased risk of NHL was associated with occupational exposure to chlorinated solvents (odds ratio (OR) = 1.4, 95% confidence interval (CI): 1.1, 1.8) and carbon tetrachloride (OR = 2.3, 95% CI: 1.3, 4.0). Those ever exposed to any organic solvent in work settings had a borderline increased risk of NHL (OR = 1.3, 95% CI: 1.0, 1.6); moreover, a significantly increased risk was observed for those with average probability of exposure to any organic solvent at medium-high level (OR = 1.5, 95% CI: 1.1, 1.9). A borderline increased risk was also found for ever exposure to formaldehyde (OR = 1.3, 95% CI: 1.0, 1.7) in work settings. Risk of NHL increased with increasing average intensity (P = 0.01), average probability (p< 0.01), cumulative intensity (P = 0.01), and cumulative probability (p < 0.01) level of organic solvent and with average probability level (P = 0.02) and cumulative intensity level of chlorinated solvent (P = 0.02). Analyses by NHL subtype showed a risk pattern for diffuse large B-cell lymphoma similar to that for overall NHL, with stronger evidence of an association with benzene exposure. Results suggest an increased risk of NHL associated with occupational exposure to organic solvents for women.

B.3.2.6.4.2. Study description and comment.

This population case-control study of NHL in Connecticut women was designed to examine possible personal and occupational risk factors for NHL. The publication of Wang et al. (2009) examined solvent exposure and adopted a JEM to assign exposure potential to nine chemicals—benzene, formaldehyde, chlorinated solvents, chloroform, carbon tetrachloride, dichloromethane, methyl chloride, and TCE. Histologically-confirmed incident cases of NHL in women aged between 21 and 84 years of age and diagnosed in Connecticut between 1996 and 2000 were identified from the Connecticut Cancer Registry, a SEER reporting site, with population controls having Connecticut address identified from random digit dialing for women <65 years of age, or by random selection from Centers for Medicare and Medicaid Service files for women aged ≥65 years old. Controls were frequency matched to cases within 5-year age groups. Face-to-face interviews were completed for 601 (72%) cases and 717 controls (69% of those identified from random digit dialing and 47% identified using Health Care Financing Administration files).

Trained interviewers administered a structured questionnaire through in-person interviews with cases and controls to collect information on diet, nutrition, and alcohol intake; reproductive factors; hair dye use; and lifetime occupational history of all jobs held ≥1 year.

Jobs were coded to standardized occupational classification and standardized industry classification titles and assigned probability and intensity of exposure to formaldehyde and nine other solvents (benzene, any chlorinated solvents, DCE, chloroform, methylene chloride, dichloroethane, methyl chloride, TCE, and carbon tetrachloride) using a JEM developed by the NCI (Dosemeci et al., 1994; Gómez et al., 1994). All jobs held up to a year before cancer diagnosis were assigned blinded as to disease status potential exposure to each exposure of interest. Lifetime exposure potential for cases and controls was based on exposure duration and a weighted score for exposure intensity and probability of each occupational and industry and defined as a cumulative exposure metric, average metric, or ever/never metric. Of the 601 cases, 77 (13%) were assigned with potential TCE exposure over their lifetime; 8 cases were assigned potential for high intensity exposure, but with low probability and the 31 cases identified with medium and high probability of exposure were considered as having low intensity exposure potential. The low exposure prevalence to TCE, overall, and few subjects identified with confidence with high TCE exposure intensity or probability implies exposure misclassification bias is likely, and likely nondifferential, notably for high exposure categories (Dosemeci et al., 1990).

Association between NHL and individual occupational solvent exposure was assessed using unconditional logistic regression model which adjusted for age, family history of hematopoietic cancer, alcohol consumption, and race. Statistical analyses treated exposure defined as a categorical variable, divided into tertiles based on the distribution of controls, in logistic regression analyses and as a continuous variable, whenever possible, to test for linear trend. Polytomous logistic regression was used to evaluate the association between histologic subtypes of NHL (DLBCL, follicular lymphoma, or CLL/small lymphocytic lymphoma) and exposure. The largest number of cases was of the cell type DLBCL.

Strength of this study is assignment of TCE exposure potential to individual subjects using a validated JEM, although uncertainty accompanied exposure assignment and TCE exposure was largely of low intensity/low probability, and no cases with medium to high intensity/probability. Resultant misclassification bias would dampen observed associations for high exposure potential categories. Low prevalence of high intensity TCE exposure would reduce the study's statistical power.

Wang R, Zhang Y, Lan Q, Holford TR, Leaderer B, Zahm SH, Boyle P, Dosemeci M, Rothman N, Zhu Y, Qin Q, Zheng T. (2009). Occupational exposure to solvents and risk of non-Hodgkin lymphoma in Connecticut women. Am J Epidmiol 189:176–185.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study evaluated multiple potential risk factors of NHL in a population-based case-control study of Connecticut women. Occupational exposure to TCE was not an a priori hypothesis.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	601 (832 eligible) cases of NHL, diagnosed between 1996 and 2000 among women, age 20–84 yrs and residents of Connecticut and histologically-confirmed, were identified from the Yale Comprehensive Cancer Center's Rapid Case Ascertainment Shared Resource, a component of the Connecticut Tumor Registry; 717 (number of eligible controls not identified) population controls were randomly identified using random digit dialing, if age <65 yrs, or from Medicare and Medicaid Service files, for women aged ≥65 yrs old and stratified by sex and 5-yr age groups.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	NHL and chronic lymphatic leukemia incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-O-2 [Codes, M-9590-9642, 9690-9701, 9740-9750].
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	All jobs held for >1 yr were assigned to standardized occupation and industry classifications. Using JEM of NCI (Dosemeci et al., 1994; Gómez et al., 1994), probability of exposure level (low, medium and high) and intensity (very low, low, medium, and high) to TCE and other solvents (benzene, any chlorinated solvents, DCE, chloroform, methylene chloride, dichloroethane, methyl chloride, carbon tetrachloride, and formaldehyde) was assigned blinded as to case or control status.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Face-to-face interview with questionnaire for detailed information about medical history, lifestyle factors, education, lifetime occupational history (all jobs held >1 yr).
Blinded interviewers	Unblinded interviews.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	None.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	601 cases (72% participation) and 717 controls (69% participation for random digit dialing controls and 47% participation for HCFA controls). Exposure prevalence, ever exposed to TCE, 77 (13%) NHL cases; medium to high TCE intensity, 13 NHL cases (2%); medium to high TCE probability, 34 cases (6%). All 34 cases with medium to high TCE probability assigned low intensity exposure.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, family history of hematopoietic cancer, alcohol consumption and race.
Statistical methods	Unconditional logistic regression.
Exposure-response analysis presented in published paper	Yes, by exposure intensity and by exposure probability.
Documentation of results	Yes.

B.3.2.6.5. Costantini et al. (2008), Miligi et al. (2006).

B.3.2.6.5.1. Costantini et al. (2008) abstract.

Background While there is a general consensus about the ability of benzene to induce acute myeloid leukemia (AML), its effects on chronic lymphoid leukemia and multiple myeloma (MM) are still under debate. We conducted a population-based case–control study to evaluate the association between exposure to organic solvents and risk of myeloid and lymphoid leukemia and MM.
Methods Five hundred eighty-six cases of leukemia (and 1,278 population controls), 263 cases of MM (and 1,100 population controls) were collected. Experts assessed exposure at individual level to a range of chemicals.
Results We found no association between exposure to any solvent and AML. There were elevated point estimates for the associations between medium/high benzene exposure and chronic lymphatic leukemia (OR: 1.8, 95% CI¼0.9–3.9) and MM (OR: 1.9, 95% CI: 0.9–3.9). Risks of chronic lymphatic leukemia were somewhat elevated, albeit with wide confidence intervals, from medium/high exposure to xylene and toluene as well.
Conclusions We did not confirm the known association between benzene and AML, though this is likely explained by the strict regulation of benzene in Italy nearly three decades prior to study initiation. Our results support the association between benzene, xylene, and toluene and chronic lymphatic leukemia and between benzene and MM with longer latencies than have been observed for AML in other studies.

B.3.2.6.5.2. Miligi et al. (2006) abstract.

BACKGROUND: A number of studies have shown possible associations between occupational exposures, particularly solvents, and lymphomas. The present investigation aimed to evaluate the association between exposure to solvents and lymphomas (Hodgkin and non-Hodgkin) in a large population-based, multicenter, case-control study in Italy. METHODS: All newly diagnosed cases of malignant lymphoma in men and women age 20 to 74 years in 1991-1993 were identified in 8 areas in Italy. The control group was formed by a random sample of the general population in the areas under study stratified by sex and 5-year age groups. We interviewed 1428 non-Hodgkin lymphoma cases, 304 Hodgkin disease cases, and 1530 controls. Experts examined the questionnaire data and assessed a level of probability and intensity of exposure to a range of chemicals. RESULTS: Those in the medium/high level of exposure had an increased risk of non-Hodgkin lymphoma with exposure to toluene (odds ratio = 1.8; 95% confidence interval = 1.1-2.8), xylene 1.7 (1.0-2.6), and benzene 1.6 (1.0-2.4). Subjects exposed to all 3 aromatic hydrocarbons (benzene, toluene, and xylene; medium/high intensity compared with none) had an odds ratio of 2.1 (1.1-4.3). We observed an increased risk for Hodgkin disease for those exposed to technical solvents (2.7; 1.2-6.5) and aliphatic solvents (2.7; 1.2-5.7). CONCLUSION: This study suggests that aromatic and chlorinated hydrocarbons are a risk factor for non-Hodgkin lymphomas, and provides preliminary evidence for an association between solvents and Hodgkin disease.

B.3.2.6.5.3. **Study description and comment.**

This series of papers of a population case-control study of lymphomas in 11 areas in Italy (Costantini et al., 2008) and occupation examines author's assigned exposure to TCE and other solvents using job-specific or industry-specific questionnaires and expert rating to cases and controls. Miligi et al. (2006) reported findings for NHL, a category that included CLL, NHL subtypes, and Hodgkin lymphoma in eight regions and Constantini et al. (2008) presented observations for specific leukemia subtypes and multiple myeloma in seven regions (eight regions for CLL). Exclusion of the regions in the original study does not appear to greatly reduce study power or to introduce a selection bias. For example, Miligi et al. (2006) included 1,428 of the 1,450 total NHL cases, the largest percentage of all lymphoma subtypes. The number of other lymphoma subtypes was much smaller compared to NHL; 304 cases of Hodgkin disease, 586 cases of leukemia, and 263 cases of multiple myeloma. All cases were identified from participating study centers and controls were randomly selected from the each area's population using stratified sampling for sex and age.

A face-to-face unblinded interview was conducted primarily at the interviewee's home with a high proportion of proxy responses among cases (19%) but not controls (5%). Bias is likely introduced by the lack of blinding of interviewers and from the high proportion of proxy interviews. A questionnaire was used to obtain information on medical history, lifestyle factors, occupational exposure, and nonoccupational solvent exposures. Industrial hygiene professionals assessed the probability and intensity of exposure to individual and classes of solvents using information provided by questionnaire. Probability was classified into three levels (low, medium, and high) with a four-category scale for intensity (very low, low, medium, and high). These qualitative scales lacked information on exposure concentrations and likely introduces misclassification bias that can either dampen or inflate observed risks given the study's use of multiple exposure groupings. "Very low level" was used for subjects with occupational exposure intensities judged to be comparable to the upper end of the normal range for the general population; "low-level intensity" when workplace exposure was judged to be low because of control measures but higher than background; "medium exposure" for occupational environments with moderate or poor control measures; and "high exposure" for workplaces lacking any control measures. Groupings of "very low/low" and "medium/high" exposure was used to examine association with NHL. Prevalence of medium to high TCE exposure among NHL cases was low, 3% for NHL cases and 2% for all leukemia subtypes. Whether temporal changes in TCE exposure concentrations were considered in assigning level and intensity is not known. Overall, this study has low sensitivity for examining TCE and lymphoma given the low prevalence of exposure, particularly to medium to high TCE intensity, the high proportion of proxy interviews among cases, particularly NHL cases (15%), and qualitative exposure assessment approach.

Costantini AS, Benvenuti A, Vineis P, Kriebel D, Tumino R, Ramazzotti V, Rodella S, et al. (2008). Risk of leukemia and multiple myeloma associated with exposure to benzene and other organic solvents: evidence from the Italian multicenter case-control study. Am J Ind Med 51:803–811.

Miligi L, Costantini AS, Benvenuti A, Kreibel D, Bolejack V, et al. (2006). Occupational exposure to solvents and the risk of lymphomas. Epidemiol 17:552–561.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study evaluated TCE and other solvent exposures and lymphoma in a large population-based, multicenter, case-control study.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	1,732 (2,066 eligible) cases of NHL, chronic lymphatic leukemia, and Hodgkin lymphoma, diagnosed between 1991 and 1993 among men and women, age 20–74 yrs and residents of eight regions in Italy, were identified from; 1,530 (2,086 eligible) population controls were randomly selected from demographic files or from sampling of National Health Service files and stratified by sex and 5-yr age groups. 586 leukemia and 263 multiple myeloma among men and women, age 20–74 in the period 1991–1993, from seven regions (eight regions for CLL) in Italy, were identified from hospital or pathology department records or a regional cancer registry; and 1,100 population controls selected from demographic files or from sampling of National Health Service files and stratified by sex and 5-yr age groups.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	NHL and Hodgkin lymphoma incidence (Miligi et al., 2006). Leukemia and multiple myeloma (Costantini et al., 2008).
Changes in diagnostic coding systems for lymphoma, particularly NHL	All NHL cases were defined following NCI Working Formulation Workgroup classification and Hodgkin lymphomas defined following the Rye classification. NHL diagnosis confirmed for 334 of 1,428 cases (23%).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	IH experts from each region using information collected on questionnaires assigned the probability of exposure level (low, medium, and high) and intensity (very low, low, medium, and high) to TCE and other solvents. Exposure was assigned blinded as to case or control status.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Face-to-face interview with questionnaire for detailed information about medical history, lifestyle factors, education, occupational history (period is not identified in published paper), and nonoccupational exposures including solvent exposure.
Blinded interviewers	Unblinded interviews.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	19% of all lymphoma cases and 5% of controls were with proxy respondents (Costantini et al., 2008).
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,732 cases (83% participation) and 1,530 controls (73% participation) (Miligi et al., 2006); no information on participation rate for leukemia or multiple myeloma cases or their controls in Costantini et al. (2008). Exposure prevalence, medium to high TCE intensity, 35 NHL cases (3%) (Miligi et al., 2006); 11 leukemia cases (2%), and 5 multiple myeloma cases (2%) (Costantini et al., 2008).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, region, education, and region.
Statistical methods	Multiple logistic regressions.
Exposure-response analysis presented in published paper	Yes, by exposure intensity and by duration (years) of exposure.
Documentation of results	Yes.

B.3.2.6.6. **Seidler et al. (2007).**

B.3.2.6.6.1. **Author's abstract.**

AIMS: To analyze the relationship between exposure to chlorinated and aromatic organic solvents and malignant lymphoma in a multi-centre, population-based case-control study. METHODS: Male and female patients with malignant lymphoma (n = 710) between 18 and 80 years of age were prospectively recruited in six study regions in Germany (Ludwigshafen/Upper Palatinate, Heidelberg/Rhine-Neckar-County, Würzburg/Lower Frankonia, Hamburg, Bielefeld/Gütersloh, and Munich). For each newly recruited lymphoma case, a gender, region and age-matched (+/-1 year of birth) population control was drawn from the population registers. In a structured personal interview, we elicited a complete occupational history, including every occupational period that lasted at least one year. On the basis of job task-specific supplementary questionnaires, a trained occupational physician assessed the exposure to chlorinated hydrocarbons (trichloroethylene, tetrachloroethylene, dichloromethane, carbon tetrachloride) and aromatic hydrocarbons (benzene, toluene, xylene, styrene). Odds ratios (OR) and 95% confidence intervals (CI) were calculated using conditional logistic regression analysis, adjusted for smoking (in pack years) and alcohol consumption. To increase the statistical power, patients with specific lymphoma subentities were additionally compared with the entire control group using unconditional logistic regression analysis. RESULTS: We observed a statistically significant association between high exposure to chlorinated hydrocarbons and malignant lymphoma (Odds ratio = 2.1; 95% confidence interval 1.1–4.3). In the analysis of lymphoma subentities, a pronounced risk elevation was found for follicular lymphoma and marginal zone lymphoma. When specific substances were considered, the association between trichloroethylene and malignant lymphoma was of borderline statistical significance. Aromatic hydrocarbons were not significantly associated with the lymphoma diagnosis. CONCLUSION: In accordance with the literature, this data point to a potential etiologic role of chlorinated hydrocarbons (particularly trichloroethylene) and malignant lymphoma. Chlorinated hydrocarbons might affect specific lymphoma subentities differentially. Our study does not support a strong association between aromatic hydrocarbons (benzene, toluene, xylene, or styrene) and the diagnosis of a malignant lymphoma.

B.3.2.6.6.2. **Study description and comment.**

This population case-control study of NHL and Hodgkin lymphoma patients in six Germany regions is part of a larger multiple-center and -country case-control study of lymphoma and environmental exposures, the EPILYMPH study (see Cocco et al. (2010) in B.3.2.6.3). A total of 710 cases and 710 controls that were matched to cases on age, sex, and region, participated in this study. Participation rates were 88% for cases and 44% for controls. Potential for selection bias may exist given the low control response rate. Strength of this study is the use of WHO classification scheme for classifying lymphomas and the high percentage of cases with histologically-confirmed diagnoses. An industrial physician blinded to case and control status

assigned exposure to specific solvents (i.e., TCE, perchloroethylene, carbon tetrachloride, etc.) using a JEM developed for the EPILYMPH investigators, a modification of Bolm-Audorff et al.(1988). Exposure prevalence to TCE among cases was 13%. A cumulative exposure score was calculated and was the sum for every job held of intensity of solvent exposure, frequency of exposure, and duration of exposure. High exposure to TCE was defined as >35 ppm-years; 3% of cases had high cumulative exposure to TCE. Intensity of TCE exposure was assessed on a semiquantitative scale with the following categories: low intensity, 2.5 ppm (0.5–5); medium intensity, 25 ppm (>5–50), high intensity, 100 ppm (>50). The frequency of exposure was the percentage of working time during which the exposure occurred based upon a 40-hour week. A semiquantitative scale was adopted for frequency of exposure with the following categories: low frequency, 3% of working time (range, 1–5%), medium frequency, 17.5 % (range, >5–30%), high frequency, 65% of working time (>30%). A cumulative Prevalence of TCE exposure among cases was 13% overall with 3% of cases identified with cumulative exposure >35 ppm-years.

Overall, the use of expert assessment for exposure and WHO classification for disease coding likely reduce misclassification bias in this study. This population case-control study, like other population case-control studies of lymphoma and TCE, has a low prevalence of TCE exposure and limits statistical power to detect risk factors.

Seidler A, Mohner M, Berger J, Mester B, Deeg E, Eisner G, Neiters A, Becker N. (2007). Solvent exposure and malignant lymphoma: a population-based case-control study in Germany. J Occup Med Toxicol 2:2. Accessed August 27, 2007, http://www.occup-med.com/content/2/1/2.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This case-control study of NHL and Hodgkin lymphomas was designed to investigate association between specific exposure and distinct lymphoma classifications which are defined by REAL and WHO classifications.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	812 male and female lymphoma patients between the ages of 18 and 80 yrs were identified from a six German study regions from 1999 to 2003. 1,602 controls were identified from population registers and matched (1:1) to cases on sex, region, and age. 710 cases and 710 controls were interviewed.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	NHL and Hodgkin lymphoma incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	WHO classification. Diagnosis confirmed by pathological report for 691 cases.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Blinded assignment of intensity and frequency of exposure to specific chlorinated hydrocarbons (includes TCE) and to aromatic hydrocarbons based upon questionnaire information on complete occupational history for all jobs of ≥1-yr duration. Exposure assessment approach based on a modification of Bolm-Audorff et al. (1988)
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Face-to-face interview with questionnaire for detailed information about medical history, lifestyle factors, and occupation. Job-task-specific supplementary questionnaire administered to subjects having held jobs of interest; e.g., painters, metal workers and welders, dry cleaners, chemical workers, shoemakers and leather workers, and textile workers.
Blinded interviewers	Unblinded interviews.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No information provided in paper.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	710 cases (87.4%) and 710 controls (44.3%). Exposure prevalence: Any TCE exposure, Cases, 13%, Controls, 15%. High cumulative exposure (>35 ppm-yr), Cases, 3%, Controls, 1%.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, region, pack years of smoking, and # grams of alcohol consumed per day.
Statistical methods	Conditional logistic regression.
Exposure-response analysis presented in published paper	Yes, by ppm-yr as continuous variable.
Documentation of results	Yes.

B.3.2.6.7. **Persson and Fredrikson (1999), Persson et al. (1993; 1989).**

B.3.2.6.7.1. **Author's abstract.**

> Non-Hodgkin's lymphoma (NHL) has been subject to several epidemiological studies and various occupational and non-occupational exposures have been identified as determinants. The present study is a pooled analysis of two earlier methodologically similar case-referent studies encompassing 199 cases of NHL and 479 referents, all alive. Exposure information, mainly on occupational agents, was obtained by mailed questionnaires to the subjects. Exposure to white spirits, thinner, and aviation gasoline as well as work as a painter was connected with increased odds ratios, whereas no increased risk was noted for benzene. Farming was associated with a decreased odds ratio and exposure to phenoxy herbicides, wood preservatives, and work as a lumberjack showed increased odds ratios. Moreover, exposure to plastic and rubber chemicals and also contact with some kinds of pets appeared with increased odds ratios. Office employment and housework showed decreased odds ratios. This study indicates the importance of investigating exposures not occurring very frequently in the general population. Solvents were studied as a group of compounds but were also separated into various specific compounds. The present findings suggest that the carcinogenic property of solvents is not only related to the aromatic ones or to the occurrence of benzene contamination, but also to other types of compounds.

B.3.2.6.7.2. **Study description and comment.**

The exposure assessment approach of Persson and Fredriksson (1999), a pooled analysis of NHL cases and referents in Persson et al. (1993), and Persson et al. (1989), was based upon self-reported information obtain from a mailed questionnaire to cases and controls. Ten of 17 main questions of the detailed multiple-page questionnaire concerned occupational exposure, with additional questions on specific job and exposure details. These studies of the Swedish population considered exposure durations of ≥1 years and those received 5–45 years before NHL diagnosis for cases and before the point in time of selection for controls. The period of TCE exposure assessed in the between 1964 and 1986, a time period similar to that of Axelson et al. (1994). Semiqualitative information about solvent exposure was obtained directly from the questionnaires. Assignment of exposure potential to individual solvents such as TCE and white spirit is not described nor does the paper describe whether assignment was done blinded as to case or control status. A five-category classification for intensity was developed although statistical analyses grouped the TCE categories as intensity scores of >2 compared to 0/1. TCE exposure prevalence among cases was 8% (16 of 199) and 7% among referents (32 of 479).

This small study of 199 NHL cases diagnosed between 1964 and 1986 at a regional Swedish hospital (Orebro) and alive at the time of data acquisition in 1986 was similar in design to other lymphoma (CLL, multiple myeloma) and occupational studies from these investigators (Flodin et al., 1987). A series of 479 referents from the same catchment area and from the same time period, identified previously from the multiple myeloma and CLL studies, served as the

source for controls in Persson and Fredrikson (1999) for the NHL analysis and in Persson et al. (1993; 1989) for the Hodgkin lymphoma analysis. Given the study's entrance date as 1964, with interviews carried out in the 1980s, some cases were deceased with information likely provided by proxy respondents. The paper does not identify the percentage of deceased cases and the magnitude of potential bias associated with proxy respondents cannot be determined. Little information is provided in the published paper on controls; however, the paper notes that 17% of eligible controls were not able or unwilling to respond to the questionnaire. Case and control series appear to differ given only subjects 40 to 80 years of age were included in the statistical analysis. Cases in Perrson et al. (1993) were histologically confirmed diagnosis of NHL; this was not so for Persson et al. (1989). Misclassification associated with misdiagnosis is not expected to be large given observation in Perrson et al. (1993) of 2% of lymphoma cases were misclassified.

Overall, the study's 20-year period between initial case and control identification and interview suggests some subjects were either survivors or information was obtained from proxy respondents. In both instances, misclassification bias is likely. No information is provided on job titles or the nature of TCE exposure, which was defined in the exposure assessment as "exposed or unexposed." Exposure prevalence to TCE in this study is higher than that found in community population studies of Miligi et al. (2006), Seidler et al. (2007), and Costantini et al. (2008).

Persson B, Fredrikson M. (1999). Some risk factors for non-Hodgkin's lymphoma. Int J Occup Med Environ Health 12:135–142.

Persson B, Fredriksson M, Olsen K, Boeryd B, Axelson O. (1993). Some occupational exposure as risk factors for malignant lymphomas. Cancer 72:1773–1778.

Persson B, Dahlander A-M, Fredriksson M, Brage HN, Ohlson C-G, Axelson O. (1989). Malignant lymphomas and occupational exposures. Br J Ind Med 46:516–520.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	These studies of Hodgkin lymphoma and NHL investigated occupational associations. Examination of TCE is not stated as a priori hypothesis.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Incident NHL and Hodgkin lymphoma cases reported to a regional cancer registry between 1975 and 1984, n = 148 (Persson et al., 1993), or identified from hospital records (Orebro Medical Center Hospital) for the period 1964 and 1986, n = 175 (Persson et al., 1989). Population controls from the same geographical area as cases were identified from previous case-control studies of leukemia and multiple myeloma and matched on age and sex. Analysis of NHL and Hodgkin lymphoma each used the same set of controls. Persson and Fredrikson (1999)—199 cases of NHL, 479 controls. Persson et al. (1993)—93 NHL and 31 Hodgkin lymphoma (90% participation); 204 controls. Persson et al. (1989)—106 NHL and 54 Hodgkin lymphoma (91%); 275 controls.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Classification system not identified in papers.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Self-reported occupational exposures as obtained from a mailed questionnaire.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Mailed questionnaire, only.
Blinded interviewers	N/A

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No information provided in paper.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Exposure prevalence to TCE Persson and Fredrikson (1999)—16 NHL cases (8%) and 32 controls (7%). Persson et al. (1993)—8 NHL cases (8%) and 5 Hodgkin lymphoma cases (16%); 18 controls (9%). Persson et al. (1989)—8 NHL cases (8%) and 7 Hodgkin lymphoma cases (13%); 14 controls (5%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Cases and controls are matched on age and sex. Statistical analyses do not control for other possible confounders.
Statistical methods	Only crude ORs are presented for TCE exposure, although logistic regression was used to examine other occupational exposure and NHL/Hodgkin lymphoma.
Exposure-response analysis presented in published paper	No.
Documentation of results	Poor, unable to determine response rate in control population, if controls were similar to cases on demographic variables such as sex and age, and whether controls were identified from same time period as cases.

B.3.2.6.8. **Nordstrom et al. (1998).**

B.3.2.6.8.1. **Author's abstract.**

To evaluate occupational exposures as risk factors for hairy cell leukemia (HCL), a population-based case-control study on 121 male HCL patients and 484 controls matched for age and sex was conducted. Elevated odds ratio (OR) was found for exposure to farm animals in general: OR 2.0, 95% confidence interval (CI) 1.2-3.2. The ORs were elevated for exposure to cattle, horse, hog, poultry and sheep. Exposure to herbicides (OR 2.9, CI 1.4-5.9), insecticides (OR 2.0, CI 1.1-3.5), fungicides (OR 3.8, CI 1.4-9.9) and impregnating agents (OR 2.4, CI 1.3-4.6) also showed increased risk. Certain findings suggested that recall bias may have affected the results for farm animals, herbicides and insecticides. Exposure to organic solvents yielded elevated risk (OR 1.5, CI 0.99-2.3), as did exposure to exhaust fumes (OR 2.1, CI 1.3-3.3). In an additional multivariate model, the ORs remained elevated for all these exposures with the exception of insecticides. We found a reduced risk for smokers with OR 0.6 (CI 0.4-1.1) because of an effect among non-farmers.

B.3.2.6.8.2. **Study description and comment.**

This population case-control of hairy cell leukemia, a B-cell lymphoid neoplasm and NHL, examined occupational organic solvent and pesticide exposures among male cases reported to the Swedish Cancer Registry between 1987 and 1992. A total of 121 cases, including 1 case one case, originally thought to have a diagnosis within the study's window, but latter learned as in 1993, and four controls per case matched on age and county of residence from the Swedish Population Registry. Occupational exposure was assessed based upon self-reported information provided in a mailed questionnaire with telephone follow-up by trained interviewer blinded to case or control status. Chemical-specific exposures of at least 1-day duration and occurring 1 year prior to case diagnosis were assigned to study subjects; however, the procedure for doing this was not described in the paper. Potential for organic solvents exposure included exposure received during leisure activities and work-related activities. Exposure prevalence to TCE among cases is 8 and 7% among controls. The low exposure prevalence and study size limit the statistical power of this study for detecting RRs <2.0.

ORs and 95% CIs are presented for chemical-specific exposures, including TCE, from logistic regression models in two separate analyses, univariate analysis and multivariate analysis adjusting for age. The OR for TCE exposure is presented only from univariate analysis. Age may not greatly confound or bias the observed association; an examination of risk estimates from univariate and multivariate analyses of the aggregated exposure category for organic solvents showed similar ORs, indicating age was not a significant source of bias in the statistical analyses because age was controlled in the study's design, a control was matching to a case on age.

Nordstrom M, Hardell L, Hagberg H, Rask-Andersen A. (1998). Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukemia evaluated in a case-control study. Br J Cancer 77:2048–2052.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Abstract—To evaluate occupational exposure as risk factors for hairy cell leukemia.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	121 cases of hairy cell leukemia in males reported to the Swedish Cancer Registry between 1987 and 1992. 484 controls (1:4 matching) identified from Swedish Population Registry and matched for age and county of residence.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Not identified in paper, likely ICD-9 (http://www.socialstyrelsen.se/, accessed February 6, 2009).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Self-reported information on occupational exposure as obtained from a mailed questionnaire to study participants. Questionnaire sought information on complete working history, other exposures, and leisure time activities with telephone interview in cases of incomplete information. Paper does not describe the procedure for assigning chemical exposures from job title information.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Mailed questionnaire.
Blinded interviewers	Follow-up telephone interview and job/exposure coding were done blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Proxy responses: 4%, cases; 1% controls.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	111 hairy cell leukemia cases, 400 controls. Response rate: 91% cases and 83% controls. Exposure prevalence among cases is 8 and 7% among controls.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Cases and controls are matched for age, sex, and county of residence. Effect measure for TCE exposure from univariate analysis presented in paper; other possible confounders or covariates not included in statistical analysis.
Statistical methods	Logistic regression.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.6.9. Fritschi and Siemiatycki (1996a), Siemiatycki (1991).

B.3.2.6.9.1. Author's abstract.

The known risk factors for lymphoma and myeloma cannot account for the current incidence rates of these cancers, and there is increasing interest in exploring occupational causes. We present results regarding lymphoma and myeloma from a large case-control study of hundreds of occupational exposures and 19 cancer sites. We examine in more detail those exposures previously considered to be related to these cancers, as well as exposures which were strongly related in our initial analyses. Lymphoma was not associated in our data with exposure to solvents or pesticides, or employment in agriculture or wood-related occupations, although numbers of exposed cases were sometimes small. Hodgkin's lymphoma was associated with exposure to fabric dust, and non-Hodgkin's lymphoma was associated with exposure to copper dust, ammonia and a number of fabric and textile-related occupations and exposures. Employment as a sheet metal worker was associated with development of myeloma.

B.3.2.6.9.2. Study description and comment.

This population study of several cancer sites included histologically-confirmed cases of NHL, Hodgkin lymphoma and myeloma ascertained from 16 Montreal-area hospitals between 1979 and 1985 and part of a larger study of 10 other cancer sites. This study relies on the use of expert assessment of occupational information on a detailed questionnaire and face-to-face interview. Fritschi and Siemiatycki (1996a) present observations of analyses examining industries, occupation, and some chemical-specific exposures, including solvents, but not TCE. Observations on TCE are found in the original report of Siemiatycki (1991).

A total of 215 NHL cases (83% response) were identified from 19 Montreal-area hospitals and while this case group is larger than that in Swedish lymphoma case-control studies, there are fewer NHL cases than other multicenter studies published since 2000. The 533 population controls (72% response), identified through the use of random digit dialing, and were used for each site-specific cancer case analyses. All controls were interviewed using face-to-face methods; however, 20% of the NHL cases were either too ill to interview or had died and, for these cases, occupational information was provided by a proxy respondent. The quality of interview conducted with proxy respondents was much lower, increasing the potential for misclassification bias, than that with the subject. The direction of this bias would diminish observed risk towards the null. Interviewers were unblinded, although exposure assignment was carried out blinded as to case and control status. The questionnaire sought information on the subject's complete job history and included questions about the specific job of the employee and work environment. Occupations considered with possible TCE exposure included machinists, aircraft mechanics, and industrial equipment mechanics. An additional specialized questionnaire was developed for certain job title of a prior interest that sought more detailed information on

tasks and possible exposures. For example, the supplemental questionnaire for machinists included a question on TCE usage.

A team of industrial hygienists and chemicals assigned exposures blinded based on job title and other information obtained by questionnaire. A semiquantitative scale was developed for 294 exposures and included TCE (any, substantial). Any exposure to TCE was 3% among cases but <1% for substantial TCE exposure; "substantial" is defined as ≥10 years of exposure for the period up to 5 years before diagnosis. The TCE exposure frequencies in this study are lower than those in more recent NHL case-control studies examining TCE. The expert assessment method is considered a valid and reliable approach for assessing occupational exposure in community-base studies and likely less biased from exposure misclassification than exposure assessment based solely on self-reported information (Fritschi et al., 2003; IOM, 2003; Siemiatycki et al., 1997).

Logistic regression models adjusted for age, ethnicity, income, and respondent status (Fritschi and Siemiatycki, 1996a) or Mantel-Haenszel χ^2 stratified on age, family income, and cigarette smoking (Siemiatycki, 1991). Odds ratios for TCE exposure are presented with 90% CIs in Siemiatycki (1991) and with 95% CIs in Fritschi and Siemiatycki (1996).

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of NHL. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The JEM, applied to the job information, was very broad since it was used to evaluate 294 chemicals. Overall, a reasonably good exposure assessment is found in this analysis; however, examination of NHL and TCE exposure is limited by statistical power considerations related to low exposure prevalence, particularly for "substantial" exposure. For the exposure prevalence found in this study to TCE and for NHL, the minimum detectable OR was 3.0 when $\beta = 0.02$ and $\alpha = 0.05$ (one-sided). The low statistical power to detect a doubling of risk and an increased possibility of misclassification bias associated with case occupational histories resulting from proxy respondents suggests this study is less sensitive than other NHL case-controls published since 2000 for examining NHL and TCE.

Fritschi L, Siemiatycki J. (1996a). Lymphoma, myeloma and occupation: Results of a case-control study. Int J Cancer 67: 498–503.

Siemitycki J. (1991). Risk Factors for Cancer in the Workplace. J Siemiatycki, Ed. Baca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study of NHL was designed to investigate association between specific exposure and cancers at 20 sites using expert assessment method for exposure assignment.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	258 histologically-confirmed NHL cases were identified among Montreal area males, aged 35–70 yrs, diagnosed in 16 Montreal hospitals between 1979 and 1985. 740 male population controls were identified from the same source population using random digit dialing methods.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	NHL.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICDO-0, 200 and 202, International Statistical Classification of Diseases for Oncology (WHO, 1977). ICDO-0 is based upon rubrics of ICD, 9th Revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of a priori interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 300 exposures, including TCE. For each exposure, a three-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1–5%; medium, >5–30%; and high, >30%).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Yes, 82% of case interviews were face-to-face; 100% of control interviews were with subject.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, ~20% of cases had proxy respondents. Interviews were completed with all control subjects.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	215 cases (83% response), 533 population controls (71%). Exposure prevalence: Any TCE exposure, 3% cases; substantial TCE exposure (exposure for ≥10 yrs and up to 5 yrs before disease onset), <1% cases.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, income, index for cigarette smoking (Siemiatycki, 1991). Age, proxy status, income, ethnicity (Fritschi and Siemiatycki, 1996a).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Unconditional logistic regression (Fritschi and Siemiatycki, 1996a).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.6.10. **Hardell et al. (1994; 1981).**

B.3.2.6.10.1. **Author's abstract.**

Results on 105 cases with histopathologically confirmed non-Hodgkin's lymphoma (NHL) and 335 controls from a previously published case-control study on malignant lymphoma are presented together with some extended analyses. No occupation was a risk factor for NHL. Exposure to phenoxyacetic acids yielded, in the univariate analysis, an odds ratio of 5.5 with a 95% confidence interval of 2.7-11. Most cases and controls were exposed to a commercial mixture of 2, 4-dichlorophenoxyacetic acid and 2, 4, 5-trichlorophenoxyacetic acid. Exposure to chlorophenols gave an odds ratio of 4.8 (2.7-8.8) with pentachlorophenol being the most common type. Exposure to organic solvents yielded an odds ratio of 2.4 (1.4-3.9). These results were not significantly changed in the multivariate analysis. Dichlorodiphenyltrichloroethane, asbestos, smoking, and oral snuff were not associated with an increased risk for NHL. The results regarding increased risk for NHL following exposure to phenoxyacetic acids, chlorophenols, or organic solvents were not affected by histopathological type, disease stage, or anatomical site of disease presentation. Median survival was somewhat longer in cases exposed to organic solvents than the rest. This was explained by more prevalent exposure to organic solvents in the group of cases with good prognosis NHL histopathology.

A number of men with malignant lymphoma of the histiocytic type and previous exposure to phenoxy acids or chlorophenols were observed and reported in 1979. A matched case-control study has therefore been performed with cases of malignant lymphoma (Hodgkin's disease and non-Hodgkin lymphoma). This study included 169 cases and 338 controls. The results indicate that exposure to phenoxy acids, chlorophenols, and organic solvents may be a causative factor in malignant lymphoma. Combined exposure of these chemicals seemed to increase the risk. Exposure to various other agents was not obviously different in cases and in controls.

B.3.2.6.10.2. **Study description and comment.**

Exposure in these case-control studies of histologically-confirmed lymphoma (NHL and Hodgkin lymphoma) (Hardell et al., 1981) or only the NHL cases only (Hardell et al., 1994) over a 4-year period, 1974–1978, in Umea, Sweden was assessed based upon information provided in a self-administered questionnaire. The questionnaire obtained information on a complete working history over the life of the subjects along with information on various other exposures and leisure time activities. Organic solvent exposures were examined secondary to this study's primary hypothesis examining phenoxy acid or chlorophenol exposures and lymphoma. The extent of recall bias related to self-reported information cannot be determined nor is information provided in the published papers misclassification bias resulting from next-of-kin interviews. Occupations were classification according to the Nordic Working Classification system. Chemical-specific exposures assignment was not described but appears to have been carried out

blinded as to case or control status. A semiquantitative classification scheme based on intensity and duration of exposure was used to categorize solvent exposure into two groupings: low grade—<1 week continuously or <1 month in total—and high grade for all other exposure scenarios. TCE exposure prevalence is similar in both studies; 4% for cases and 1% for controls. The low exposure prevalence and small numbers of cases with TCE exposure (n = 4) limits the statistical power of these analyses and results in wide CIs around the estimated OR for TCE exposure (95% CI, 1.3–42).

The Rappaport Classification was used to identify NHL and Hodgkin lymphoma cases. The Rappaport Classification was in widespread use until the 1970s and was based on a cell's pathologic characteristics. Equivalence of NHL groupings according to Rappaport Classification system to ICDA-8 groupings, also in use during this time period, is 200 "Lymphosarcoma and reticulum-cell sarcoma" and 202 "Other neoplasms of lymphoid tissue."

Hardell L, Eriksson M, Degerman A. (1994). Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-Hodgkin's lymphoma. Cancer Res 54:2386–2389.

Hardell L, Eriksson M, Lenner P, Lundgren E. (1981). Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Br J Cancer 43:169–176.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	NHL cases from a case-control study of lymphoma (NHL and Hodgkin lymphoma) are analyzed separately to evaluate herbicide and organic solvents exposure.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	105 cases of histologically-confirmed NHL among males aged 25–85 yrs admitted to local hospital's oncology department between 1974 and 1978. A total of 335 male controls identified from the Swedish Population Registry, for living cases, and from the Swedish Registry for Causes of Death, for dead cases. Controls matched to cases by age, residence municipality, and year of death, for dead cases.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Rappaport Classification; equivalent to ICDA-8 Codes, 200, and 202.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Self-reported information on occupational exposure as obtained by questionnaire, with a telephone interview for incomplete or unclear information. Questionnaire sought information on complete working history, other exposures and leisure time activities. Paper does not describe the procedure for assigning chemical exposures from job title information.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	No information in paper.
Blinded interviewers	Follow-up telephone interview was done blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No information in paper.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	105 NHL cases, 332 controls. Response rates could not be calculated given insufficient information in paper. Prevalence of TCE exposure, 4% cases, 1% controls.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Cases and controls matched on sex, age, place of residence, and vital status. Deceased controls are matched to deceased cases on year of death.
Statistical methods	Mantel-Haenszel stratified by age and vital status.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.7. Childhood Leukemia

B.3.2.7.1. Shu et al. (2004; 1999)

B.3.2.7.1.1. Author's abstract.

Ras proto-oncogene mutations have been implicated in the pathogenesis of many malignancies, including leukemia. While both human and animal studies have linked several chemical carcinogens to specific ras mutations, little data exist regarding the association of ras mutations with parental exposures and risk of childhood leukemia. Using data from a large case control study of childhood acute lymphoblastic leukemia (ALL; age <15 years) conducted by the Children's Cancer Group, we used a case-case comparison approach to examine whether reported parental exposure to hydrocarbons at work or use of specific medications are related to ras gene mutations in the leukemia cells of children with ALL. DNA was extracted from archived bone marrow slides or cryopreserved marrow samples for 837 ALL cases. We examined mutations in K-ras and N-ras genes at codons 12, 13, and 61 by PCR and allele-specific oligonucleotide hybridization and confirmed them by DNA sequencing. We interviewed mothers and, if available, fathers by telephone to collect exposure information. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived from logistic regression to examine the association of parental exposures with ras mutations. A total of 127 (15.2%) cases had ras mutations (K-ras 4.7% and N-ras 10.68%). Both maternal (OR 3.2, 95% CI 1.7-6.1) and paternal (OR 2.0, 95% CI 1.1-3.7) reported use of mind-altering drugs were associated with N-ras mutations. Paternal use of amphetamines or diet pills was associated with N-ras mutations (OR 4.1, 95% CI 1.1-15.0); no association was observed with maternal use. Maternal exposure to solvents (OR 3.1, 95% CI 1.0-9.7) and plastic materials (OR 6.9, 95% CI 1.2-39.7) during pregnancy and plastic materials after pregnancy (OR 8.3, 95% CI 1.4-48.8) were related to K-ras mutation. Maternal ever exposure to oil and coal products before case diagnosis (OR 2.3, 95% CI 1.1-4.8) and during the postnatal period (OR 2.2, 95% CI 1.0-5.5) and paternal exposure to plastic materials before index pregnancy (OR 2.4, 95% CI 1.1-5.1) and other hydrocarbons during the postnatal period (OR 1.8, 95% CI 1.0-1.3) were associated with N-ras mutations. This study suggests that parental exposure to specific chemicals may be associated with distinct ras mutations in children who develop ALL.

Parental exposure to hydrocarbons at work has been suggested to increase the risk of childhood leukemia. Evidence, however, is not entirely consistent. Very few studies have evaluated the potential parental occupational hazards by exposure time windows. The Children's Cancer Group recently completed a large-scale case-control study involving 1842 acute lymphocytic leukemia (ALL) cases and 1986 matched controls. The study examined the association of self-reported occupational exposure to various hydrocarbons among parents with risk of childhood ALL by exposure time window, immunophenotype of ALL, and age at diagnosis. We found that maternal exposure to solvents [odds ratio (OR), 1.8; 95% confidence interval (CI), 1.3-2.5] and paints or thinners (OR, 1.6; 95% CI, 1.2-2.2) during the preconception period (OR, 1.6; 95% CI, 1.1-2.3) and during pregnancy (OR, 1.7; 95% CI, 1.2-2.3) and to plastic materials during the postnatal period (OR, 2.2; 95% CI, 1.0-4.7) were related to an increased risk of childhood ALL. A positive association between ALL and paternal exposure to plastic

materials during the preconception period was also found (OR, 1.4; 95% CI, 1.0-1.9). The ALL risk associated with parental exposures to hydrocarbons did not vary greatly with immunophenotype of ALL. These results suggest that the effect of parental occupational exposure to hydrocarbons on offspring may depend on the type of hydrocarbon and the timing of the exposure.

B.3.2.7.1.2. **Study description and comment.**

Parent hydrocarbon occupational exposure in this case-control study of acute lymphatic leukemia in children <15 years of age was assessed from telephone questionnaire to mothers and, whenever available, fathers of cases and controls who were part of the large-scale incidence study by the Children's Cancer/Oncology Group. A recent paper examines hydrocarbon exposures and relationship with the ras proto-oncogene (Shu et al., 2004). Nearly 50% of childhood leukemia cases in the United States were treated by a Children's Cancer Group hospital or institution and between January 1, 1989 and June 15, 1993, the study period, a total of 2,081 incident childhood leukemia cases were identified with 1,914 interviews with mothers. Controls were randomly selected using a random digit dialing procedure and matched to cases on age, race, and geographic location. Using structured questionnaires, parents or a surrogate when unavailable were asked about job title, industry, duties, starting and stopping date for all jobs held by the father for >6 months beginning at age 18 years and by the mother for all jobs held at least 6 months in the period from 2 year prior to the index pregnancy to date of diagnosis of leukemia case or the reference date of the controls. The questionnaire sought information on specific exposures to solvents (carbon tetrachloride, TCE, benzene, toluene, and xylene), plastic materials, paints, pigments or thinners, and oil or coal products. Exposure quantitative was not possible. Statistical analyses use self-reported exposure to specific hydrocarbons as defined as a dichotomous variable (yes/no). The potential for misclassification bias is greater with exposure assessment based upon self-reports compared to that by expert assessment (Teschke et al., 2002). Exposure information was linked to start and stop data of the relevant job to determine the timing of exposure related to specific windows of possible susceptibility for ALL. The author's do not describe jobs associated with possible TCE exposure.

The father's questionnaire was completed for 1,801 of the 2,081 eligible cases and 1,813 of the 2,597 eligible controls. Of the 1,618 matched sets, direct interview with fathers were obtained for 83% of cases and 68% of controls. Maternal interview were completed for 1,914 of the 2,081 eligible cases (92%). The low prevalence of any exposure to TCE, 1% for mothers (15 cases of 1,842 matched pairs with maternal exposure information) and 8% for fathers (136 cases out 1,618 matched pairs), limits the statistical power of this study to detect low to moderate risk.

Shu Xo, Perentesis JP, Wen W, Buckley JD, Boyle E, Ross, JA, Robison LL. (2004). Parental exposure to medications and hydrocarbons and ras mutations in children with acute lymphoblastic leukemia: A report from the Children's Oncology Group. Cancer Epidemiol Biomarkers Prev 13:1230–1235.

Shu XO, Stewart P, Wen W-Q, Han D, Potter JD, Buckley JD, Heineman E, Robison LL. (1999). Parental occupational exposure to hydrocarbons and risk of acute lymphocytic leukemia in offspring. Cancer Epidemiol Markers Prev 8:783–291.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Shu et al. (2004; 1999) examine possible association with a number of maternal and paternal exposures among cases and controls identified from the Children's Cancer/Oncology Group. The Children's Cancer/Oncology Group is an association of >120 centers in the United States, Canada, and Australia who collaboratively carry out research on risk factors and treatment of childhood cancers.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	848 children with acute lymphatic leukemia of ages 0–9 yrs of age at diagnosis from 1980 to 1993 and ≤14 yrs old at diagnosis between 1994 and 2000 were identified from cancer care centers in Québec, Canada. Controls are concurrently identified from population, from 1980 to 1993, from family allowance files and from 1994 to 2000, from universal health insurance files; and, matched (1:1 matching ratio) to cases on sex and age at the time of diagnosis (calendar date). Participation rates- 93.1% cases (790 of 849 eligible cases); 86.2% controls (790 of 916 eligible controls).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Childhood leukemia incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD, 9th revision, Code 204.0.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Telephone interviews of mothers of cases and controls using structured questionnaire were administered to obtain information on general risk factors and potential confounders. Questionnaire also sought information on a complete job history, for the mother from 18 yrs of age to the end of pregnancy and included for each job, job title, dates of employment, type of industry, and location of employer. Statistical analyses based on self-reported occupational exposure to hydrocarbons as defined by broad groups and individual hydrocarbons.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Telephone interview, >99% response.
Blinded interviewers	Telephone interviews were not blinded, but exposure assignment and coding was carried out blinded to case and control status by chemists and industrial hygienists.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	100% of cases and controls had maternal history provided by direct interview with mothers. 13% of cases and 30% of controls had paternal information provided by proxy respondent (e.g., through maternal interview).
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	15 cases (2% exposure prevalence) and 9 controls (1% exposure prevalence) with maternal TCE exposure. 136 cases (8% exposure prevalence) and 104 controls (13% exposure prevalence) with paternal TCE exposure.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Child's age at time of diagnosis, sex, and calendar year of diagnosis, maternal age and level of schooling.
Statistical methods	Conditional logistic regression By two time periods; 2 yrs before pregnancy up to birth, during specific pregnancy period. By level of exposure; Level 1 (some exposure) compared to no exposure, and Level 2 (greater exposure potential) compared to no exposure.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes.

B.3.2.7.2. Costas et al. (2002), MDPH (1997b).

B.3.2.7.2.1. Author's abstract.

> A 1981 Massachusetts Department of Public Health study confirmed a childhood leukemia cluster in Woburn, Massachusetts. Our follow-up investigation attempts to identify factors potentially responsible for the cluster. Woburn has a 130-year industrial history that resulted in significant local deposition of tannery and chemical manufacturing waste. In 1979, two of the city's eight municipal drinking water wells were closed when tests identified contamination with solvents including trichloroethylene. By 1986, 21 childhood leukemia cases had been observed (5.52 expected during the seventeen year period) and the case-control investigation discussed herein was begun. Nineteen cases and 37 matched controls comprised the study population. A water distribution model provided contaminated public water exposure estimates for subject residences. Results identified a non-significant association between potential for exposure to contaminated water during maternal pregnancy and leukemia diagnosis, (odds RATIO=8.33, 95% CI 0.73–94.67). However, a significant dose-response relationship (P<0.05) was identified for this exposure period. In contrast, the child's potential for exposure from birth to diagnosis showed no association with leukemia risk. Wide confidence intervals suggest cautious interpretation of association magnitudes. Since 1986, expected incidence has been observed in Woburn including 8 consecutive years with no new childhood leukemia diagnoses.

B.3.2.7.2.2. Study description and comment.

Exposure in this case-control study of childhood leukemia over a 20-year period in Woburn, Massachusetts was assessed based upon the potential for a residence at the time of diagnosis to receive water from wells G and H, wells with a hydraulic mixing model of Murphy (Murphy, 1990), which described the town's water distribution system. Monitoring of wells G and H in 1979 showed the presence of several VOCs; TCE and perchloroethylene (PERC) were found to exceed drinking water guidelines, at 267 and 21 ppb, respectively. Low levels of other contaminates were detected including chloroform, 1,2-DCE methyl chloroform, trichlorotrifluoroethane, and inorganic arsenic. The Murphy model described the water flow through Woburn during the lifetime of wells G and H. The model uses data describing the physical layout of Woburn's municipal water system and information regarding the pumping cycles of wells G and H and other active uncontaminated wells that supplied the municipal water system. Model accuracy showed distribution of water from wells G and H to a block area with predicted mixture concentrations with an average error within 10% of the know concentration. Nearly 70% of the model predictions were within 20% of the know validation concentrations. An exposure value for cases and controls by exposure period was the sum of the model-predicted water concentration for each residence in Woburn as assigned to a hydrologically-distinct area along the water distribution network. Both cumulative and average exposure estimates were derived using the model.

Costas K, Knorr RS, Condon SK. (2002). A case-control study of childhood leukemia in Woburn, Massachusetts: the relationship between leukemia incidence and exposure to public drinking water. Sci Total Environ 300:23–25.

Massachusetts Department of Public Health (MDPH). (1997b). Woburn Childhood Leukemia Follow-up Study. Volumes I and II. Final Report.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes, "this follow-up investigation attempts to identify factors potentially responsible for the leukemia cluster in Woburn, MA" and the primary exposure of concern for investigation is "the potential consumption of contaminated water from Wells G and H by Woburn residents."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	21 cases of leukemia diagnosed in children <19 yrs between 1969 and 1989 who were residents of Woburn Massachusetts. Cases diagnosed from 1982 and latter were provided by the Massachusetts Cancer Registry. Cases diagnosed prior to 1982 were identified from local pediatric health professionals and by contacting all greater-Boston childhood oncology centers that treated children with leukemia. Two controls for each case were randomly selected from Woburn Public School records on a geographically basis and matched to cases on race, sex and date of birth (± 3 months).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Childhood leukemia incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-O (Acute Lymphatic Leukemia, Acute Myelogenous Leukemia, and Chronic Myelogenous Leukemia).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	In-person interviewers with mothers and fathers of cases and controls using questionnaire to gather information regarding demographics, residential information for the mother and child, occupational history, maternal medical and reproductive history, child's medical history, and lifestyle questions. The father's questionnaire contained questions concerning military and occupational history and also included duplicate questions on maternal occupational history, child's medical history, and lifestyle habits. A hydraulic mixing computer model describing Woburn's water distribution system was utilized to assign an exposure index expressed as cumulative number of months a household received contaminated drinking water from Wells G and H. Exposure Index = fraction of time during month when water from Wells G and H reached the user area + fraction of water from Wells G and H supplied to user area. No quantitative measures of TCE and other volatile organic solvents concentrations were included in hydraulic mixing model.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Personal interviews with cases and controls; 19 of 21 cases (91%) and 38 of possible 54 controls (70%) were interviewed.
Blinded interviewers	Interviewers were not blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	One parent interviewed for 21% of cases and 11% of controls.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Participation rates- 93.1% cases (790 of 849 eligible cases); 86.2% controls (790 of 916 eligible controls).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Composite covariates used to control for SES, maternal smoking during pregnancy, maternal age at birth of child, and maternal alcohol consumption during pregnancy.
Statistical methods	Conditional logistic regression.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes and includes information in MDPH Final Report (1997b).

B.3.2.7.3. McKinney et al. (1991).

B.3.2.7.3.1. Author's abstract.

OBJECTIVE--To determine whether parental occupations and chemical and other specific exposures are risk factors for childhood leukemia. DESIGN--Case-control study. Information on parents was obtained by home interview. SETTING--Three areas in north England: Copeland and South Lakeland (west Cumbria); Kingston upon Hull, Beverley, East Yorkshire, and Holderness (north Humberside), and Gateshead. SUBJECTS--109 children aged 0-14 born and diagnosed as having leukemia or non-Hodgkin's lymphoma in study areas during 1974-88. Two controls matched for sex and date and district of birth were obtained for each child. MAIN OUTCOME MEASURES--Occupations of parents and specific exposure of parents before the children's conception, during gestation, and after birth. Other adults living with the children were included in the postnatal analysis. RESULTS--Few risk factors were identified for mothers, although preconceptional association with the food industry was significantly increased in case mothers (odds ratio 2.56; 95% confidence interval 1.32 to 5.00). Significant associations were found between childhood leukemia and reported preconceptional exposure of fathers to wood dust (2.73, 1.44 to 5.16), radiation (3.23, 1.36 to 7.72), and benzene (5.81, 1.67 to 26.44); ionizing radiation alone gave an odds ratio of 2.35 (0.92 to 6.22). Raised odds ratios were found for paternal exposure during gestation, but no independent postnatal effect was evident. CONCLUSION--These results should be interpreted cautiously because of the small numbers, overlap with another study, and multiple exposure of some parents. It is important to distinguish periods of parental exposures; identified risk factors were almost exclusively restricted to the time before the child's birth.

B.3.2.7.3.2. Study description and comment.

A population case-control study of ALL and NHL in children of <14 years of age and residing in three areas in the United Kingdom was carried out to identify possible risk factors for the region's observed increased background childhood leukemia rates. The Sellafield nuclear reprocessing plant was located in one of the areas and one hypothesis was an examination of parental radiation exposure and childhood lymphoma. Unblinded face-to-face interviews with cases, identified from regional tumor registries, and controls, identified using regional birth registers, used a structured questionnaire to ascertain a complete history of employment and exposure to specific substances and radiation from both child's biological parents, preferred, although, in the absence of one parent, surrogate information by the other parent was obtained from the date of first employment to end of the study period or, if earlier, the date the parent ceased seeing the child. The questionnaire additionally sought information on maternal and paternal exposure to 22 known chemical carcinogens. McKinney et al. (1991) noted that exposures were highly correlated. Information on job title and industry as reported in the questionnaire was coded independently by experts to occupational groupings and titles using a national classification scheme from the Office of Population Census and Surveys and is a

strength of this study. The category of metal refining industry and occupations was one of nine occupational groups identified a priori for hypothesis testing. Statistical analyses are based on exposure as defined by industry, occupational title, or chemical-specific exposure.

Interviewers with one or both parents were carried out for 109 of 151 eligible cases (72%) and with 206 of 269 eligible controls (77%), and the low exposure prevalence; no information was presented on the number of surrogate interviews, or, where only one parent responded for both parents. The low prevalence of TCE exposure, five discordant pairs (one subject with exposure and the matched subject without exposure) identified with maternal TCE exposure and 16 discordant pairs with paternal preconceptional TCE exposure, greatly limited the statistical power of this study.

McKinney PA, Alexander FE, Cartwright RA, Parker L. (1991). Parental occupations of children with leukemia in west Cumbria, north Humberside, and Gateshead. BMJ 302:681–687.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study examines a number of risk factors (specific chemicals and occupational groups) as possibly associated with the high background rate of acute lymphatic leukemia and NHL in children ≤14 yrs in the three regions. 22 individual chemicals and 7 occupational groups for a priori hypothesis testing.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	151 case children identified from two tumor registries (Yorkshire and Northern Region). No information provided in paper on reporting accuracy of these registries. 269 population controls identified from District health authority birth registers and matched to cases on age, sex, and region of residency at time of case diagnosis. Participation rates- 72% of cases (n = 109) and 77% of controls (n = 206).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Childhood leukemia incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	No information provided in published paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Face-to-face interviews of mothers of cases and controls using structured questionnaire were administered to obtain information on general risk factors and potential confounders. Questionnaire also sought information on a maternal and paternal complete job history, from first employment to end of study and included for job title, dates of employment, and industry. Questionnaire administered to both parents, and, if one parent was unavailable, information was provided by proxy. Questionnaire also sought information on 22 specific chemicals. Expert assignment of occupation based upon National classification system. Statistical analyses industry of employment, job or occupation, and specific exposures.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	No, face-to-face interview with 72% of case parents and 77% of control parents.
Blinded interviewers	Face-to-face interviews were not blinded. Expert assignment of occupation was carried out blinded.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No information provided in paper on percentage of proxy interviews.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Exposure prevalence to TCE—maternal exposure, 2 cases (2%) and 3 controls (2%); paternal exposure, 9 cases (9%) and 7 controls (4%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Cases and control matched on age, sex, and region of residency at time of case diagnosis.
Statistical methods	Discordant pair analysis.
Exposure-response analysis presented in published paper	No.
Documentation of results	Limited reporting of ORs for job title and occupations.

B.3.2.7.4. Lowengart et al. (1987)

B.3.2.7.4.1. Author's abstract.

> A case-control study of children of ages 10 years and under in Los Angeles County was conducted to investigate the causes of leukemia. The mothers and fathers of acute leukemia cases and their individually matched controls were interviewed regarding specific occupational and home exposures as well as other potential risk factors associated with leukemia. Analysis of the information from the 123 matched pairs showed an increased risk of leukemia for children whose fathers had occupational exposure after the birth of the child to chlorinated solvents [odds ratio (OR) = 3.5, P = .01], spray paint (OR = 2.0, P = .02), dyes or pigments (OR = 4.5, P = .03), methyl ethyl ketone (CAS: 78-93-3; OR = 3.0, P = .05), and cutting oil (OR = 1.7, P = .05) or whose fathers were exposed during the mother's pregnancy with the child to spray paint (OR = 2.2, P = .03). For all of these, the risk associated with frequent use was greater than for infrequent use. There was an increased risk of leukemia for the child if the father worked in industries manufacturing transportation equipment (mostly aircraft) (OR = 2.5, P = .03) or machinery (OR = 3.0, P = .02). An increased risk was found for children whose parents used pesticides in the home (OR = 3.8, P = .004) or garden (OR = 6.5, P = .007) or who burned incense in the home (OR = 2.7, P = .007). The risk was greater for frequent use. Risk of leukemia was related to mothers' employment in personal service industries (OR = 2.7, P = .04) but not to specified occupational exposures. Risk related to fathers' exposure to chlorinated solvents, employment in the transportation equipment-manufacturing industry, and parents' exposure to household or garden pesticides and incense remains statistically significant after adjusting for the other significant findings.

B.3.2.7.4.2. Study description and comment.

Self-assessed parental exposure to chemical classes and to individual chlorinated solvents was assigned in this case-control study of leukemia in children ≤10 years old using information obtained through telephone interviews with mothers and fathers of cases and controls. Interviews were carried out for 79% of case mothers (159 or 202 cases) and 81% (124 of 154) case fathers. The number of potential controls was not identified in the paper, although it was reported that interviews were carried out for 136 referent mothers and 87 referent fathers. Mothers served as proxy respondents for paternal exposures in roughly 20% of cases and 30% of controls. The complete occupational history was sought for the period 1 year before the case diagnosis date, if the case was older than 2 years, 6 months before the diagnosis date, if the case was between the ages of 1 and 2 years, and the same as the date of diagnosis of the case was <1 year old. Questions on specific occupational exposures such as solvents or degreasers, metals, and other categories were included on the questionnaire, with self-reported information used to assign exposure potential. Exposure is defined only as a dichotomous variable (yes/no). In this study using a matched-pair design in the statistical analyses, there were six case-control pairs of paternal cases but not controls and three case-control pairs with paternal controls but not cases

with TCE exposure before pregnancy or during pregnancy. Few mothers reported exposure to chlorinated solvents. A strength of the study is the ability to examine exposure at a number of developmental periods, preconception, during pregnancy, and postnatal. Misclassification bias is likely strong in this study, introduced through the large number of proxy respondents and exposure assessment based upon self-reported information. Misclassification resulting from proxy information will dampen observed risks, where as misclassification of self-reported exposures may bias observed risks in either direction. For this reason and because of the low prevalence of exposure nature of exposure assessment approach, this study provides little information on childhood leukemia risks and TCE exposure.

Lowengart RA, Peters JM, Cicioni C, Buckley J, Bernstein L, Preston-Martin S, Rappaport E. (1987). Childhood leukemia and parents' occupational and home exposures. JNCI 79:39–46.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This case-control study of children ≤10 yrs of age was conducted to identify possible risk factors of childhood leukemia. TCE exposure was one of many occupational exposures assessed in this study.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	202 cases of acute lymphatic leukemia in children ≤10 yrs of age at time of diagnosis from 1980 through 1984 were identified from the Los Angeles County Cancer Surveillance Program, a population-based cancer registry. Controls were identified from among friends of cases with additional controls selected using random digit dialing from the same population as cases and were matched to cases on age, sex, race, and Hispanic origin. 123 cases (61% response rate) and 123 controls (not able to calculate response rate since number of possible controls not identified in paper).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Telephone questionnaire sought information on maternal and paternal preconception, pregnancy, and postnatal (up to 1 yr before case diagnosis) exposures, including a full occupational history (job title, employers, and dates of employments) and on the child's exposure from birth to 1 yr before case diagnosis. Parents also provide self-reported information on specific exposures or occupational activities. Occupations grouped according to hydrocarbon exposure potential using definition of Zack et al. (1980).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Telephone interview with 159 of 202 (79%) case mothers and 124 of 202 case fathers (61%). Of controls, interviews were obtained from 136 mothers (65 friends of cases, 71 population controls) and 87 fathers.
Blinded interviewers	Unblinded interviews.

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 19% of paternal exposure information on cases was provided by the mother. 43 of 130 control mothers provided information on paternal exposures (33%).
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Paternal TCE exposure 1 yr before pregnancy, 1/0 discordant pairs During pregnancy, 6/3 discordant pairs After delivery 8/3 discordant pairs. No information is provided in paper on maternal TCE exposure.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, race, and Hispanic origin.
Statistical methods	Discordant pair analysis.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.8. Melanoma Case-Control Studies

B.3.2.8.1. Fritschi and Siemiatycki (1996b), Siemiatycki (1991).

B.3.2.8.1.1. Author's abstract.

OBJECTIVES: Associations between occupational exposures and the occurrence of cutaneous melanoma were examined as part of a large population based case-control study of 19 cancer sites. METHODS: Cases were men aged 35 to 70 years old, resident in Montreal, Canada, with a new histologically confirmed cutaneous melanoma (n = 103). There were two control groups, a randomly selected population control group (n = 533), and a cancer control group (n = 533) randomly selected from among subjects with other types of cancer in the large study. Odds ratios for the occurrence of melanoma were calculated for each exposure circumstance for which there were more than four exposed cases (85 substances, 13 occupations, and 20 industries) adjusting for age, ethnicity, and number of years of schooling. RESULTS: Significantly increased risk of melanoma was found for exposure to four substances (fabric dust, plastic dust, trichloroethylene, and a group containing paints used on surfaces other than metal and varnishes used on surfaces other than wood), three occupations (warehouse clerks, salesmen, and miners and quarrymen), and two industries (clothing and non-metallic mineral products). CONCLUSIONS: Most of the occupational circumstances examined were not associated with melanoma, nor is there any strong evidence from previous research that any of those are risk factors. For the few occupational circumstances which were associated in our data with melanoma, the statistical evidence was weak, and there is little or no supporting evidence in the scientific literature. On the whole, there is no persuasive evidence of occupational risk factors for melanoma, but the studies have been too small or have involved too much misclassification of exposure for this conclusion to be definitive.

B.3.2.8.1.2. Study description and comment.

Fritschi and Siemiatycki (1996b) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and melanoma conducted in Montreal, Quebec (Canada) and part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 124 newly diagnosed cases of melanoma (ICD-O, 172), confirmed on the basis of histology reports, between 1979 and 1985; 103 of these participated in the study interview (83.1% participation). One control group (n = 533) consisted of patients with other forms of cancer recruited through the same study procedures and time period as the melanoma cancer cases. A population-based control group (n = 533, 72% response), frequency matched by age strata, was drawn using electoral lists and random digit dialing. Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents. The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job

activities, and any additional information that could furnish clues about exposure from the interviews.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Fritschi and Siemiatycki (1996b) present observations of logistic regression analyses examining industries, occupation, and some chemical-specific exposures, but not TCE. Observations on TCE from Mantel-Haenszel analyses are found in the original report of Siemiatycki (1991). Any exposure to TCE was 6% among cases (n = 8) and 4% for substantial TCE exposure (n = 4); "substantial" is defined as ≥10 years of exposure for the period up to 5 years before diagnosis.

Logistic regression models adjusted for age, ethnic origin, SES, Quetlet as an index of body mass, and respondent status (Fritschi and Siemiatycki, 1996b) or Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, Quetlet, ethnic origin, and respondent status (Siemiatycki, 1991). Odds ratios for TCE exposure are presented with 90% CIs in Siemiatycki (1991) and 95% CIs in Fritschi and Siemiatycki (1996b).

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of melanoma. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The JEM, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Fritschi L, Siemiatycki J. (1996b). Melanoma and occupation: Results of a case-control study. 1996. Occup Environ Med 53:168–173.

Siemiatycki J. (1991). Risk Factors for Cancer in the Workplace. J Siemiatycki, Ed. Boca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	124 melanoma cases were identified among male Montreal residents between 1979 and 1985 of which 103 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of other cancer cases identified in the larger study (n = 533). Participation rate: cases, 83.1%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-O, 172 (malignant neoplasm of skin).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of a priori interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a three-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1–5%; medium, >5–30%; and high, >30%).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	99 cases (76.7% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 8% cases (n = 8); substantial TCE exposure (exposure for ≥10 yrs and up to 5 yrs before disease onset), 4% cases (n = 4).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, education, and ethnic origin (Fritschi and Siemiatycki, 1996b). Age, family income, cigarette smoking, and ethnic origin (Siemiatycki, 1991).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Fritschi and Siemiatycki, 1996b).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.9. Pancreatic Cancer Case-Control Studies

B.3.2.9.1. Kernan et al. (1999).

B.3.2.9.1.1. Author's abstract.

Background The relation between occupational exposure and pancreatic cancer is not well established. A population-based case-control study based on death certificates from 24 U.S. states was conducted to determine if occupations/ industries or work-related exposures to solvents were associated with pancreatic cancer death.
Methods The cases were 63,097 persons who died from pancreatic cancer occurring in the period 1984±1993. The controls were 252,386 persons who died from causes other than cancer in the same time period.
Results Industries associated with significantly increased risk of pancreatic cancer included printing and paper manufacturing; chemical, petroleum, and related processing; transport, communication, and public service; wholesale and retail trades; and medical and other health-related services. Occupations associated with significantly increased risk included managerial, administrative, and other professional occupations; technical occupations; and sales, clerical, and other administrative support occupations.
Potential exposures to formaldehyde and other solvents were assessed by using a job exposure matrix developed for this study. Occupational exposure to formaldehyde was associated with a moderately increased risk of pancreatic cancer, with ORs of 1.2, 1.2, 1.4 for subjects with low, medium, and high probabilities of exposure and 1.2, 1.2, and 1.1 for subjects with low, medium, and high intensity of exposure, respectively.
Conclusions The findings of this study did not suggest that industrial or occupational exposure is a major contributor to the etiology of pancreatic cancer. Further study may be needed to confirm the positive association between formaldehyde exposure and pancreatic cancer.

B.3.2.9.1.2. Study description and comment.

Kernan et al. (1999) reported data from a case-control study of occupational exposures and pancreatic cancer, coding usual occupation as noted on death certificates to assign potential TCE exposure to cases and controls. Deaths from pancreatic cancer from 1984 to 1993 were identified from 24 U.S. state and frequency-matched to nonpancreatitis or other pancreatic disease deaths by state, race, sex, and age (5-year groups); 63,097 pancreatic cancer deaths (case series) and 252,386 controls were selected for analysis.

Exposure assessment in this study group occupational (n = 509) and industry (n = 231) codes into 16 broad occupational and 20 industrial categories. Additionally, a JEM of Gomez et al. (1994) was applied to develop exposure surrogates for 11 chlorinated hydrocarbons, including TCE, and two larger groupings, all chlorinated hydrocarbons and organic solvents. A qualitative surrogate (ever exposed/never exposed) for TCE exposure is developed and no information is provided on death certifications on employment duration to examine exposure-response patterns.

Kernan et al. (1999) report mortality ORs from logistic regression for TCE exposure intensity and probability of exposure.

Overall, this is a large study that examined specific exposures using a generic JEM. Errors resulting from exposure misclassification are likely, not only introduced by the generic JEM, but through the use of usual occupation as coded on death certificates, which may not fully represent an entire occupational history.

Kernan GJ, Ji B-T, Dosemeci M, Silverman DT, Balbus J, Zahm SH. (1999). Occupational risk factors for pancreatic cancer: A case-control study based on death certificates from 24 U.S. states. Am J Ind Med 36:260–270.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between pancreatic cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	63,097 pancreatic cancer cases were identified using death certificates from 24 U.S. states between 1984 and 1993. 63,097 noncancer, nonpancreatitis or other pancreatic disease deaths (controls) identified from the same source population and frequency-matched to cases by state, race, sex, and age (1:4 matching).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-9, 157 (malignant neoplasm of pancreas).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Usual occupation coded on death certificate coded to 1980 U.S. census classification system for occupation and industry. 509 occupation codes and 231 industry codes grouped into 16 broad occupational and 20 industrial categories based on similarity of occupational exposures. JEM of Gomez et al. (1994) used to assign exposure surrogates for 11 chlorinated hydrocarbons, including TCE, and two broad categories, chlorinated hydrocarbons and organic solvents.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	This study did not use interviews, information reported on death certificate used to infer potential exposure.
Blinded interviewers	No interviews were conducted in this study.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Exposure prevalence: Any TCE exposure (Low intensity exposure or higher), 14% cases (n = 9,068); High TCE exposure, 2% cases (n = 1,271).

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, metropolitan status, region of residence, and marital status.
Statistical methods	Logistic regression.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.10. Prostatic Cancer Case-Control Studies

B.3.2.10.1. Aronson et al. (1996), Siemiatycki (1991).

B.3.2.10.1.1. Author's abstract.

A population-based case-control study of cancer and occupation was carried out in Montréal, Canada. Between 1979 and 1986, 449 pathologically confirmed cases of prostate cancer were interviewed, as well as 1,550 cancer controls and 533 population controls. Job histories were evaluated by a team of chemist/hygienists using a checklist of 294 workplace chemicals. After preliminary evaluation, 17 occupations, 11 industries, and 27 substances were selected for multivariate logistic regression analyses to estimate the odds ratio between each occupational circumstance and prostate cancer with control for potential confounders. There was moderate support for risk due to the following occupations: electrical power workers, water transport workers, aircraft fabricators, metal product fabricators, structural metal erectors, and railway transport workers. The following substances exhibited moderately strong associations: metallic dust, liquid fuel combustion products, lubricating oils and greases, and polyaromatic hydrocarbons from coal. While the population attributable risk, estimated at between 12% and 21% for these occupational exposures, may be an overestimate due to our method of analysis, even if the true attributable fraction were in the range of 5–10%, this represents an important public health issue.

B.3.2.10.1.2. Study description and comment.

Aronson et al. (1996) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and prostate cancer conducted in Montreal, Quebec (Canada) and was part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 557 newly diagnosed cases of prostate cancer (ICD-O, 185), confirmed on the basis of histology reports, between 1979 and 1985; 449 of these participated in the study interview (80.6% participation). One control group consisted of patients with other forms of cancer recruited through the same study procedures and time period as the prostate cancer cases. A population-based control group (n = 533, 72% response), frequency-matched by age strata, was drawn using electoral lists and random digit dialing. Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents. The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these

exposure dimensions was categorized into none, any, or substantial exposure. Aronson et al. (1996) presents observations of logistic regression analyses examining industries, occupation, and some chemical-specific exposures, but not TCE. Observations on TCE from Mantel-Haenszel analyses are found in the original report of Siemiatycki (1991). Any exposure to TCE was 2% among cases (n = 11) and <2% for substantial TCE exposure (n = 7); "substantial" is defined as ≥10 years of exposure for the period up to 5 years before diagnosis.

Logistic regression models adjusted for age, education, and ethnicity (Aronson et al., 1996) or Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, coffee, and ethnic origin (Siemiatycki, 1991). Odds ratios for TCE exposure are presented with 90% CIs in Siemiatycki (1991) and 95% CIs in Aronson et al. (1996).

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of prostate cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The JEM, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Aronson KJ, Siemiatycki J, Dewar R, Gérin M. (1996). Occupational risk factors for prostate cancer: Results from a case-control study in Montréal, Canada. Am J Epidemiol 143:363–373.

Siemitycki J. (1991). Risk Factors for Cancer in the Workplace. J Siemiatycki, Ed. Boca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	557 prostate cancer cases were identified among male Montreal residents between 1979 and 1985 of which 449 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of other cancer cases identified in the larger study. Participation rate: cases, 83.1%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-O, 185 (malignant neoplasm of prostate).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of a priori interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a three-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1–5%; medium, >5–30%; and high, >30%).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	449 cases (80.6% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 2% cases (n = 11); substantial TCE exposure (exposure for ≥10 yrs and up to 5 yrs before disease onset), <2% cases (n = 7).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, ethnic origin, SES, Quetlet as an index of body mass, and respondent status (Aronson et al., 1996). Age, family income, cigarette smoking, ethnic origin, and respondent status (Siemiatycki, 1991).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Aronson et al., 1996).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.11. RCC Case-Control Studies—Arnsberg Region of Germany

A series of studies (including Henschler et al. (1995), discussed in cohort study section) have been conducted in an area with a long history of TCE use in several industries. The main importance of these studies is that there is considerable detail on the nature of exposures, which made it possible to estimate the order of magnitude of exposure even though there were no direct measurements.

B.3.2.11.1. Brüning et al. (2003).

B.3.2.11.1.1. Author's abstract.

BACKGROUND: German studies of high exposure prevalence have been debated on the renal carcinogenicity of trichloroethylene (TRI). METHODS: A consecutive hospital-based case-control study with 134 renal cell cancer (RCC) cases and 401 controls was conducted to reevaluate the risk of TRI in this region which were estimated in a previous study. Exposure was self-assessed to compare these studies. Additionally, the job history was analyzed, using expert-based exposure information. RESULTS: The logistic regression results, adjusted for age, gender, and smoking, confirmed a TRI-related RCC risk in this region. Using the database CAREX for a comparison of industries with and without TRI exposure, a significant excess risk was estimated for the longest held job in TRI-exposing industries (odds ratio (OR) 1.80, 95% confidence interval (CI) 1.01-3.20). Any exposure in "metal degreasing" was a RCC risk factor (OR 5.57, 95% CI 2.33-13.32). Self-reported narcotic symptoms, indicative of peak exposures, were associated with an excess risk (OR 3.71, 95% CI 1.80-7.54). CONCLUSIONS: The study supports the human nephrocarcinogenicity of trichloroethylene.

B.3.2.11.1.2. Study description and comment.

This study is a second case-control follow-up of renal cell cancer in the Arnsberg area of Germany, which was intended to deal with some of the methodological issues present in the two earlier studies. The major advantage of studies in the Arnsberg area is the high prevalence of exposure to TCE because of the large number of companies doing the same kind of industrial work. An interview questionnaire procedure for self-assessment of exposures similar to the one used by Vamvakas et al. (1998) was used to obtain detailed information about solvents used, job tasks, and working conditions, as well as the occurrence of neurological symptoms. The industry and job title information in the subjects' job histories were also analyzed by two schemes of expert-rated exposure assignments for broad groups of jobs. The CAREX database from the European Union, for industry categories, and the British JEM developed by Pannett et al. (1985), for potential exposure to chemical classes or specific chemical, but not TCE, was adopted in an attempt to obtain a potentially less biased assessment of exposures.

Exposure prevalences for employment in industries with potential TCE and perchloroethylene exposures was high in both cases (87%) and controls (79%) using the CAREX

approach, but much lower using the JEM approach for potential exposure to degreasing agents (12% cases, 9% controls), self-reported exposure to TCE (18% cases, 10% controls), and TCE exposure with any symptom occurrence (14% cases, 4% controls). Both the CAREX and British JEM rating approaches are very broad and they have potentially high rates of misclassification of exposure intensity in job groupings and industry groupings. In an attempt to avoid reporting biases associated with the legal proceeding for compensation, analyses were conducted on self-reported exposure to selected agents (yes or no). The regional use of TCE and perchloroethylene (tetrachloroethylene) were so widespread that most individuals recognized the local abbreviations. If individuals claimed to be exposed when they were not, it would reduce the finding of a relationship if one existed. Similarly, subjects were grouped by frequency of perceived symptoms (any, less than daily, daily) associated with TCE or perchloroethylene exposure. Overreporting would also introduce misclassification and reduce evidence of any relationship. Self-reporting of exposure to chemicals in case-control studies, generally, is considered unreliable since, within the broad population, workers rarely know specific chemicals to which they have potential exposure. However, in cohort studies and case-control studies in which one industry dominates a local population such as in this study, this is less likely because the numbers of possible industries and job titles are much smaller than in a broad population. The Arnsberg area studies focused on a small area where one type of industry was very prevalent, and that industry used primarily just two solvents: TCE and tetrachloroethylene. As a result, it was common knowledge among the workers what solvent an individual was using, and, for most, it was TCE. Self-reported TCE exposure is considered to be less biased compared to possible misclassification bias associated with using the CAREX exposure assessment approach which identified approximately 90% of all cases as holding a job in an industry using TCE or perchloroethylene (see above discussion).

Some subjects in Brüning et al. (2003) are drawn from the underlying Arnsberg population as studied by Vamvakas et al. (1998) (reviewed below) and TCE exposures to these subjects would be similar—substantial, sustained high exposures to TCE at 400–600 ppm during hot dip cleaning and >100 ppm overall. However, the larger ascertainment area outside the Arnsberg region for case and control identification may have resulted in a lower exposure prevalence compared to Vamvakas et al. (1998).

Brüning T, Pesch B, Wiesenhütter B, Rabstein S, Lammert M, Baumüller A, Bolt H. (2003). Renal cell cancer risk and occupational exposure to trichloroethylene: results of a consecutive case-control study in Arnsberg, Germany. Am J Ind Med 23:274–285.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract—study aim was to "reevaluate the risk of TRI in this region which were estimated in a previous study."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	162 RCC cases identified from September 1999 to April 2000 and who had undergone nephrectomy between 1992 and 2000 (a time period preceding that adopted in Vamvakas et al., (1998)]) from a regional hospital urology department in Arnsberg, Germany; 134 of the recruited cases were interviewed. 401 hospital controls were interviewed between 1999 and 2000 from local surgery departments or geriatric departments and frequency matched to cases by sex and age. 134 of 162 (83%) cases; response rate among controls could not be calculated lacking information on the number of eligible controls.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	N/A
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Face-to-face interview with subjects or their next of kin using a structured questionnaire with questions to obtain information on a complete job history by job title, supplemental information on job tasks with suspected exposure to specific agents, medical history, and personal habits. Questionnaires also sought self-reported information on duration and frequency of exposure to TCE and perchloroethylene, and, for these individuals, frequency of narcotic symptoms as a marker of high peak exposure. Jobs titles were coded according to a British classification of occupations and industries with potential chemical-specific exposures identified for each occupation using CAREX, a carcinogen exposure database or the British JEM of Pannett et al. (1985) for chemical groupings (e.g., degreasing agents, organic solvents).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	100% of cases or their NOK and 100% controls with face-to-face interviews.
Blinded interviewers	No information on whether interviewers were blinded.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 17% of case interviews with next-of-kin; all controls were alive at time of interview.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	CAREX Job-exposure-matrix 117 cases with TCE exposure (87% exposure prevalence among cases). 316 controls with TCE exposure (79% exposure prevalence among controls). Self-reported TCE exposure 25 cases with TCE exposure (18% exposure prevalence among cases). 38 controls with TCE exposure (9.5% exposure prevalence among controls).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and tobacco smoking.
Statistical methods	Conditional logistic regression.
Exposure-response analysis presented in published paper	Yes, duration of exposure as 4 categories (no, <10 yrs, 10–<20 yrs, and 20+ yrs).
Documentation of results	Yes.

B.3.2.11.2. **Pesch et al. (2000b).**

B.3.2.11.2.1. **Author's abstract.**

BACKGROUND: This case-control study was conducted to estimate the renal cell cancer (RCC) risk for exposure to occupation-related agents, besides other suspected risk factors. METHODS: In a population-based multicentre study, 935 incident RCC cases and 4298 controls matched for region, sex, and age were interviewed between 1991 and 1995 for their occupational history and lifestyle habits. Agent-specific exposure was expert-rated with two job-exposure matrices and a job task-exposure matrix. Conditional logistic regression was used to calculate smoking adjusted odds ratios (OR). RESULTS: Very long exposures in the chemical, rubber, and printing industries were associated with risk for RCC. Males considered as 'substantially exposed to organic solvents' showed a significant excess risk (OR = 1.6, 95% CI : 1.1-2.3). In females substantial exposure to solvents was also a significant risk factor (OR = 2.1, 95% CI : 1.0-4.4). Excess risks were shown for high exposure to cadmium (OR = 1.4, 95% CI : 1.1-1.8, in men, OR = 2.5, 95% CI : 1.2-5.3 in women), for substantial exposure to lead (OR = 1.5, 95% CI : 1.0-2.3, in men, OR = 2.6, 95% CI : 1.2-5.5, in women) and to solder fumes (OR = 1.5, 95% CI : 1.0-2.4, in men). In females, an excess risk for the task 'soldering, welding, milling' was found (OR = 3.0, 95% CI : 1.1-7.8). Exposure to paints, mineral oils, cutting fluids, benzene, polycyclic aromatic hydrocarbons, and asbestos showed an association with RCC development.
CONCLUSIONS: Our results indicate that substantial exposure to metals and solvents may be nephrocarcinogenic. There is evidence for a gender-specific susceptibility of the kidneys.

B.3.2.11.2.2. **Study description and comment.**

This multicenter study of RCC and bladder cancer and in Germany, which included the Arnsberg region plus four others, identified two case series from participating hospitals, 1,035 urothelial cancer cases and 935 RCC cases with a single population control series matched to cases by region, sex, and age (1:2 matching ratio to urothelial cancer cases and 1:4 matching ratio to RCC cases). A strength of the study was the high percentage of interviews with RCC cases within 2 months of diagnosis (88.5%), reducing bias associated with proxy or next-of-kin interview, and few cases diagnoses confirmed by sonography only (5%). In all, 935 (570 males, 365 females) RCC cases were interviewed face-to-face with a structured questionnaire.

Two general JEMs, British and German, were used to assign exposures based on subjects' job histories reported in an interview. Researchers also asked about job tasks associated with exposure, such as metal degreasing and cleaning, and use of specific agents (organic solvents chlorinated solvents, including specific questions about carbon tetrachloride, TCE, and tetrachloroethylene) to evaluate TCE potential using a JTEM. A category of "any use of a solvent" mixes the large number with infrequent slight contact with the few noted earlier who have high intensity and prolonged contact. Analyses examining TCE exposure using either

the JEM of JTEM assigned a cumulative TCE exposure index of none to low, medium high and substantial, defined as the product of exposure probability x intensity x duration with the following cutpoints: none to low, <30th percentile of cumulative exposure scores; medium, 30th–<60th percentile; high, 60th–<90th percentile; and, substantial, ≥90th percentile. The use of the German JEM identified approximately twice as many cases with any potential TCE exposure (42%) compared to the JTEM (17%) and, in both cases, few cases identified with substantial exposure, 6% by JEM and 3% by JTEM. Pesch et al. (2000b) noted "exposure indices derived from an expert rating of job tasks can have a higher agent-specificity than indices derived from job titles." For this reason, the JTEM approach with consideration of job tasks is considered as a more robust exposure metric for examining TCE exposure and RCC due to likely reduced potential for exposure misclassification compared to TCE assignment using only job history and title.

While this case-control study includes the Arnsberg area, several other regions are included as well, where the source of the TCE and chlorinated solvent exposures are much less well defined. Few cases were identified as having substantial exposure to TCE and, as a result, most subjects identified as exposed to TCE probably had minimal contact, averaging concentrations of about 10 ppm or less (NRC, 2006).

Pesch B, Haerting J, Ranft U, Klimpet A, Oelschägel, Schill W, and the MURC Study Group. (2000b). Occupational risk factors for renal cell carcinoma: agent-specific results from a case-control study in Germany. Int J Epidemiol 29:1014–1024.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This case-control study was conducted to estimate RCC risk for exposure to occupational-related agents; chlorinated solvents including TCE were identified as exposures of a priori interest.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	935 RCC cases were identified from hospitals in a five-region area in Germany between 1991 and 1995. Cases were confirmed histologically (95%) or by sonography (5%) and selected without age restriction. 4,298 population controls identified from local residency registries in the five-region area were frequency matched to cases by region, sex, and age. Participation rate: cases, 88%; controls, 71%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	N/A
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	A trained interviewer interviewed subjects using a structured questionnaire which covered occupational history and job title for all jobs held longer than 1 yr, medical history, and personal information. Two general JEMs, British and German, were used to assign exposures based on subjects' job histories reported in an interview. Researchers also asked about job tasks associated with exposure, such as metal degreasing and cleaning, and use of specific agents (organic solvents chlorinated solvents, including specific questions about carbon tetrachloride, TCE, and tetrachloroethylene) and chemical-specific exposure were assigned using a JTEM. Exposure index for each subject is the sum over all jobs of duration × probability × intensity. A four category grouping was used in statistical analyses defined by exposure index distribution of controls: no-low; medium, 30^{th} percentile; high, 60^{th} percentile; substantial, 90^{th} percentile.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Interviewers carried out face-to-face interview with all cases and controls. All cases were interviewed in the hospital; 88.5% of cases were interviewed within 2 months after diagnosis. All controls had home interviews.
Blinded interviewers	No, by nature of interview location.

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	JEM: 391 cases with TCE exposure index of medium or higher (42% exposure prevalence among cases). JTEM: 172 cases with TCE exposure index of medium or higher (18% exposure prevalence among cases). No information is presented in paper on control exposure prevalence.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, study center, and smoking.
Statistical methods	Conditional logistic regression.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes.

B.3.2.11.3. Vamvakas et al. (1998).

B.3.2.11.3.1. Author's abstract.

> A previous cohort-study in a cardboard factory demonstrated that high and prolonged occupational exposure to trichloroethene (C2HCl3) is associated with an increased incidence of renal cell cancer. The present hospital-based case/control study investigates occupational exposure in 58 patients with renal cell cancer with special emphasis on C2HCl3 and the structurally and toxicologically closely related compound tetrachloroethene (C2Cl4). A group of 84 patients from the accident wards of three general hospitals in the same area served as controls. Of the 58 cases, 19 had histories of occupational C2HCl3 exposure for at least 2 years and none had been exposed to C2Cl4; of the 84 controls, 5 had been occupationally exposed to C2HCl3 and 2 to C2Cl4. After adjustment for other risk factors, such as age, obesity, high blood pressure, smoking and chronic intake of diuretics, the study demonstrates an association of renal cell cancer with long-term exposure to C2HCl3 (odds ratio 10.80; 95% CI: 3.36-34.75).

B.3.2.11.3.2. Study description and comment.

In a follow-up to Henschler et al. (1995) (discussed below), a case-control study was conducted in the Arnsberg region of Germany where there has long been a high prevalence of small enterprises manufacturing small metal parts and goods, such as nuts, lamps, screws, and bolts. Both cases and controls were identified from hospital records; cases from of a large regional hospital in North Rhine Wetphalia during the period 1987 and 1992 and controls who were admitted to accident wards during 1993 at three other regional hospitals. Control selection was carried out independent of cases demographic risk factors (i.e., controls were not matched to cases). Controls may not be fully representative of the case series (NRC, 2006); they were selected from a time period after case selection, which may introduce bias if TCE use changes over time resulted in decreased potential for exposure among controls, and use of accident ward patients may be representative of the target population.

Exposures to TCE resulted from dipping metal pieces into vats, with room temperatures up to 60°C, and placing the wet parts on tables to dry. Some work rooms were noted to be small and poorly ventilated. These conditions are likely to result in high inhalation exposure to TCE (100–500 ppm). Cherrie et al. (2001) estimated the long-term exposures to be approximately 100 ppm. Some of the cases included in this study were also pending legal compensation. As a result, there had been considerable investigation of the exposure situation by occupational hygienists from the Employer's Liability Insurance Association and occupational physicians, including walk-through visits and interviews of long-term employees. The legal action could introduce a bias, a tendency to overreport some of the subjective reports by the subjects. However, the objective working conditions were assessed by knowledgeable professionals, who

corroborated the presence of the poorly controlled hot dip tanks, extensive use of TCE for all types of cleaning, and the process descriptions.

NRC (2006) discussed a number of criticisms in the literature on Vamvakas et al. (1998) by Green and Lash (1999), Cherrie et al. (2001), and Mandel (2001) and noted the direction of possible bias would be positive or negative depending on the specific criticism. Overall, cases in this study substantial, sustained exposures to high concentrations of TCE at 400–600 ppm during hot dip cleaning and >100 ppm overall and observations can inform hazard identification although the magnitude of observed association is uncertain give possible biases.

Vamvakas S, Brüning T, Thomasson B, Lammert M, Baumüller A, Bolt HM, Dekant W, Birner G, Henschler D, Ulm K. (1998). Renal cell cancer risk and occupational exposure to trichloroethylene: results of a consecutive case-control study in Arnsberg, Germany. Am J Ind Med 23:274–285.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes. From introduction—study aim was designed to investigate further the role of occupation exposure to TCE/perchloroethylene in the formation of renal cancer.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	73 RCC cases that had undergone nephrectomy between December 1987 and May 1992 from a hospital urology department in Arnsberg, Germany were contacted by mail; 58 of the recruited cases were. 112 controls identified from accident wards of three area hospitals were interviewed during 1993. Controls underwent abdominal sonography to exclude kidney cancer. 62 of 73 (85%) cases and 84 of 112 (75%) of controls participated in study.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	N/A
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Face-to-face interview with subjects or, if deceased, with their next of kin or former colleagues using a structured questionnaire with questions to obtain information on job tasks with selected exposure to specific agents and to self-reported selected exposures. A supplemental questionnaire on job conditions was administered to subjects reporting exposure to TCE and perchloroethylene. Subjects with TCE exposures were primarily exposed through degreasing operations in small businesses. Self-reported TCE exposure was ranked using a semiquantitative scale based upon total exposure time and frequency/duration of self-reported acute prenarcotic symptoms. **Cherrie et al. (2001) estimated that the machine cleaning exposures to TCE were ~400–600 ppm, with long-term average TCE exposure as ~100 ppm.**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Personal physicians interviewed 100% of cases or their NOK/former colleague and 100% controls.
Blinded interviewers	Interviewers were not blinded nor was developments of exposure assessment semiquantitative scale.
CATEGORY F: PROXY RESPONDENTS	

>10% proxy respondents	No information provided in paper on number of cases with NOK interviews or interviews with former colleagues; all controls were alive and interviewed by their personal physician.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	19 cases with TCE or perchloroethylene exposure (33% exposure prevalence) and 1 control with perchloroethylene exposure.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, obesity, high blood pressure, smoking, and diuretic use.
Statistical methods	Mantel-Haenszel χ^2.
Exposure-response analysis presented in published paper	Yes, semiquantitative scale of 4 categories (no, +, ++, +++).
Documentation of results	No information on number of eligible controls or number interviews with case NOK or former colleagues.

B.3.2.12. RCC Case-Control Studies—Arve Valley Region of France

A case-control study was conducted in the Arve Valley to examine the a priori hypothesis of an association with RCC and TCE exposure. The Arve Valley, like the Arnsburg Region in Germany, has a long history of TCE use in the screw-cutting industry. The Arve Valley, situated in the Rhône-Alpes region of eastern France is a major metalworking sector with around 800 small and medium-sized firms specializing in "screw-cutting" or the machining of small mechanical parts from bars, in small, medium, and large series on conventional automatic lathes or by digital control. This industry evolved around the time of World War I from the region's expertise in clock-making. A major point of this study is that it was designed as a follow-up study to the German renal cell cancer case-control studies but in a different population with similar exposure patterns and with high prevalence of exposure to TCE. For this reason, there is considerable detail on the nature of exposure, which made it possible to estimate the order of magnitude of exposure, even though there were not direct measurements.

B.3.2.12.1. Charbotel et al.(2009), Charbotel et al. (2007) Charbotel et al. (2006).

B.3.2.12.1.1. Charbotel et al. (2009) abstract.

Abstract Background– Several studies have investigated the association between trichloroethylene (TCE) exposure and renal cell cancer (RCC) but findings were inconsistent. The analysis of a case control study has shown an increased risk of RCC among subjects exposed to high cumulative exposure. The aim of this complementary analysis is to assess the relevance of current exposure limits regarding a potential carcinogenic effect of TCE on kidney.
Methods– Eighty-six cases and 316 controls matched for age and gender were included in the study. Successive jobs and working circumstances were described using a detailed occupational questionnaire. An average level of exposure to TCE was attributed to each job period in turn. The main occupational exposures described in the literature as increasing the risk of RCC were assessed as well as non-occupational factors. A conditional logistic regression was performed to test the association between TCE and RCC risk. Three exposure levels were studied (average exposure during the eight-hour shift): 35 ppm, 50 ppm and 75 ppm. Potential confounding factors identified were taken into account at the threshold limit of 10% (p = 0.10) (body mass index [BMI], tobacco smoking, occupational exposures to cutting fluids and to other oils).
Results– Adjusted for tobacco smoking and BMI, the odd-ratios associated with exposure to TCE were respectively 1.62 [0.77–3.42], 2.80 [1.12–7.03] and 2.92 [0.85–10.09] at the thresholds of 35 ppm, 50 ppm and 75 ppm. Among subjects exposed to cutting fluids and TCE over 50 ppm, the OR adjusted for BMI, tobacco smoking and exposure to other oils was 2.70 [1.02–7.17].
Conclusion– Results from the present study as well as those provided in the international literature suggest that current French occupational exposure limits for TCE are too high regarding a possible risk of RCC.

B.3.2.12.1.2. **Charbotel et al. (2007) abstract.**

Background: We investigated the association between exposure to trichloroethylene (TCE) and mutations in the von Hippel-Lindau (VHL) gene and the subsequent risk for renal cell carcinoma (RCC).
Methods: Cases were recruited from a case-control study previously carried out in France that suggested an association between exposures to high levels of TCE and increased risk of RCC. From 87 cases of RCC recruited for the epidemiological study, 69 were included in the present study. All samples were evaluated by a pathologist in order to identify the histological subtype and then be able to focus on clear cell RCC. The majority of the tumor samples were fixed either in formalin or Bouin's solutions. The majority of the tumors were of the clear cell RCC subtype (48 including 2 cystic RCC). Mutation screening of the 3 VHL coding exons was carried out. A descriptive analysis was performed to compare exposed and non exposed cases of clear cell RCC in terms of prevalence of mutations in both groups.
Results: In the 48 cases of RCC, four VHL mutations were detected: within exon 1 (c.332G>A, p.Ser111Asn), at the exon 2 splice site (c.463+1G>C and c.463+2T>C) and within exon 3 (c.506T>C, p.Leu169Pro). No difference was observed regarding the frequency of mutations in exposed vs. unexposed groups: among the clear cell RCC, 25 had been exposed to TCE and 23 had no history of occupational exposure to TCE. Two patients with a mutation were identified in each group.
Conclusion: This study does not confirm the association between the number and type of VHL gene mutations and exposure to TCE previously described.

B.3.2.12.1.3. **Charbotel et al. (2006) abstract.**

Background: We investigated the association between exposure to trichloroethylene (TCE) and mutations in the von Hippel-Lindau (VHL) gene and the subsequent risk for renal cell carcinoma (RCC).
Methods: Cases were recruited from a case-control study previously carried out in France that suggested an association between exposures to high levels of TCE and increased risk of RCC. From 87 cases of RCC recruited for the epidemiological study, 69 were included in the present study. All samples were evaluated by a pathologist in order to identify the histological subtype and then be able to focus on clear cell RCC. The majority of the tumor samples were fixed either in formalin or Bouin’s solutions. The majority of the tumors were of the clear cell RCC subtype (48 including 2 cystic RCC). Mutation screening of the 3 VHL coding exons was carried out. A descriptive analysis was performed to compare exposed and non-exposed cases of clear cell RCC in terms of prevalence of mutations in both groups.
Results: In the 48 cases of RCC, four VHL mutations were detected: within exon 1 (c.332G>A, p.Ser111Asn), at the exon 2 splice site (c.463+1G>C and c.463+2T>C) and within exon 3 (c.506T>C, p.Leu169Pro). No difference was observed regarding the frequency of mutations in exposed vs. unexposed groups: among the clear cell RCC, 25 had been exposed to TCE and 23 had no history of

occupational exposure to TCE. Two patients with a mutation were identified in each group.
Conclusion: This study does not confirm the association between the number and type of VHL gene mutations and exposure to TCE previously described.

To test the effect of the exposure to trichloroethylene (TCE) on renal cell cancer (RCC) risk, a case–control study was performed in the Arve Valley (France), a geographic area with a high frequency and a high degree of such exposure. Cases and controls were selected from various sources: local general practitioners and urologists practicing in the area and physicians (urologists and oncologists) from other hospitals of the region who might treat patients from this area. Blinded telephone interviews with cases and controls were administered by a single trained interviewer using occupational and medical questionnaires. The analysis concerned 86 cases and 316 controls matched for age and gender. Three approaches were developed to assess the link between TCE exposure and RCC: exposure to TCE for at least one job period (minimum 1 year), cumulative dose number of ppm of TCE per job period multiplied by the number of years in the job period) and the effect of exposure to peaks. Multivariate analysis was performed taking into account potential confounding factors. Allowing for tobacco smoking and Body Mass Index, a significantly 2-fold increased risk was identified for high cumulative doses: odds ratio (OR) = 2.16 (1.02–4.60). A dose-response relationship was identified, as was a peak effect; the adjusted OR for highest class of exposure-plus-peak being 2.73 (1.06–7.07). After adjusting for exposure to cutting fluids the ORs, although still high, were not significant because of lack of power. This study suggests an association between exposures to high levels of TCE and increased risk of RCC. Further epidemiological studies are necessary to analyze the effect of lower levels of exposure.

B.3.2.12.1.4. **Study description and comment.**

Cases in the population-based, case-control study were obtained retrospectively from regional medical practitioners or from teaching hospitals from 1993 to 2002, and prospectively from 2002 to mid-2003. One case was excluded from analysis because it was not possible to find a control subject. Controls were either selected from the same urology practice as cases or, for cases selected from teaching hospitals, from among patients of the case's general practitioner. Telephone interviews of 87 RCC cases and 316 controls matched for age and sex by a trained interviewer were used to obtain information on occupational and medical history for the case-control analysis of Charbotel et al. (2006). Of the 87 RCC cases, 67 cases provided consent for mutational analysis of which 48 cases were diagnosed with clear cell RCC, suitable for mutational analysis of the *VHL* gene (Charbotel et al., 2007). Tissue samples were paraffin-embedded or frozen tissues and ability to fully sequence the *VHL* gene depended on type of the fixative procedure; only 26 clear cell RCC cases (34% of 73 clear cell RCC cases in the case-control study) could full sequencing of the *VHL* gene occur.

Two occupational questionnaires were administered to both cases and controls, a questionnaire developed specifically to evaluate jobs and exposure potential in the screw-cutting

industry and a more general one for any other jobs. Interviewers were essentially blinded to subject status as case or control for the occupational questionnaires given the medical questionnaire was administered afterwards (Fevotte et al., 2006). The medical questionnaire included familial kidney disease and medical history, BMI, and history of smoking. A task/TCE-Exposure Matrix was designed using information obtained from questionnaires and routine atmospheric monitoring of workshops or biological monitoring (U-TCA) of workers carried out since the 1960s. Questionnaires were used to elicit from each subject the main tasks associated with each job, working conditions, activities, or jobs that might involve TCE exposures and possible exposure to other occupational risk factors for RCC.

The JEM linked to corresponding TCE-exposure levels using available industrial hygiene monitoring data on atmospheric TCE levels and from biological measurement on workers. Estimates reflected task duration, use of protective equipment, and distance from TCE source, as well, as both dermal and inhalation exposure routes. Estimated TCE intensities for jobs associated with open cold degreasing were 15–18 ppm, 120 ppm for jobs working near open hot degreasing machines, with up to 300 ppm for work directly above tank and for job and intensities of 300–600 ppm for emptying, cleaning, and refilling degreasers. Eight local physicians with knowledge of working conditions corroborated the working conditions for individual job periods after 1980 in screw-cutting shops. Overall, there was good agreement (72%) between physician and the JEM. Three exposure surrogates were assigned to each case and control: TWA exposure (Charbotel et al., 2009), cumulative exposure (Charbotel et al., 2006), and cumulative exposure with and without peak exposure (Charbotel et al., 2006).

An 8-hour TWA exposure concentration was developed for each job period from 1924 to 2003 and was the product of the task-specific estimated TCE intensity and duration of task. A subject's lifetime 8-hour TWA was the sum of each job period specific estimated TWA. Exposure peak, daily exposure reaching ≥200 ppm for at least 15 minutes, was assessed as an additive factor and was defined by frequency (seldom exposed, few times yearly to frequently exposure, few time weekly).

Over the study period, 19% (295 of 1,486) job periods were assessed as having TCE exposure with an 8-hour TWA of <35 ppm for 72% of exposed jobs and >75 ppm for 5% of exposed jobs. Exposure prevalence to TCE peaked in the 1970s with roughly 20% of job periods with TCE exposure and 8% of subjects identified with >75 ppm. By the 1990s, exposure prevalence had not only decreased to 7% but also exposure intensity, only 5% of job periods with >75 ppm.

Cumulative TCE exposure was the sum of 8-hour TWAs overall job periods with statistical analysis using four categories: no, low, medium, and high. These were defined as low, 5–150 ppm-years; medium, 155–335 ppm-year; and high, >335 ppm-years (HSIA, 2005). Analyses were also carried out examining peak exposure, classified as yes/no and without

assignment of quantitative level, as additional exposure to average TCE concentration; 33 subjects were exposed to peaks and very few to high peaks.

The high exposure prevalence and strong approach for exposure assessment provides Charbotel et al. (2009; 2006) more statistical power and ability to assess association of RCC and TCE exposure. However, the low participation rate, inability to fully sequence the *VHL* gene in all clear cell RCC cases, the lower background prevalence of mutations (15% in this study compared to roughly 50% in other series) in Charbotel et al. (2007) suggest a relative insensitivity of assay used and lack of a positive control limits the mutational analysis. These methodological limitations introduce bias with greater uncertainties for evaluating consistency of findings with somatic *VHL* mutations observed in other TCE-exposed RCC cases (Brauch et al., 1999; Brüning et al., 1997b). TCE exposure prevalence (>5 ppm-year) in Charbotel et al. (2006) was 43% among cases and is higher than that observed in other population-based case-control studies of RCC and TCE (e.g., Pesch et al., 2000a). While some subjects had jobs with exposures to high concentrations of TCE during the 1970s and 1980s, a large percentage of jobs were to TCE concentrations of <35 ppm (8-hour TWA). Jobs with high TCE concentrations also were identified as having frequent exposure to peak TCE concentrations, particularly before 1980. Peak TCE estimates in this study were judged to be lower than those in German studies of the Arnsberg region (Vamvakas et al., 1998; Henschler et al., 1995) but higher than those of Hill Air Force Base civilian workers (Blair et al., 1998; Stewart et al., 1991) due to a lower frequency of degreasing tasks in Blair et al. (1998) cohort and to slower technological changes in degreasing process in the French case-control study (Fevotte et al., 2006).

Charbotel B, Fevotte J, martin JL, Bergeret A. (2009). Cancer du rein et expositions au trichloroethylene: les valeurs limites d'exposition professionnelle fraçaises en vigueur sont-elles adaptées. Rev Epidemiol Sante Publique 57:41–47.

Charbotel B, Fevotte J, Hours M, Martin J-L, Bergeret A. (2006). Case-control study on renal cell cancer and occupational exposure to trichloroethylene. Part II: Epidemiological Aspects. Ann Occup Hyg 50:777–787.

Fevotte J, Charbotel B, Muller-Beaute P, Martin J-L, Hours, Bergeret A. (2006). Case-control study on renal cell cancer and occupational exposure to trichloroethylene. Part I: Exposure assessment. Ann Occup Hyg 50:765–775.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes. From abstract—study aim was to "test the effect of TCE exposure on renal cell cancer."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	117 cases of RCC patients were identified retrospectively from 1993 to June 2002, and prospectively from June 2002 to June 2003 from patients of urology practices and hospital urology and oncology departments in the region of Arve Valley, France. 404 controls were identified from the same urology practice or from the same general practitioner, for cases identified from hospital records and matched on residency in the geographic study area at time of case diagnosis, sex, and year of birth. Controls sought medical treatment for conditions other than kidney or bladder cancer. Case definition included clear cell and other subtypes of RCC including chromophil, chromophobe and collecting duct carcinomas. 87 or 117 (74%) cases and 316 of 404 (78%) controls participated in study.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	N/A

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Occupational questionnaires sought information for each study subject a complete job history and was followed-up with either a questionnaire specific for jobs and exposures in the screw-cutting industry or a General Occupational Questionnaire, whichever was more applicable to subject. Questionnaires also sought self-reported information on potential TCE exposures. A medical questionnaire seeking information on medical history and familial kidney disease was administered after occupational questionnaires. Jobs titles were coded according to standardized classification of occupations and 1,486 job periods grouped into three categories (screw-cutting, nonscrew-cutting but job with possible TCE exposure, and no TCE exposure). An estimated 8-hr TWA was assigned to each job and job period using a JTEM. RCC and TCE was examined using three exposure approaches: exposure to at least 5 ppm for at least one job period (minimum 1 yr), cumulative dose or $\sum$ (TCE ppm per job × years) using quantitative ranking levels (no exposure, low, medium, and high), and potential for peak defined as any exposure 200+ ppm. **TCE concentrations associated with quantitative ranking are low, 5–150 ppm-yrs; medium, 155–335 ppm-yrs; high, >335 ppm-yrs.**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Telephone interviews were conducted by a trained interviewer.
Blinded interviewers	The paper notes interviewers were blinded “as far as possible” since medical questionnaire was administered after the occupational questionnaires.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 22% of cases were dead at time of interview compared to 7% of controls.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	37 cases with TCE exposure (43% exposure prevalence), 110 controls with TCE exposure (35% exposure prevalence). 16 cases with high level confidence TCE exposure (27% exposure prevalence), 37 controls with high level confidence TCE exposure (16%).

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, tobacco smoking, and BMI (Charbotel et al., 2006). Age, sex tobacco smoking, BMI, and exposure to cutting or petroleum oils (Charbotel et al., 2009).
Statistical methods	Conditional logistic regression on matched pairs.
Exposure-response analysis presented in published paper	Yes, cumulative exposure as four categories (no, low, medium and high exposure) and cumulative exposure plus peaks.
Documentation of results	Yes.

B.3.2.13. RCC Case-Control Studies in Other Regions

B.3.2.13.1. Moore et al. (2010)

B.3.2.13.1.1. Author's abstract.

Trichloroethylene (TCE) is a suspected renal carcinogen. TCE-associated renal genotoxicity occurs predominantly through glutathione S-transferase (GST) conjugation and bioactivation by renal cysteine beta-lyase (CCBL1). We conducted a case-control study in Central Europe (1,097 cases and 1,476 controls) specifically designed to assess risk associated with occupational exposure to TCE through analysis of detailed job histories. All jobs were coded for organic/chlorinated solvent and TCE exposure (ever/never) as well as the frequency and intensity of exposure based on detailed occupational questionnaires, specialized questionnaires, and expert assessments. Increased risk was observed among subjects ever TCE exposed [odds ratio (OR) = 1.63; 95% confidence interval (95% CI), 1.04-2.54]. Exposure-response trends were observed among subjects above and below the median exposure [average intensity (OR = 1.38; 95% CI, 0.81-2.35; OR = 2.34; 95% CI, 1.05-5.21; P(trend) = 0.02)]. A significant association was found among TCE-exposed subjects with at least one intact GSTT1 allele (active genotype; OR = 1.88; 95% CI, 1.06-3.33) but not among subjects with two deleted alleles (null genotype; OR = 0.93; 95% CI, 0.35-2.44; P(interaction) = 0.18). Similar associations for all exposure metrics including average intensity were observed among GSTT1-active subjects (OR = 1.56; 95% CI, 0.79-3.10; OR = 2.77; 95% CI, 1.01-7.58; P(trend) = 0.02) but not among GSTT1 nulls (OR = 0.81; 95% CI, 0.24-2.72; OR = 1.16; 95% CI, 0.27-5.04; P(trend) = 1.00; P(interaction) = 0.34). Further evidence of heterogeneity was seen among TCE-exposed subjects with >or=1 minor allele of several CCBL1-tagging single nucleotide polymorphisms: rs2293968, rs2280841, rs2259043, and rs941960. These findings provide the strongest evidence to date that TCE exposure is associated with increased renal cancer risk, particularly among individuals carrying polymorphisms in genes that are important in the reductive metabolism of this chemical, and provides biological plausibility of the association in humans.

B.3.2.13.1.2. Study description and comment.

The hospital case-control study of kidney cancer in men and women who were residents in areas of the sevens study centers evaluated nonoccupational and occupational risk factors and included a detailed exposure assessment for chlorinated organic solvents, including TCE. Histologically-confirmed incident cases of RCC (ICD-O-2, Code C.64) between 20 and 79 years of age and diagnosed between 1999 and 2003 at seven participating hospitals were eligible as cases, with hospital in-patient or out-patient controls admitted to the same hospital centers but with non-tobacco-related conditions, excluding genitourinary cancers, and frequency-matched to cases by sex and age, and by study center. The final study population included 1,097 cases and 1,476 controls for a participation rate, depending on study center of 90–98% and 90–96% for cases and controls, respectively. As part of the study, blood samples obtained from 925 cases

and 1,192 controls were assayed for deletion of the GSTT1 polymorphism and genetic variation across the renal cysteine β-lyase (CCBL1) gene.

Face-to-face interviews were conducted using standard questionnaires that asked about lifestyle habits and personal, familial medical history, and for each job held ≥1 year. For specific jobs or industries with likely exposure to know or suspected occupational carcinogens of interest, a specialized occupation questionnaires were used to gather more detailed information. For every job in a subject's work history, an exposure assessment team from each center, with extensive knowledge of industries in the region and blinded to case or control status, evaluated the frequency and intensity of exposure to organic and chlorinated solvents based on the general and job-specific questionnaires. The general category of aliphatic chlorinate organic solvents included perchloroethylene, methylene chloride, carbon tetrachloride, 1, 1, 1-trichloroethane, and TCE. Subjects identified as exposed to organic solvents were reevaluated by the team at a later date to confirm assignment as an attempt to reduce exposure misclassification. The reevaluation was performed blinded to case and controls status. For each exposed job, the frequency, intensity, and confidence of exposure to TCE, organic solvents, and chlorinated solvents. While TCE exposure was correlated with both chlorinated solvents and organic solvents exposure, it was not associated with other co-exposures. Exposure frequency was coded into three categories, representing the average percentage of a working day exposure was likely (1–4.9, 5–30, >30%), with midpoint weights for cumulative exposure calculations of 0.025, 0.175, and 0.50, respectively, and assuming a log-normal exposure distribution. TCE intensity was also coded into three categories (0–<5, 5–50, >50 ppm) with midpoint weights for cumulative exposure calculations of 2.5, 25, and 75 ppm, respectively. Exposure surrogates developed included cumulative exposure, the product of the midpoints for intensity and frequency and multiplied by duration. Average exposure intensity was a second exposure surrogate and defined as the quotient of cumulative exposure and duration. Last, confidence of exposure that represented the expected percentage of workers that would be exposed in that job was categorized as possible (<40%), probable (40–89%), or definite (≥90%). Among subjects with probable exposure (high confidence TCE exposure), the median intensity score was 0.076 ppm [25th and 75th percentile range among cases, 0.83–7.25 ppm] and median cumulative exposure scores were 1.58 (25th and 75th percentiles, 0.77–2.87 ppm-year) and 1.95 ppm-years (25th and 75th percentiles, 0.83–7.25 ppm-year) among cases and controls, respectively.

Association between RCC and organic solvents, chlorinated solvents, and TCE exposure for jobs with any confidence level and for holding a job with probable or definite exposure was assessed using unconditional logistic regression to estimate ORs and 95% CIs. All statistical models included covariates for sex, age, and study center. Analyses were also modeled to account for a 20-year lag. Almost all TCE exposure occurred at least 20 years before RCC onset and Moore et al. (2010) did not report these findings as OR estimates were similar to those from the models using unlagged exposure surrogate.

The strong exposure approach in Moore et al. (2010) and examination of exposure probability or confidence are strengths of the study. TCE used did not appear widespread as exposure prevalence was low, 6 % of cases had held a job of any exposure probability, compared to 29% of cases identified with any exposure to organic solvents. The percentage of cases was even lower, 4%, for higher confidence TCE exposure. Additionally, evaluation of GST polymorphisms provides assessment of susceptibility factors.

Moore LE, Buffetta P, Karami S, Brennan P, Stewart PS, Hung R, et al. (2010). Occupational trichloroethylene exposure and renal carcinoma risk: Evidence of genetic susceptibility by reductive metabolism gene variants. Cancer Res 20:6527–6536.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Study hypotheses of investigating risk association with occupation TCE exposure and kidney (excluding pelvis) cancers through analysis of job histories and use of detailed exposure assessment method.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cases: 1,097 histologically-confirmed RCC cases in males and females, 20–79 yrs of age, 1999–2003, identified through seven hospital centers in four countries (Czech Republic, Poland, Romania, Russia). Controls: 1,476 in-patient or out-patient hospital controls admitted to same hospital as case with nontobacco-related conditions and frequency matched to cases by sex and age, and by study center.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	RCC incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-0-2 [Codes C.54].
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Job-specific questionnaire for job ≥1 year. Exposure assessment team from each center with knowledge of region's industries to assess frequency, intensity and confidence of exposure to TCE and organic solvent group (perchloroethylene, methylene chloride, carbon tetrachloride, and 1,1,1-trichloroethane). Exposure surrogates of frequency (three categories based on percentage of day), intensity (three groups), cumulative exposure (product of intensity, duration, frequency), and average exposure intensity (cumulative exposure score divided by the number of years exposed). Exposure confidence score (possible, probably, definite) defined as percentage of workers exposed at a job.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	In-person interview using questionnaire.
Blinded interviewers	No information in published paper if interviewers were blinded. Exposure assessment assigned blinded.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No proxy interviews.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Cases: 90–99% participation rate; Controls: 90–96% participation rate. Exposure prevalence, ever exposed to TCE (6% of cases holding TCE job, any confidence level; 4% of cases with probable or definite exposure).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and center. Place of residence, tobacco smoking, BMI, and hypertension also examined but did not alder OR estimate by >10%, and thus, were not included in final models.
Statistical methods	Unconditional logistic regression.
Exposure-response analysis presented in published paper	Test for trend reported for years, hours, cumulative and average intensity of exposure.
Documentation of results	Yes, study was well documented with supplemental material available on publisher's webpage.

B.3.2.13.2. **Parent et al. (2000a), Siemiatycki (1991).**

B.3.2.13.2.1. **Author's abstract.**

BACKGROUND: Little is known about the role of workplace exposures on the risk of renal cell cancer. METHODS: A population-based case-control study was undertaken in Montreal to assess the association between hundreds of occupational circumstances and several cancer sites, including the kidney. A total of 142 male patients with pathologically confirmed renal cell carcinoma, 1900 controls with cancer at other sites and 533 population-based controls were interviewed. Detailed job histories and relevant data on potential confounders were obtained. A group of chemists-hygienists evaluated each job reported and translated them into a history of occupational exposures using a checklist of 294 substances. Multivariate logistic regression models using either population, cancer controls, or a pool of both groups were used to estimate odds ratios. RESULTS: There were some indications of excess risks among printers, nursery workers (gardening), aircraft mechanics, farmers, and horticulturists, as well as in the following industries: printing-related services, defense services, wholesale trade, and retail trade. Notwithstanding the low precision of many of the odds ratio estimates, the following workplace exposures showed some evidence of excess risk: chromium compounds, chromium (VI) compounds, inorganic acid solutions, styrene-butadiene rubber, ozone, hydrogen sulphide, ultraviolet radiation, hair dust, felt dust, jet fuel engine emissions, jet fuel, aviation gasoline, phosphoric acid and inks. CONCLUSIONS: For most of these associations there exist no, or very little, previous data. Some associations provide suggestive evidence for further studies.

B.3.2.13.2.2. **Study description and comment.**

This population case-control study of histologically-confirmed kidney cancer among males who resided in the Montreal Metropolitan area relies on the use of expert assessment of occupational information on a detailed questionnaire and face-to-face interview and was part of a larger study of 10 other site-specific cancers and occupational exposures (Parent et al., 2000a; Siemiatycki, 1991). Interviewers were unblinded, although exposure assignment was carried out blinded as to case and control status. The questionnaire sought information on the subject's complete job history and included questions about the specific job of the employee and work environment. Occupations considered with possible TCE exposure included machinists, aircraft mechanics, and industrial equipment mechanics. An additional specialized questionnaire was developed for certain job title of a prior interest that sought more detailed information on tasks and possible exposures. For example, the supplemental questionnaire for machinists included a question on TCE usage. A team of industrial hygienists and chemicals assigned exposures blinded based on job title and other information obtained by questionnaire. A semiquantitative scale was developed for 300 exposures and included TCE (any, substantial). Parent et al. (2000a) presents observations of analyses examining job title, occupation, and some chemical-

specific exposures, but not TCE. Observations on TCE are found in the original report of Siemiatycki (1991). Any exposure to TCE was 3% among cases but <1% for substantial TCE exposure; "substantial" is defined as >10 years of exposure for the period up to 5 years before diagnosis. The TCE exposure frequencies in this study are lower than those in Brüning et al. (2003) and Charbotel et al. (2006), studies conducted in geographical areas with a high prevalence of industries using TCE. The expert assessment method is considered a valid and reliable approach for assessing occupational exposure in community-base studies and likely less biased from exposure misclassification than exposure assessment based solely on self-reported information (Fritschi et al., 2003; IOM, 2003; Siemiatycki et al., 1987). For example, Dewar et al. (1994) examine sensitivity of JEM of Siemiatycki et al. (1987) to exposure assessment by chemists and industrial hygienists using interview information and evaluation of job histories. Specific solvents are not examined, although, a sensitive 84% and specificity of 97% was found for the JEM for general solvent exposure.

This population study of several cancer sites included histologically-confirmed cases of kidney cancer (ICD-O 189, malignant neoplasm of kidney and other and unspecified urinary organs) ascertained from 16 Montreal-area hospitals between 1979 and 1985. A total of 227 eligible kidney cancer cases were identified were identified from 19 Montreal-area hospitals; 177 cases participated in the study (78% response). One control group (n = 1,295) consisted of patients with other forms of cancer (excluding lung cancer and other intestinal cancers) recruited through the same study procedures and time period as the rectal cancer cases. A population-based control group (n = 533), frequency matched by age strata, was drawn using electoral lists and random digit dialing. All controls were interviewed using face-to-face methods; however, 20% of the all cancer cases in the larger study were either too ill to interview or had died and, for these cases, occupational information was provided by a proxy respondent. The quality of interview conducted with proxy respondents was much lower, increasing the potential for misclassification bias, than that with the subject. The direction of this bias would diminish observed risk towards the null.

Statistical analysis are considered valid; logistic regression model, which included terms for respondent status, age, smoking, and BMI in Parent et al. (2000a) and Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, and ethnic origin in Siemiatycki (1991). Odds ratios are presented with 90% CIs in Siemiatycki (1991) and 95% CIs in Parent et al. (2000a).

Overall, exposure assessment in this study adopted a superior approach, using expert knowledge and use of a JEM. However, examination of NHL and TCE exposure is limited by statistical power considerations related to low exposure prevalence, particularly for "substantial" exposure. For the exposure prevalence found in this study to TCE and for kidney cancer, the minimum detectable OR was 3.0 when $\beta = 0.02$ and $\alpha = 0.05$ (one-sided). The low statistical power to detect a doubling of risk and an increased possibility of misclassification bias

associated with case occupational histories resulting from proxy respondents suggests a decreased sensitivity in this study for examining kidney cancer and TCE.

Parent M-E, Hua Y, Siemiatycki J. (2000a). Occupational risk factors for renal cell carcinoma in Montreal. Am J Ind Med 38:609–618.

Siemiatycki J. (1991). Risk Factors for Cancer in the Workplace. Baca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	277 kidney cancer cases were identified among male Montreal residents between 1979 and 1985 of which 177 (147 RCCs) were interviewed. 740 male population controls were identified from the same source population using random digit dialing; 533 were interviewed. A second control series consisted of all other cancer controls excluding lung and bladder cancer cases. Participation rate: cases, 78%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD 189 (malignant neoplasm of the kidney and other and unspecified urinary organs) (Siemiatycki, 1991). ICD 189.0, RCC (Parent et al., 2000a).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of a priori interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 300 exposures, including TCE. For each exposure, a three-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1–5%; medium, >5–30%; and high, >30%).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	100% of cases and controls were interviewed face-to-face by a trained interviewer. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 16% of cases, 13% of population controls, and 22% of cancer controls had proxy respondents.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	177 cases (78% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 2% cases; substantial TCE exposure (exposure for ≥10 yrs and up to 5 yrs before disease onset), 1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, income, index for cigarette smoking (Siemiatycki, 1991). Age, smoking, BMI, and proxy status (Parent et al., 2000b).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Parent et al., 2000a).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.13.3. **Dosemeci et al. (1999).**

B.3.2.13.3.1. **Author's abstract.**

BACKGROUND: Organic solvents have been associated with renal cell cancer; however, the risk by gender and type of solvents is nuclear. METHODS: We evaluated the risk of renal cell carcinoma among men and women exposed to all organic solvents-combined, all chlorinated aliphatic hydrocarbons (CAHC)-combined, and nine individual CAHC using *a priori* job exposure matrices developed by NCI in a population-based case-control study in Minnesota, U.S. We interviewed 438 renal cell cancer cases (273 men and 165 women) and 687 controls (462 men and 225 women). RESULTS: Overall, 34% of male cases and 21% of female cases were exposed to organic solvents in general. The risk of renal cell carcinoma was significantly elevated among women exposed to all organic solvents combined (OR = 2.3; 95% CI = 1.3-4.2), to CAHC combined (OR = 2.1; 95% CI = 1.1-3.9), and to trichloroethylene (TCE) (OR = 2.0; 95% CI = 1.0-4.0). Among men, no significant excess risk was observed among men exposed to any of these nine individual CAHCs, all CAHCs-combined, or all organic solvents-combined. DISCUSSION: These observed gender differences in risk of renal cell carcinoma in relation to exposure to organic solvents may be explained by chance based on small numbers, or by the differences in body fat content, metabolic activity, the rate of elimination of xenobiotics from the body, or by differences in the level of exposure between men and women, even though they have the same job title.

B.3.2.13.3.2. **Study description and comment.**

Dosemeci et al. (1999) reported data from a population-based case-control study of the association between occupation exposures and renal cancer risk. The investigators identified newly diagnosed patients with histologically confirmed RCC from the Minnesota Cancer Surveillance System from July 1, 1988 to December 31, 1990. The study was limited to white cases, and age and gender-stratified controls were ascertained using random digit dialing (for subjects ages 20–64) and from Medicare records (for subjects 65–85 years). Of the 796 cases and 796 controls initially identified, 438 cases (273 men, 165 women) and 687 controls (462 men, 225 women) with complete personal interviews were included in the occupational analysis.

Data were obtained using in-person interviews that included demographic variables, residential history, diet, smoking habits, medical history, and drug use. The occupational history included information about the most recent and usual industry and occupation (coded using the standard industrial and occupation codes, Department of Commerce), job activities, hire and termination dates, and full/part time status. A JEM developed by the NCI (Gómez et al., 1994) was used with the coded job data assign occupational exposure potential for 10 chlorinated aromatic hydrocarbons and organic solvents, and includes TCE.

Dosemeci et al. (1999) adopted logistic regression methods to evaluate renal cancer and occupational exposures. Odds ratios were adjusted for age, smoking, hypertension, and use of drugs for hypertension, and BMI.

Strengths of this study include the use of incident cases of renal cancer from a defined population area, with confirmation of the diagnosis using histology reports. The occupation history was based on usual and most recent job, in combination with a relatively focused JEM. In contrast to the type of exposure assessment that can be conducted in cohort studies within a specific workplace; however, exposure measurements, based on personal or workplace measurement, were not used, and a full lifetime job history was not obtained.

Dosemeci M, Cocco P, Chow W-H. (1999). Gender differences in risk of renal cell carcinoma and occupational exposures to chlorinated aliphatic hydrocarbons. Am J Ind Med 36:54–59.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes. From abstract—study aim was to evaluate effect of organic solvents on RCC risk using a priori JEMs.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	796 white males and females identified through the Minnesota Cancer Surveillance System with histological confirmed RCC between July 1, 1988 and December 31, 1990. Interviews were obtained for 690 subjects, of which 241 were with next-of-kin and excluded; 438 cases (273 males and 165 females) were included in analysis. 707 white population controls identified through random digit dialing, and matched to cases, aged 20–65 yrs old, by age and sex using a stratified random sample or, for cases aged 65–85, from Health Care Financing Administration list. 687 controls (462 males and 225 females) are included in the analysis. Participation rate: cases, 87%; controls, 86%. Occupational analysis: cases, 55%, controls 83%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence
Changes in diagnostic coding systems for lymphoma, particularly NHL	N/A
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	A trained interviewer blinded to case and control status interviewed subjects at home using a questionnaire which covered occupational, residential, and medical histories; demographic information; and personal information. Occupational history included self-reporting of the most recent job and usual occupation and industry, employment dates, and focused on 13 specific occupations or industries. Occupation and industry were coded according to a standard occupational classification or standard industrial classification with potential chemical-specific exposures to TCE and eight other chlorinated hydrocarbons identified using the JEM of Dosemeci et al. (1999) and Gomez et al. (1994).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	All cases and controls had face-to-face interviews.
Blinded interviewers	Yes, interviewers were blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	

>10% proxy respondents	No, subjects with next-of-kin interviews were excluded from the analysis.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	55 cases with TCE exposure (13% exposure prevalence among cases). 69 controls cases with TCE exposure (10% exposure prevalence among controls).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, smoking, BMI, and hypertension/ use of diuretics/use of anti-hypertension drugs.
Statistical methods	Logistic regression.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.14. Other Cancer Site Case-Control Studies

B.3.2.14.1. Siemiatycki (1991), Siemiatycki et al. (1987).

B.3.2.14.1.1. Author's abstract.

A multi-cancer site, multi-factor, case-referent study was undertaken to generate hypotheses about possible occupational carcinogens. About 20 types of cancer were included. Incident cases among men aged 35-70 years and diagnosed in any of the major Montreal hospitals were eligible. Probing interviews were carried out for 3,726 eligible cases. The interview was designed to obtain detailed lifetime job histories and information on potential confounders. Each job history was reviewed by a team of chemists who translated it into a history of occupational exposures. These occupational exposures were then analyzed as potential risk factors in relation to the sites of cancer included. For each site of cancer analyzed, referents were selected from among the other sites in the study. The analysis was carried out in stages. First a Mantel-Haenszel analysis was undertaken of all cancer-substance associations, stratifying on a limited number of covariates, and, then, for those associations which were noteworthy in the initial analysis, a logistic regression analysis was made taking into account all potential confounders. This report describes the fieldwork and analytical methods.

B.3.2.14.1.2. Study description and comment.

Siemiatycki (1991) reported data from a case-control study of occupational exposures and several site-specific cancers, including lung and pancreas, conducted in Montreal, Quebec (Canada). Other cases included in this study were cancers of the bladder, colon, rectum, esophagus prostate, and lymphatic system (NHL); a description of the other case series are found in other sections in this appendix. The investigators identified 1,082 newly diagnosed cases of lung cancer (ICD-O, 162) and 165 newly diagnosed cases of pancreatic cancer (ICD-O, 157), confirmed on the basis of histology reports, between 1979 and 1985; 857 lung cancer (79.2%) and 117 pancreatic cancer cases (70.7%) participated in the study interview. One control group consisted of patients with other forms of cancer recruited through the same study procedures and time period as the melanoma cancer cases. The control series for lung cancer cases excluded other lung cancer cases; the control series for pancreatic cancer cases excluded all lung cancer cases. Additionally, a population-based control group (n = 533, 72% response), frequency-matched by age strata, was drawn using electoral lists and random digit dialing. Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents. The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Any exposure to TCE was 2% among cases (n = 21 lung cancer cases, 2 pancreatic cancer cases) and 1% for substantial TCE exposure (n = 9 lung cancer cases); "substantial" is defined as ≥10 years of exposure for the period up to 5 years before diagnosis. None of the pancreatic cancer cases was identified with "substantial" exposure to TCE.

Mantel-Haenszel χ^2 analyses examined occupation exposures and lung cancer stratified on age, family income, cigarette smoking, ethnic origin, alcohol consumption, and respondent status or pancreatic cancer stratified on age, income, cigarette smoking, and respondent status (Siemiatycki, 1991). Odds ratios for TCE exposure in Siemiatycki (1991) are presented with 90% CIs.

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The JEM, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Siemitycki J. (1991). Risk Factors for Cancer in the Workplace. J Siemiatycki, Ed. Boca Raton: CRC Press.

Siemiatycki J, Wacholder S, Richardson L, Dewar R, Gérin M. (1987). Discovering carcinogens in the occupational environment. Scand J Work Environ Health 13:486–492.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	1,082 lung cases were identified among male Montreal residents between 1979 and 1985 of which 857 were interviewed; 165 cases were identified among male Montreal residents between 1979 and 1985 of which 117 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of other cancer cases identified in the larger study. Participation rate: lung cancer cases, 79.2 %, pancreatic cancer cases, 70.7%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-O, 122 (malignant neoplasm of trachea, bronchus and lung). ICD-O, 157 malignant neoplasm of pancreas.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of a priori interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a three-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1–5%; medium, >5–30%; and high, >30%).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	857 lung cancer cases (79.2% response), 117 pancreatic cancer cases (70.7% response); 533 population controls (72% response). Exposure prevalence: Any TCE exposure, 2% cancer cases (n = 21 lung cancer cases and 2 pancreatic cancer cases); substantial TCE exposure (exposure for ≥10 yrs and up to 5 yrs before disease onset), 1% lung cancer cases (n = 9), no pancreatic cancer cases assigned "substantial" TCE exposure.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Lung cancer—age, family income, cigarette smoking, ethnic origin, alcohol consumption, and respondent status. Pancreatic cancer—age, income, cigarette smoking, and respondent status.
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3. Geographic-Based Studies

B.3.3.1. Coyle et al. (2005)

B.3.3.1.1. Author's abstract.

Purpose. To investigate the role of environment in breast cancer development, we conducted an ecological study to examine the association of releases for selected industrial chemicals with breast cancer incidence in Texas.
Methods. During 1995–2000, 54,487 invasive breast cancer cases were reported in Texas. We identified 12 toxicants released into the environment by industry that: (1) were positively associated with breast cancer in epidemiological studies, (2) were Environmental Protection Agency (EPA) Toxics Release Inventory (TRI) chemicals designated as carcinogens or had estrogenic effects associated with breast cancer risk, and (3) had releases consistently reported to EPA TRI for multiple Texas counties during 1988–2000. We performed univariate, and multivariate analyses adjusted for race and ethnicity to examine the association of releases for these toxicants during 1988–2000 with the average annual age-adjusted breast cancer rate at the county level.
Results. Univariate analysis indicated that formaldehyde, methylene chloride, styrene, tetrachloroethylene, trichloroethylene, chromium, cobalt, copper, and nickel were positively associated with the breast cancer rate. Multivariate analyses indicated that styrene was positively associated with the breast cancer rate in women and men (b = 0.219, p =0.004), women (b = 0.191, p=0.002), and women ‡ 50 years old (b = 0.187, p=0.002).
Conclusion. Styrene was the most important environmental toxicant positively associated with invasive breast cancer incidence in Texas, likely involving women and men of all ages. Styrene may be an important breast carcinogen due to its widespread use for food storage and preparation, and its release from building materials, tobacco smoke, and industry.

B.3.3.1.2. Study description and comment.

Residential address in 254 Texas counties at time of cancer diagnosis was the exposure surrogate in this ecologic study of invasive breast cancer in over a 5-year period (1995–2000). Incident breast cancer cases in males and females were identified from Texas Cancer Registry. During the 5-year period, 54,487 cases were diagnosed, of which 53,910 were in females (99%). The association between median average annual age-adjusted breast cancer rates for women and men, all women, women <50 years old, and women ≥50 years old and 12 hazardous air pollutants identified as exposures of interested were examined using nonparametric tests (Mann-Whitney U test) and linear regression analyses. The 12 hazardous air pollutants were: carbon tetrachloride, formaldehyde, methylene chloride, styrene, perchloroethylene, TCE, arsenic, cadmium, chromium, cobalt, copper, and nickel. On-site atmospheric release data on individual hazardous air pollutants was identified from EPA's Toxics Release Inventory (TRI) for a 13-year period, 1998–2000 with an exposure surrogate as the annual total release in pounds/year for the 12 hazardous air pollutants.

Coyle et al. (2005) compared average annual age-adjusted breast cancer rate for counties reporting a release to that rate for non-reporting counties using Mann-Whitney U test. Additionally, multiple linear regression analyses was used to determine the association of the average annual age-adjusted breast cancer rates with the 12 hazardous air pollutants, adjusting for race and ethnicity when associated with the study's outcome variable.

While this study provides insight on cancer rates in studied population, TCE and other hazardous air pollutant exposures are poorly defined and the exposure surrogate unable to distinguish subjects more with higher exposure potential from those with low or minimal exposure potential. Some information may be provided through examination of inter-county release rates; however, no information is provided by Coyle et al. (2005). Furthermore, the ecologic design of the study does not address residential history or other information on an individual-subject level and is subject to bias from "ecologic fallacy" or improper inference about individual-level associations based on aggregate-level analysis. Overall, this study is not able to identify risk factors (etiologic exposures), has low sensitivity for examining TCE, and provides little weight in an overall weight of evidence evaluation of TCE and cancer.

Coyle YM, Hynan LS, Euhus DM, Minhajuddin ATM. (2005). An ecological study of the association of environmental chemicals on breast cancer incidence in Texas. Breast Cancer Res Treat.92:107–114.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Hypothesis of this study was to evaluate breast risks in Texas counties and hazardous air pollutants.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cases are incident breast cancers in males and females over a 5-yr period (1995–2000) in subjects residing in Texas and reported to the Texas Cancer Registry.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Residence in Texas county as time of diagnosis is exposure surrogate. Annual release by county of 12 hazardous air pollutants (carbon tetrachloride, formaldehyde, methylene chloride, styrene, perchloroethylene, TCE, arsenic, cadmium, chromium, cobalt, copper, and nickel) are obtained from EPA's TRI database.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	54,487 incident breast cancer cases in males and females.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and race/ethnicity.
Statistical methods	Mann-Whitney U test (nonparametric) to compared average annual age-adjusted breast cancer rate between counties reported hazardous air pollutant release to that for non-reporting counties. Linear logistic regression
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3.2. Morgan and Cassady (2002)

B.3.3.2.1. Author's abstract.

In response to concerns about cancer stemming from drinking water contaminated with ammonium perchlorate and trichloroethylene, we assessed observed and expected numbers of new cancer cases for all sites combined and 16 cancer types in a California community (1988 to 1998). The numbers of observed cancer cases divided by expected numbers defined standardized incidence ratios (SIRs) and 99% confidence intervals (CI). No significant differences between observed and expected numbers were found for all cancers (SIR, 0.97; 99% CI, 0.93 to 1.02), thyroid cancer (SIR, 1.00; 99% CI, 0.63 to 1.47), or 11 other cancer types. Significantly fewer cases were observed than expected for cancer of the lung and bronchus (SIR, 0.71; 99% CI, 0.61 to 0.81) and the colon and rectum (SIR, 0.86; 0.74 to 0.99), whereas more cases were observed for uterine cancer (SIR, 1.35; 99% CI, 1.06 to 1.70) and skin melanoma (SIR, 1.42; 99% CI, 1.13 to 1.77). These findings did not identify a generalized cancer excess or thyroid cancer excess in this community.

B.3.3.2.2. Study description and comment.

Residential address in 13 census tracts in Redlands (San Bernardino County, California) at time of cancer diagnosis was the exposure surrogate in this ecologic study of cancer incidence over a 10-year period (1988–1998). Seventeen cancers in adults (all cancers, bladder, brain and other nervous system, breast [females only], cervix, colon and rectum, Hodgkin lymphoma, kidney and renal pelvis, leukemia [all], liver and bile duct, lung and bronchus, NHL, melanoma, ovary, prostate, thyroid and uterus) and three site-specific incident cancers in children under 15 years of age (leukemia [all], brain/CNS, and thyroid) were identified from the Desert Sierra Cancer Surveillance Program, a regional cancer registry reporting to the California Cancer Registry, with expected numbers of site-specific cancer using age-race annual site-specific cancer incidence rates between 1988 and 1992 to 1990 census-reported information on population size and demographics. The use of the Desert Sierra Cancer Surveillance Program rates which include the studied population would inflate the number of site-specific cancer expected; however, the potential magnitude of bias is likely minimal given the Redlands populations was estimated as 2% of the total population of the regional cancer registries ascertainment area (Morgan and Cassady, 2002). This is a record-based study and information on personal habits and potential risk factors other than race, sex, and age are lacking for individual subjects.

Morgan and Cassidy (2002) identified TCE and perchlorate from drinking water as exposures of interest. Limited monitoring data from the 1,980 identified TCE concentrations in Redlands wells as between 0.09 and 97 ppb TCE and drinking water concentrations as below the maximum contaminant level (5 ppb) since 1991. The paper lacks information if water monitoring represented wells in the 13-census tract study area. Furthermore, the paper does not

include information on water treatment and distribution networks to provide an estimate of TCE concentration in finished tap water to individual homes. These authors noted their inability to identify higher or lower exposed subjects, as well, as minimally exposed subjects as a source of uncertainty. No data are presented on perchlorate concentrations in well or drinking water. The assumption of residence in 13 census tracts is insufficient as a surrogate of potential exposure to TCE and perchlorate in the absence of exposure modeling and data on water distribution patterns. Exposure misclassification bias is highly likely and of a nondifferential nature which would dampen observed associations.

While this study provides insight on cancer rates in studied population, TCE exposure is poorly defined and the exposure surrogate unable to distinguish subjects more with higher exposure potential from those with low or minimal exposure potential. Furthermore, the ecologic design of the study does not address residential history or other information on an individual-subject level and is subject to bias from "ecologic fallacy" or improper inference about individual-level associations based on aggregate-level analysis. Morgan and Cassidy (2002) furthermore discuss the relatively high education and income levels in the Redlands population compared with the average for the referent population may lead to lower tobacco use and higher than average access to health care, biases that would dampen risks for lung and other tobacco-related cancers, but may also increase risks for colon and cervical cancers. Overall, this study is not able to identify risk factors (etiologic exposures), has low sensitivity for examining TCE, and provides little weight in an overall weight of evidence evaluation of TCE and cancer.

Morgan JW, Cassady RE. (2002). Community cancer assessment in response to long-time exposure to perchlorate and trichloroethylene in drinking water. J Occup Environ Med 44:616–621.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Hypothesis of this study was to evaluate cancer risks in a California community, not to evaluate TCE and cancer explicitly.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cases are incident cancers over a 10-yr period (1988–1989) in subjects residing in 13 Redlands (California) census tracts at time of diagnosis. 17 site-specific cancers are identified in adults and 3 site-specific cancers in children <15 yrs old. Cancer cases identified from Desert Sierra Cancer Surveillance Program (DSCSP), a regional cancer registry. Annual age-race-site specific cancer rates from DSCSP for 1988 and 1992 and age-race-sex specific population estimates for 1990.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Residence in a 13-census tract area of Redlands, California is exposure surrogate. No data are presented on TCE or perchlorate concentrations in treated drinking water supplied to residents.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	3,098 incident cancers, the largest number from 536 breast cancer and fewest number from Hodgkin disease.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and race/ethnicity.
Statistical methods	SIR with indirect standardization of estimated expected numbers of site-specific cancers adjusted for population growth; 90% CIs presented in tables.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3.3. Cohn et al. (1994b)

B.3.3.3.1. Author's abstract.

A study of drinking water contamination and leukemia and non-Hodgkin's lymphoma (NHL) incidence (1979-1987) was conducted in a 75-town study area. Comparing incidence in towns in the highest trichloroethylene (TCE) stratum (>5 microg/L) to towns without detectable TCE yielded an age-adjusted rate ratio (RR) for total leukemia among females of 1.43 (95% CI 1.07-1.90). For females under 20 years old, the RR for acute lymphocytic leukemia was 3.26 (95% CI 1.27-8.15). Elevated RRs were observed for chronic myelogenous leukemia among females and for chronic lymphocytic leukemia among males and females. NHL incidence among women was also associated with the highest TCE stratum (RR = 1.36; 95% CI 1.08-1.70). For diffuse large cell NHL and non-Burkitt's high-grade NHL among females, the RRs were 1.66 (95% CI 1.07-2.59) and 3.17 (95% CI 1.23-8.18), respectively, and 1.59 (95% CI 1.04-2.43) and 1.92 (95% CI 0.54-6.81), respectively, among males. Perchloroethylene (PCE) was associated with incidence of non-Burkitt's high-grade NHL among females, but collinearity with TCE made it difficult to assess relative influences. The results suggest a link between TCE/PCE and leukemia/NHL incidence. However, the conclusions are limited by potential misclassification of exposure due to lack of individual information on long-term residence, water consumption, and inhalation of volatilized compounds.

B.3.3.3.2. Study description and comment.

This expanded study of a previous analysis of TCE and perchloroethylene in drinking water in a 27-town study area (Fagliano et al., 1990)examined leukemia and NHL incidence from 1979 to 1987 in residents and TCE and other VOCs in drinking water delivered to 75 municipalities. Exposure estimates were developed from data generated by a mandatory monitoring program for 4 trihalomethane chemicals and 14 other volatile organic chemicals in 1984–1985 for public water supplies and from historical monitoring data conducted in 1978–1984 by the New Jersey Department of Environmental Protection and Energy and the New Jersey Department of Health, which was the mean of monthly averages for this period. The average and maximum concentration of TCE and other chemicals were estimated by considering together, for the period prior to 1985, details of the distribution system size, well or surface water use, patterns of water purchases among systems, and significant changes in water supply, and for years after 1985, samples of finished water from the plant and samples taken from the distribution system under the assumption of homogeneous mixing. The number of distribution system samples for each supply varied from 2 to 50. Additionally, a dilution factor assuming complete mixing was used to adjust for water purchased from another source. A single summary average and maximum concentration for each contaminate for a municipality was assigned to all cases residing in that municipality at the time of cancer diagnosis. Concentrations of TCE and perchloroethylene were highly correlated (r = 0.63). A ranking of municipalities was the same

when using average or maximum concentration and the maximum concentration of TCE or perchloroethylene used in statistical analyses was grouped into three strata: <0.1 (referent group), 0.1–5, >5–20, and >20 ppb.

Incident cases of NHL and forms of leukemia reported to the New Jersey State Cancer Registry were identified from 1979 and 1987. Incidence rate ratios were estimated using Poisson regression models fitted to age- and sex-specific numbers of cases by exposure strata and the stratum-specific population. Statistical treatment considered exposure to other drinking water contaminants, atmospheric emissions of hazardous air pollutants as reported to U.S. EPA's TRI by municipality and two socioeconomic variables measured as municipal—average annual household income and percentage of high school graduates. None of the water trihalomethane or VOCs other than perchloroethylene was shown to be associated with childhood leukemia or adult lymphomas. Furthermore, neither average income, education, nor TRI release data were associated with NHL or leukemia except in one exception, TRI release was shown to modify the effects of TCE and high-grade non-Burkett's lymphoma in females.

This ecological study is subject to known biases and confounding as introduced through its study design (NRC, 1997). Exposure estimates are crude (averages), do not consider individual differences in drinking water patterns, and assigns group exposure levels to all subjects without consideration of residential history. Potential for misclassification bias is likely great in this study as is the potential for bias. This study does attempt to examine three possible confounding exposures, although these are crudely defined, and some potential for residual confounding is possible given the study's use of aggregated data.

Cohn P, Klotz J, Bove F, Berkowitz M, Fagliano J. (1994b). Drinking water contamination and the incidence of leukemia and non-Hodgkin's lymphoma. Environ Health Perspect 102:556–561.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study was designed to further examine drinking water contaminates and lymphoma; a previous study of TCE and perchloroethylene in drinking water found a statistically significant association with leukemia among females residing in a 27-town study area (Fagliano et al., 1990).
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Incident cases of various forms of leukemia (all leukemia, acute lymphocytic, chronic lymphocytic, acute myelogenous, chronic myelogenous, other specified and unspecified leukemia) and NHL (total, low-grade, intermediate-grade [total and diffuse large cell a B-cell lymphoma], high-grade including non-Burkett's lymphoma) from 1979 to 1987 are identified from New Jersey State Cancer Registry. Subjects grouped in lowest exposure category are referents.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Average and maximum concentration of TCE and other chemicals were estimated by considering together, for the period prior to 1985, details of the distribution system size, well or surface water use, patterns of water purchases among systems, and significant changes in water supply, and for years after 1985, samples of finished water from the plant and samples taken from the distribution system under the assumption of homogeneous mixing. No difference in municipality ranking by average or maximum concentration. Three grouped categories of maximum concentration in statistical analysis are <0.1 (referent), 0.1–5, >5 ppb (U.S. EPA Maximum Contaminant Level for TCE and perchloroethylene).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,190 leukemia cases (663 males, 527 females), 119 cases assigned >5.0 ppb TCE. 1,658 NHL cases (841 males, 817 females), 165 cases assigned >5.0 ppb TCE.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and sex.
Statistical methods	Poisson regression fitted to the age-and sex-specific count of cases in towns grouped by exposure strata and weighted by the logarithm of the strata-specific population.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes.

B.3.3.4. Vartiainen et al. (1993)

B.3.3.4.1. Author's abstract.

Concentrations up to 212 μg/l of trichloroethene (TCE) and 180 μg/l of tetrachloroethene (TeCE) were found in the drinking water from two villages in Finland. To evaluate a possible exposure, urine sample from m95 and 21 inhabitants in these villages and from two control groups of 45 and 15 volunteers were collected. Dichloroacetic acid (DCA) and trichloroacetic acid (TCA), the metabolites of TCE and TeCE, were also analyzed. The individuals using contaminated water in one of the villages excreted TCE an average 19 μg/d (<1 – 110 μg/d) and in the other 7.9 μg/d (<1 – 50 μg/d), while the controls excreted an average 2.0 μg/d (<1 – 6.4 μg/d) or 4.0 μg/d (<1 – 13 μg/d). No increased incidence rates were found in the municipalities in question for total cancer, liver cancer, non-Hodgkin's lymphomas, Hodgkin's disease, multiple myeloma, or leukemia.

B.3.3.4.2. Study description and comment.

This published study of two separate analyses: (1) urinary biomonitoring of 106 subjects from two Finish municipalities, Hausjärvi and Hattula and (2) calculation of total cancer and site-specific cancer incidence between 1953 and 1991 in Hausjärvi and Hattula residents. Limited exposure monitoring data are presented in the paper. TCE concentrations in drinking water from Oitti are lacking other than noting TCE and perchloroethylene were 100–200 μg/L in 1992. TCE concentrations in drinking water from Hattula were <10 μg/L in December 1991; however, samples (number unknown) taken 6 months later contained 212 and 66 μg/L TCE. These two municipalities discontinued use of these sources for drinking water in August 1992.

Cancer incidence for six sites (all cancers, liver cancer, NHL, Hodgkin lymphoma, multiple myeloma, and leukemia) between 1953 and 1991 in Hausjärvi and Hattula residents was obtained from the Finnish Cancer Registry. A total of 1,934 cancers were observed during the study period. Standardized incidence ratios for each municipality were calculated using site-specific cancer incidence rates from the Finnish population for the entire time period and for three shorter periods, 1953–1971, 1972–1981, and 1982–1991. The paper does not identity the source for or size of Hausjärvi and Hattula population estimates and if temporal changes in population estimates were considered in the statistical analysis. This study, using record systems, did not include information obtained directly from subjects and lacks information on personal and lifestyle factors that may introduce bias or confounding.

This study provides little information in an overall weight-of-evidence analysis on cancer risks and TCE exposure. A major limitation is its lack of exposure assessment to TCE and perchloroethylene. While this study provides some information on cancer incidence in the two towns over a 40-year period, this study is not able to identify potential risk factors and exposures.

Vartiainen T, Pukkala E, Rienoja T, Strandman T, Kaksonen K. (1993). Population exposure to tri- and tetrachloroethene and cancer risk: two cases of drinking water pollution. Chemosphere 27:1171–1181.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Study aim was: (1) to determine if residents of two villages in Finland had exposure to TCE and perchloroethylene as indicated from urinary biomonitoring; (2) identify biomarker for low-level exposure; and (3) to determine cancer incidence in Hausjärvi and Hattula, two municipalities in Finland. This study could not identify potential risk factors.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cancer incidence cases identified from Finnish Cancer Registry. Site-specific cancer rates for the Finnish population was used a referent.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Residence in two municipalities is the exposure surrogate in this ecologic study. The paper lacks exposure assessment to TCE and perchloroethylene in drinking water in Hausjärvi and Hattula.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	3,846 cancer cases; 1,942 from Hausjärvi and 1,904 from Hattula.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and sex.
Statistical methods	SIR with cancer incidence rates in Finnish population as referent.
Exposure-response analysis presented in published paper	No.
Documentation of results	Cancer incidence analysis is not well documented.

B.3.3.5. Mallin (1990)

B.3.3.5.1. Author's abstract.

Cancer maps from 1950 through 1979 revealed areas of high mortality from bladder cancer for both males and females in several northwestern Illinois counties. In order to further explore this excess, a bladder cancer incidence study was conducted in the eight counties comprising this region. Eligible cases were those first diagnosed with bladder cancer between 1978 and 1985. Age adjusted standardized incidence ratios were calculated for each county and for 97 zip codes within these counties. County results revealed no excesses. Zip code results indicated elevated risks in a few areas, but only two zip codes had significantly elevated results. One of these zip codes had a significant excess in males (standardized incidence ratio = 1.5) and females (standardized incidence ratio = 1.9). This excess was primarily confined to one town in this zip code, in which standardized incidence ratios were significantly elevated in males (1.7) and females (2.6). Further investigation revealed that one of four public drinking water wells in this town had been closed due to contamination; two wells were within a half mile (0.8 km) of a landfill site that had ceased operating in 1972. Tests of these two wells revealed traces of trichloroethylene, tetrachloroethylene, and other solvents. Further investigation of this cluster is discussed.

B.3.3.5.2. Study description and comment.

This ecologic study of bladder cancer incidence and mortality among white residents in nine Illinois counties between 1978 and 1985 was carried out to further investigate a previous finding of elevated bladder cancer mortality rates in some counties. The study lacks exposure assessment to subjects and potential sources of exposure was examined in a post hoc manner in one case only, for a community with an observed elevated bladder cancer incidence. The limited exposure examination focused on groundwater contamination and proximity of Superfund sites to the community, lacked assignment of exposure surrogates to individual study subjects, and findings are difficult to interpret given the lack of exposure assessment for the other eight counties.

Histologically-confirmed incident bladder cancer cases were identified from hospital records in eight of the nine counties. Since the nine-county area bordered on neighboring states of Wisconsin and Iowa, incident bladder cancer cases were also ascertained from the Wisconsin Cancer Reporting System and Iowa's State Health Registry. No information is provided in the paper on completeness of ascertainment of bladder cancer cases among residents or on the source for identifying bladder cancer deaths. Expected numbers of incident cancers calculated using age-specific rates for white males and females from the SEER program (incidence) or the U.S. population (mortality), and the census data on population estimates for the nine-county area. Statistical analyses adopt indirect standardization methods to calculate SMR and SIRs for a community and SIRs for individual postal zip codes. The use of records and absence of

information collected from subject personal interviews precluded examination of possible confounders other than age and race.

This ecological study is subject to known biases and confounding as introduced through its study design (NRC, 1997). Ecological studies like this study are subject to bias known as "ecological fallacy" since variables of exposure and outcome measured on an aggregate level may not represent association at the individual level. Consideration of this bias is important for diseases with more than one risk factor, such as the site-specific cancers evaluated in this assessment. Lack of information on smoking is another uncertainty. While this study provides insight on bladder cancer rates in the studied communities, it does not provide any evidence on cancer and TCE exposure. For this reason, this study provides little weight in an overall weight-of-evidence analysis.

Mallin K. (1990). Investigation of a bladder cancer cluster in Northwestern Illinois. Amer J Epidemiol 132:S96–S106.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The hypothesis of study was to "further exposure a previous finding of bladder cancer excess in several northwestern Illinois counties." (from abstract).
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Incident cancer cases diagnosed between 1978 and 1985 were identified in residents in nine northwestern Illinois counties from the Illinois Cancer Registry, the Wisconsin Cancer Reporting System or the Iowa State Health Registry. Source for deaths in subjects residing at the time of death in the 9 counties was not identified in the published paper. Expected number of bladder cancer derived using: (1) SEER age-race-sex specific incidence rates and (2) age-race-sex specific mortality rates of the U.S. population for 1978–1981 and for 1982–1985 and census estimates of population for each county or postal zip code area.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence and mortality.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	This is a health survey and lacks exposure assessment to communities and to individual subjects. Monitoring of volatile organic chemicals including TCE in two municipal drinking water wells for 1982–1988 in a community with elevated bladder cancer rates was identified in paper; TCE concentrations were <15 ppb. It is not known whether monitoring data are representative of exposure to study subjects.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	712 bladder cancer incident cases and 222 bladder cancer deaths among white males and female residents in nine northwestern Illinois counties.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and sex.
Statistical methods	SIR with cancer incidence rates from SEER program and mortality rates of U.S. population as referents.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3.6. Isacson et al. (1985)

B.3.3.6.1. Author's abstract.

> With data from the Iowa Cancer Registry, age-adjusted sex-specific cancer incidence rates for the years 1969-1981 were determined for towns with a population of 1,000–10,000 and a public water supply from a single stable ground source. These rates were related to levels of volatile organic compounds and metals found in the finished drinking water of these towns in the spring of 1979. Results showed association between 1,2 dichloroethane and cancers of the colon and rectum and between nickel and cancers of the bladder and lung. The effects were most clearly seen in males. These associations were independent of other water quality and treatment variables and were not explained by occupational or other sociodemographic features including smoking. Because of the low levels of the metals and organics, the authors suggest that they are not causal factors, but rather indicators of possible anthropogenic contamination of other types. The data suggest that water quality variables other than chlorination and trihalomethanes deserve further consideration as to their role in the development of human cancer.

B.3.3.6.2. Study description and comment.

This ecologic study of cancer incidence at six sites (bladder, breast, colon, lung, prostate, rectum) and chlorinated drinking water uses monitoring data from finished public drinking water supplies to infer exposure to residents of Iowa towns of 1,000–10,000 population sizes. Towns were included if they received water from a single major source (surface water, wells of <150 feet depth, or wells ≥50 feet depth) prior to 1965. Water monitoring for VOCs, trace elements, and heavy metals was carried in Spring, 1979, as part of a larger nationwide collaborative study of bladder cancer and artificial sweeteners (Hoover and Strasser, 1980), and samples analyzed using proton-induced x-ray emission for trihalomethanes, TCE, perchloroethylene, 1,2-dichloroethane, 1,1,1-trichloroethane, carbon tetrachloride, 1,2-DCE, and 43 inorganic elements. 1,1,1-Trichloroethane was the most frequently detected VOC in both surface and groundwater; TCE, perchloroethylene, and 1,2-dichloroethane were more frequently detected in shallow wells than in deep (>150 feet) wells.

Cancer incidence was obtained for the period 1969 and 1981 with age-adjusted site-specific cancer incidence rates for males and females calculated separately for four VOCs (1,2-dichloroethane, TCE, perchloroethylene, and 1,1,1-trichloroethane) in finished groundwater supplies using the direct standardization method. Using the address at the time of diagnosis, each cancer patient was classified into one of two groups: (1) residing within the city limits and, thus, drinking the municipality's water; or (2) residing outside the city limits and consuming water from a private source. Age-adjusted incidence rates are reported by group study town into two TCE water concentrations categories of <0.15 and ≥0.15 μg/L.

This ecological study on drinking water exposure and cancer provides little information in a weight-of-evidence analysis of TCE and cancer. Exposure estimates are crude (averages),

do not consider individual differences in drinking water patterns or other sources of exposure, and assigns group exposure levels to all subjects. Potential for misclassification bias is likely great in this study, likely of a nondifferential nature, and dampen observations.

Isacson P, Bean JA, Splinter R, Olson DB, Kohler J. (1985). Drinking water and cancer incidence in Iowa. III. Association of cancer with indices of contamination. Amer J Epidemiol 121:856–869.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This ecological study was designed to examine consistency with the hypothesis of an association between cancer and chlorinated water through examination of other water contaminants besides water chlorination byproducts and trihalomethanes.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Subjects are incident cases of cancer of the bladder, breast, prostate, lung rectum, and stomach reported to the Iowa Cancer Registry between 1969 and 1981 and, who resided in towns with a 1970 population of 1,000–10,000 and a public drinking water supply coming solely from a single major source (wells) prior to 1965. Age-adjusted site-specific incidence rates are calculated using the direct method and the 1970 Iowa population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	As part of another epidemiologic study on water chlorination and bladder cancer, finished drinking water samples from treatment plant were collected in Iowa municipalities with populations of 1,000 or larger in Spring 1979 and analyzed using proton induced x-ray emission for 4 trihalomethanes (chloroform, chlorodibromomethane, bromoform, dibromochloromethane), 7 VOCs (TCE, perchloroethylene, 1,1,1-trichloroethane, carbon tetrachloride, 1,2-dichloroethane, and cis- and trans-1,2-DCE) and 43 inorganic elements, including metals. The predominant contaminant was 1,1,1-tri-chloroethane; detectable levels of TCE were found in approximately 20% of sampled municipalities. Study towns were ranked into two categories of TCE in finished water, <0.15 µg/L and ≥0.15 µg/L in the statistical analysis.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	11,091 cancer cases of which ~20% of cases resided in municipality with finished water TCE concentration of ≥0.15 µg/L. Bladder, 852 cases Breast (female), 1,866 cases Colon, 2,032 cases Lung 1,828 cases Prostate, 1,823 cases Rectum, 824 cases
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and sex.
Statistical methods	Age-adjusted site-specific mortality rates calculated using direct standardization method and 1970 Iowa population.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3.7. Studies in the Endicott Area of New York

A series of health statistics reviews and exposure studies have been conducted in an area with a history of VOCs, including TCE, detected in municipal wells used to supply drinking water to residents of Endicott, Broome County, New York. These studies were carried out by staff the NYS DOH with support from the ATSDR. Early health surveys examined cancer incidence among Broome County residents between 1976 and 1980 or 1981 and 1990, with focused analyses of cancer incidence among residents of Endicott Village and other nearby towns, childhood leukemia in the Town of Union and possible etiologic factors, and adult leukemia deaths and employment in the shoe and boot manufacturing industry (NYSDOH, 2005; Forand, 2004). Two recent studies focused on cancer incidence or birth outcomes among Village of Endicott residents living in a geographically defined area with VOC exposure potential as documented from indoor and soil vapor monitoring (ATSDR, 2008b, 2006a).

The Village of Endicott is a mixed residential, commercial, and industrial community with a rich industrial heritage, and a number of VOCs were used at industrial locations in and around Endicott, as well as been disposed at area landfills (ATSDR, 2006b). Three wells provide drinking water to the Village of Endicott: Ranney, which supplied most of the water used by the Endicott Municipal Water Works since it was first placed in service in 1950; and, South Street, where two wells resided. The Endicott Municipal Water Supply operates on a grid-water system, neighborhoods closest to the wells are usually supplied at a greater rate from nearby wells as compared to wells farther away (ATSDR, 2006b).

Routine monitoring of the Ranney well in the early 1980s detected VOCs at levels above New York State drinking water guidelines (ATSDR, 2006b). A groundwater-contaminated plume northwest of the Ranney Well was found in a lower aquifer from which the municipal drinking supply is drawn. Several sources were initially recognized as contributing to contamination of the wellfield with a supplemental remedial investigation concluding that the Endicott Village Landfill was the source of the VOCs in the Endicott Wellfield water supply (ATSDR, 2006b). Groundwater samples collected from monitoring wells installed during previous investigations, wells installed as part of the supplemental remedial investigation, the Purge well, and the Ranney well contained many VOCs. Remediation efforts starting in the 1980s have reduced contamination in this well to current maximum contaminant levels. Water monitoring of the South Street wells (wells 5 and 28) has been carried out for VOCs since 1980 and 1981, respectively (ATSDR, 2006b). Detection limits for VOCs from the South Street wells varied from 0.5 to 1.0 µg/L; 1,1-dichloroethane had the highest detection frequency, in 44% of all samples, and TCE was detected in 3 of 116 samples obtained between 1980 and 2004 (ATSDR, 2006b).

An upper aquifer with a contaminant plume containing VOCs was also identified and sampling data indicated that there were multiple sources of vapor contamination, including a former IBM facility located in the Village (NYSDEC, 2008; U.S. EPA, 2005d). This

groundwater contaminant plume flows directly beneath the center of the Village of Endicott and serves as a source of soil vapor contamination. Findings of a 2002 investigation indicated that vapor migration had resulted in detectable levels of contaminants in indoor air structures, including locations in the Village of Endicott and Town of Union. Of soil gas and indoor air monitoring at >300 properties in an area south of the IBM Endicott facility, TCE was the most commonly found contaminant in indoor air, at levels ranging from 0.18 to 140 $\mu g/m^3$ (NYSDEC, 2008). This area is identified as the Eastern study area in the health statistics review of ATSDR (2008b, 2006a). Other contaminants besides TCE detected in soil gas and indoor air less frequently and at lower levels included tetrachloroethylene, cis-1,2-dichloroethene, 1,1,1-trichloroethane, 1,1-DCE, 1,1-dichloroethane, and Freon 113. Vapor-intrusion contamination was also identified in a neighborhood adjacent to the Eastern area, call the Western study in the health statistic review, and perchloroethylene and its degradation byproducts were detected by vapor monitoring. Perchloroethylene levels generally ranged from 0.1 to 3.5 $\mu g/m^3$ of air (ATSDR, 2006a).

B.3.3.7.1. ATSDR (2008b, 2006a)

B.3.3.7.1.1. ATSDR (2006a) executive summary.

> ***Background*** The New York State Department of Health (NYS DOH) conducted this Health Statistics Review because of concerns about health issues associated with environmental contamination in the Endicott area. Residents in the Endicott area may have been exposed to volatile organic compounds (VOCs) through a pathway known as soil vapor intrusion. Groundwater in the Endicott area is contaminated with VOCs as a result of leaks and spills associated with local industry and commercial businesses. In some areas of Endicott, VOC contamination from the groundwater has contaminated the adjacent soil vapor which has migrated through the soil into structures through cracks in building foundations (soil vapor intrusion). Trichloroethene (TCE), tetrachloroethene (PCE) and several other VOCs have been found in the soil vapor and in the indoor air of some structures.
> ***Conclusions*** This health statistics review was conducted because of concerns that exposure to VOCs through vapor intrusion may lead to adverse health effects. Although this type of study cannot prove whether there is a causal relationship between VOC exposure in the study area and the increased risk of several health outcomes observed, it does serve as a first step in providing guidance for further health studies and interventions. The elevated rates of several cancers and birth outcomes observed will be evaluated further to try to identify additional risk factors which may have contributed to these adverse health outcomes.
>
> Limitations in the current study included limited information about the levels of VOCs in individual homes, the duration of the exposure, the amount of time residents spent in the home each day and the multiple exposures and exposure pathways that likely existed among long term residents of the Endicott area. In addition, personal information such as medical history; dietary and lifestyle choices such as smoking and drinking; and occupational exposures to chemicals

were not examined. Future evaluations of cancer and birth defects and VOC exposures in the area should take these factors into account. The small population size of the study area also limited the ability to detect meaningful elevations or deficits in disease rates, especially for certain rare cancers and birth outcomes.

This study represents the first step in a step-wise approach to addressing health outcome concerns related to environmental contamination in Endicott, NY. Follow-up will consist of further reviewing of the cancer and birth outcome data already collected. Additional efforts will include reviewing individual case records of kidney and testicular cancers, heart defects, Down syndrome and term low birth weight births. In addition, we will review spontaneous fetal deaths among residents of the area. The information gained, along with the results of this Health Statistics Review, will be used to assess if a follow up epidemiologic study is feasible. Any follow-up study should be capable of accomplishing one of two goals: either to advance the scientific knowledge about the relationship between VOC exposure and health outcomes; or as part of a response plan to address community concerns. While not mutually exclusive, the distinction between these goals must be considered when developing a follow-up approach. Any plans for additional study will need to address other risk factors for these health outcomes such as smoking, occupation and additional information on environmental exposures. As in the past, NYS DOH will solicit input from the community.

B.3.3.7.1.2. **ATSDR (2008b) executive summary.**

This follow-up investigation was conducted to address concerns and to provide more information related to elevated cancers and adverse birth outcomes identified in the initial health statistics review entitled “Health Statistics Review: Cancer and Birth Outcome Analysis, Endicott Area, Town of Union, Broome County, New York” (2006a).

The initial health statistics review was carried out to address concerns about health issues among residents in the Endicott area who may have been exposed to volatile organic compounds (VOCs) through a pathway known as soil vapor intrusion. The initial health statistics review reported a significantly elevated incidence of kidney and testicular cancer among residents in the Endicott area. In addition, elevated rates of heart defects and low birth weight births were observed. The number of term low birth weight births, a subset of low birth weight births, and the number of small for gestational age (SGA) births were also significantly higher than expected.

The purpose of this follow-up investigation was to gather more information and conduct a qualitative examination of medical and other records of individuals identified with adverse birth outcomes and cancers found to be significantly elevated. Quantitative analyses were also carried out for two additional birth outcomes, conotruncal heart defects (specific defects of the heart’s outflow region), and spontaneous fetal deaths (stillbirths), and for cancer incidence accounting for race.

Cancer Incidence Adjusting for Race: Because a higher percentage of the population in the study area was white compared to the comparison population, we examined the incidence of cancer among whites in the study area compared to

the incidence in the white population of New York State, excluding New York City. Cancer incidence among whites was evaluated for the years 1980-2001. Results: Limiting the analysis of cancer to only white individuals had little effect on overall cancer rates or standardized incidence ratios compared to those of the entire study area population analyzed previously. The only difference was the lung cancer which had been borderline non-significantly elevated was not borderline significantly elevated.

Cancer Case Record Review: We reviewed medical and other records of individuals with kidney and testicular cancers to try to determine smoking, occupational and residential histories. A number of preexisting data sources were used including: hospital medical records; cancer registry records; death certificates; newspaper obituaries; Motor Vehicle records; and city and telephone directories. Results: The case record review did not reveal any unusual patterns in terms of age, gender, year of diagnosis, cell type, or mortality rate among individuals with kidney or testicular cancer. There was some evidence of an increased prevalence of smoking among those with kidney cancer and some indication that several individuals diagnosed with testicular and kidney cancer may have been recent arrivals to the study area.

Conclusions/Recommendations: The purpose of the additional analyses reported in the draft for public comment follow-up report was to provide information on certain cancers and reproductive outcomes which were elevated in the initial health statistics review. Although these additional analyses could not determine whether there was a causal relationship between VOC exposures in the study area and the increased risk of several health outcomes that were observed, they did provide more information to help guide additional follow-up. The March 2007 public comment report provided a list of follow-up options for consideration and stated, "Although an analytical (case-control) epidemiologic study of cancer or birth defects within this community is not recommended at this time, we describe several follow up options for discussion with the Endicott community. A case-control study would be the preferable method for progressing with this type of investigation, but the potentially exposed population in the Endicott area is too small for conducting a study that would be likely to be able to draw strong conclusions about potential health risks.

Alternative follow-up options were discussed at meetings with Endicott stakeholders and were the subject of responses to comments on the draft report. From these discussions and written responses, NYS DOH has noted community interest in two possible options for future activities: a health statistics review based on historic outdoor air emissions modeling, and a multi-site epidemiologic study examining cancer outcomes in communities across the state with VOC exposures similar to Endicott. NYS DOH has considered these comments and examined whether these options would be able to accomplish one of two goals: either to advance the scientific knowledge about the relationship between VOC exposure and health outcomes or to be part of a response plan to address community concerns.

An additional health statistics review using historic outdoor air emission modeling results to identify and study a larger population of residents potentially exposed to TCE is not likely to meet either of these goals at this time. Because of

the limitations of the health statistics review for drawing conclusions about cause and effect, conducting an additional health statistics review is not likely to increase our understanding of whether exposures in the Endicott area are linked to health outcomes. Limitations with the available historic outdoor air data also would make it difficult to accurately define the appropriate boundaries for the exposure area. ATSDR historic outdoor air emissions modeling activity was unable to model TCE due to a lack of available records.

A multi-site epidemiologic study of health outcomes in communities across the state with VOC exposures similar to Endicott offers some promise of meeting the goal of advancing the scientific knowledge about the relationship between VOC exposures and health outcomes. The community has indicated its preference that such a study focus on cancer outcomes. Given the complex issues involved in conducting such a study (e.g., tracking down cases or their next of kin after many years, participants' difficulty in accurately remembering possible risk factors from many years ago, and the long time period between exposure to a carcinogen and the onset of cancer), we do not consider a multisite case-control study of cancer as the best option at this time. An occupational cancer study is a better option than a community-based study because it can better incorporate information about past workplace exposures and could use corporate records to assist in finding individual employees many years after exposure.

Heart defects have been associated with TCE exposure in other studies. Given the shorter latency period, and thus the shorter time period in which other risk factors could come into play, a multi-site study of heart defects has some merit as a possible option. Currently, NYS DEC and NYS DOH are investigating many communities around New York State which could have VOC exposure patterns similar to Endicott, and thus could be included in such a multi-site epidemiologic study. However, in most of these communities exposure information sufficient to identify a study population is not yet available. NYS DOH will continue to evaluate these areas as additional exposure information becomes available, with the goal of identifying other communities for possible inclusion in a multi-site epidemiologic study of heart defects.

NYS DOH will continue to keep the Endicott community and stakeholders informed about additional information regarding other communities with exposures similar to those that occurred in the Endicott area. NYS DOH staff will be available as needed to keep interested Endicott area residents up-to-date on the feasibility of conducting a multi-site study that includes the Endicott area.

B.3.3.7.1.3. **Study description and comment.**

Health statistics review conducted by NYS DOH because of concerns about possible exposures to VOCs in Endicott area groundwater and vapor intrusion into residences examined cancer incidence between 1980 and 2001 and birth outcomes among residents living in a study area defined by soil vapor sampling and exposure modeling. The reviews were supported by ATSDR and conclusions presented in final reports (ATSDR, 2008b, 2006a) have received external comment, but the studies have not been published in the open peer-reviewed literature. Testing of soil gas and indoor air of >300 properties, including 176 residences (location not identified) for VOCs detected TCE levels ranging from 0.18 to 140 $\mu g/m^3$; other VOCs less

commonly detected included perchloroethylene, 1,1-dichloroethane, 1,1-DCE, 1,2-DCE, vinyl chloride, 1,1,1-trichloroethane, methylene chloride, and Freon 113. A model was developed to predict VOC presence in soil vapor based on measured results ("Groupwater Vapor Project, Endicott, New York: Summary of findings, working draft. Cited in ATSDR," 2006). Subsequent sampling and data collection verified this model. Initial study area boundaries were determined based on the extent of the probable soil vapor contamination >10 $\mu g/m^3$ of VOCs as defined by the model. Contour lines of modeled VOC soil vapor contamination levels, known as isopleths, were mapped using a GIS. This study area is referred to as the Eastern study area in ATSDR (2008b, 2006a). Additional sampling west of the initial study area identified further contamination with the contaminant in this area primarily identified as perchloroethylene at levels ranging from 0.1 to 3.5 $\mu g/m^3$ in an area referred to as the Western study area (ATSDR, 2008b, 2006a). The source of perchloroethylene contamination was not known. A digital map of the 2000 Census block boundaries was overlaid on these areas of contamination. The study areas were then composed of a series of blocks combined to conform as closely to the areas of soil vapor contamination as possible.

Incident cancer cases for 18 sites, including cancer in children ≤19 years, between 1980 and 2001 and obtained from the New York State Cancer Registry and addresses were geocoded to identify cases residing in the study area. The observed numbers of site-specific cancers were compared to that expected calculated using age-, sex-, and year-specific cancer incidence rates for New York State exclusive of New York City and population estimates from 1980, 1990, and 2000 Censuses. Expected numbers of site-specific cancer did not include adjustment for race in (ATSDR, 2006a); however, race was examined in the 2008 follow-up, study which compared cancer incidence among the white residents in the study area to that of whites in New York State (ATSDR, 2008b). Over the 22-year period, a total of 347 incident cancers were observed among residents in the study area, 339 of these were in white residents. Less than six cases of cancers in children ≤19 years old were identified and ATSDR (2006a) did not present a SIR for this grouping, similar to their treatment of other site-specific cancers with less than six observed cases.

The follow-up analysis by ATSDR (2008b) reviewed medical records of kidney and testicular cancer cases for smoking and occupational and residential histories, and restricted the statistical analysis to white residents, given the few numbers of observed cancers in the small population of nonwhite residents. Limiting the analysis to only white individuals in the study area had little effect on overall cancer rates or SIR estimates (ATSDR, 2006a). As observed in ATSDR (2006a), statistically significant excess risks were observed for kidney cancer in both sexes and testicular cancer in males. In addition, lung cancer estimate risks in males and in males and females were of the same magnitude in both analyses, but CIs excluded a risk of 1.0 in the ATSDR (2008b) analyses, which adjusted for race. Review of medical records for the 15 kidney and 6 testicular cancer cases provided limited information about personal exposures

and potential risk factors because of incomplete reporting in records. The record review did not reveal any unusual patterns in either kidney or testicular cancer in terms of age, year of diagnosis, anatomical site, cell type, or mortality rate. Occupational history suggested possible workplace chemical exposure for roughly half of the 13 kidney cancer cases and none of the testicular cancer cases whose medical records included occupational history. For smoking, half of the nine kidney cancer cases and some (number not identified) of the three testicular cancer cases with such information in medical records were current or former smokers; smoking habits were not reported for the other cases. Last, examination of city and phone directories revealed that while half the kidney cancer cases as long term Endicott residents, several cases of testicular cancer were among residents who recently moved into the Endicott area.

These health surveys are descriptive; they provide evidence of cancer rates in a geographical area with some documented exposures to several VOCs including TCE, but are unable to identify possible etiologic factors for the observed elevations in kidney, testicular, or lung cancers. The largest deficiency is the lack of exposure assessment, notably historical exposure, to individual subjects. Review of city and phone directories suggests some kidney and testicular cancer cases were among recently-arrived residents, a finding inconsistent with a cancer latent period; however, of greater importance is the finding of cancers among subjects with long residential history. On the other hand, the population in the study areas has declined over the past 20 years (ATSDR, 2006a) and residents who may have moved from the study area were not included, introducing potential bias if cancer risks differed in these individuals. The medical history review suggests several risk factors, including smoking and occupational exposure, as important to kidney and testicular cancer observations. Lacking information for all subjects, there is uncertainty regarding the additive effect of other potential risk factors such as smoking to residential exposures. For this reason, while excesses in several incident cancers are observed in these reports, potential etiological risk factors are ill-defined, and the weight these studies contribute in the overall weight-of-evidence analysis is limited.

ATSDR (Agency for Toxic Substances and Disease Registry). (2006a). Health Consultation. Cancer and Birth Outcome Analysis, Endicott Area, Town of Union, Broome County, New York. Health Statistics Review. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. May 26, 2006.

ATSDR (Agency for Toxic Substances and Disease Registry). (2008b). Health Consultation. Cancer and Birth Outcome Analysis, Endicott Area, Town of Union, Broome County, New York. Health Statistics Review Follow-Up. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. May 15, 2008.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This health statistics review examined incidence for 18 types of cancer in residents living in the Village of Endicott at the time of diagnosis. This study was not designed to identify possible etiologic factors.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Subjects are incident cases of cancer of the 18 types of cancers including childhood cancer (all cancers in children ≤19 yrs of age) reported to the New York Cancer Registry between 1980 and 2001 among residents in two areas of the Village of Endicott, New York. The expected number of cancer cases for the period was calculated using cancer incidence rates for New York State exclusion of New York City and population estimates from 1980, 1990, and 2000 Censuses.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD 9th Revision.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	This geographic-based study does not develop quantitative estimates of exposure, rather study boundaries are defined using soil gas and indoor air monitoring data and computer modeling. Testing of soil gas and indoor air of >300 properties, including 176 residences (location not identified) in the Eastern study area for VOCs detected TCE levels ranging from 0.18 to 140 µg/m^3; other VOCs less commonly detected included perchloroethylene, 1,1-dichloroethane, 1,1-DCE, 1,2-DCE, vinyl chloride, 1,1,1-trichloroethane, methylene chloride, and Freon 113. A model was developed to predict VOC presence in soil vapor based on measured results (["Groupwater Vapor Project, Endicott, New York: Summary of findings, working draft. Cited in ATSDR," 2006]). Subsequent sampling and data collection verified this model. Initial study area boundaries were determined based on the extent of the probable soil vapor contamination >10 µg/m^3 of VOCs as defined by the model. Additional sampling west of the initial study area identified further contamination with the contaminant in this area primarily identified as perchloroethylene at levels ranging from 0.1 to 3.5 µg/m^3 in an area referred to as the Western study area. The study areas were then composed of a series of blocks combined to conform as closely to the areas of soil vapor contamination as possible. Cancer incident cases in residents at the time of diagnosis in the two areas were included in the study.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No information.
>50% cohort with full latency	No information.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Record study.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Record study.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	347 total cancers in males and females among an estimated population size of 3,540 (1980)–3,002 (2000).

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and sex (ATSDR, 2006a). Age, sex, and race (ATSDR, 2008b). Medical record review of 15 kidney and 6 testicular cancer cases provided limited information on smoking, work history, and residential history for a small percentage of these cases (ATSDR, 2008b).
Statistical methods	
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3.8. Studies in Arizona

B.3.3.8.1. Studies of West Central Phoenix Area, Maricopa County, Arizona.

B.3.3.8.1.1. Aickin et al. (1992), Aickin (2004).

B.3.3.8.1.1.1. Aickin et al. (1992) author's abstract.

Reports of a suspected cluster of childhood leukemia cases in West Central Phoenix have led to a number of epidemiological studies in the geographical area. We report here on a death certificate-based mortality study, which indicated an elevated rate ratio of 1.95 during 1966-1986, using the remainder of the Phoenix standard metropolitan statistical area (SMSA) as a comparison region. In the process of analyzing the data from this study, a methodology for dealing with denominator variability in a standardized mortality ratio was developed using a simple linear Poisson model. This new approach is seen as being of general use in the analysis of standardized rate ratios (SRR), as well as being particularly appropriate for cluster investigations.

B.3.3.8.1.1.2. Aickin (2004) author's abstract.

BACKGROUND AND OBJECTIVES: Classical statistical inference has attained a dominant position in the expression and interpretation of empirical results in biomedicine. Although there have been critics of the methods of hypothesis testing, significance testing (P-values), and confidence intervals, these methods are used to the exclusion of all others. METHODS: An alternative metaphor and inferential computation based on credibility is offered here. RESULTS: It is illustrated in three datasets involving incidence rates, and its advantages over both classical frequentist inference and Bayesian inference, are detailed. CONCLUSION: The message is that for those who are unsatisfied with classical methods but cannot make the transition to Bayesianism, there is an alternative path.

B.3.3.8.1.1.3. Study description and comment.

This study by staff of Arizona Department of Health Services of leukemia mortality or incidence rates among children ≤19 years old living at the time a death in West Central Phoenix in Maricopa County assume residence in the defined geographical area as a surrogate of undefined exposures. Aickin et al. (2004) adopted a classical statistical approach, linear Poisson regression, to estimate age-, sex- and calendar year adjusted RRs for leukemia mortality between 1966 and 1986 among children ≤19 years old living in the study area at the time of death. Leukemia mortality rates for the rest of Maricopa County, excluding the study area and three additional geographic areas previously identified with hazardous waste contamination, were selected as the referent (Aickin et al., 1992). Aickin (2004) adopted inferential or Bayesian approaches to test whether childhood leukemia incidence between 1966 and 1986 would confirm the mortality analysis observation.

Both studies use residence at time of diagnosis or death in the study area, West Central Phoenix, Arizona, as the exposure surrogate; specific exposures such as drinking water contaminants are not examined nor is information on parental factors considered in the analysis. Some information on potential exposures in the community-at-large may be obtained from reports prepared by the AZ DHS of epidemiologic investigations of cancer mortality rates among residents of this area. Aickin et al. (1992) is the published finding on childhood leukemia. Past exposure to the population of West Central Phoenix to environmental contaminants has been difficult to quantify because of a paucity of environmental monitoring data (ADHS, 1990). Community concerns about the environment focused on TCE found in drinking water in late 1981: air pollution, from benzene emission from a nearby major gasoline storage and distribution facility, and pesticide residues. Two wells that occasionally supplemented the water supply in West Central Phoenix were closed after TCE was detected at the wellhead. The levels of TCE measured at the time contamination was detected were 8.9 and 29.0 ppb (report does not identify the number of samples nor concentration ranges). The period over which contaminated water had been supplied from these wells was not known nor whether significant exposure to the population occurred after mixing with surface water. Other compounds identified in the contaminated plume besides TCE included 1,1-DCE, trans-1,2-DCE, chloroform, and chromium. The exposure assessment in the AZ DHS reports is inadequate to describe exposure potential to TCE to subjects of Aickin et al. (1992) and Aickin (2004). Moreover, potential etiologic factors for the observed elevated estimated RR for childhood leukemia bases are not examined. While these studies support an inference of elevated childhood leukemia rates in residents of West Central Phoenix, these studies provide little information on childhood leukemia and TCE exposure and contribute little weight in the overall weight-of-evidence analysis of cancer and TCE.

Aickin M, Chapin CA, Flood TJ, Englender SJ, Caldwell GG. (1992). Assessment of the spatial occurrence of childhood leukemia mortality using standardized rate ratios with a simple linear Poisson model. Int J Epidemiol 21:649–655.

Aickin M. (2004). Bayes without priors. J Clin Epidemiol 57:4–13.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Aickin et al. (1992) illustrated a methodologic approach to reduce variability in rate ratios from small-sized populations. Childhood leukemia mortality in a geographically-defined area in central Phoenix, Arizona, was the case study adopted to illustrate methodologic approach. The analysis was not designed to examine possible etiologic factors. The purpose of Aickin (2004) "was to determine whether a 1.95 standardized mortality ratio [19] for leukemia in West Central Phoenix (compared to the remainder of Maricopa County) would be confirmed in an incidence study" [p. 8].
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Leukemia deaths among children ≤19 yrs of age between the years 1966 and 1986 and with addresses on death certificates in the geographically-defined study area were identified from Arizona death tapes. Referent group is childhood leukemia mortality rate of all other Maricopa residents excluding the study area and three other areas with identified hazardous waste contamination (Aickin et al., 1992). Incident cases of childhood leukemia (≤19 yrs) among residents living in study area were identified from the Arizona Cancer Registry and from cancer registry and medical record reviews at 13 area hospitals (ADHS, 1990).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer mortality (Aickin et al., 1992). Cancer incidence (Aickin, 2004).
Changes in diagnostic coding systems for lymphoma, particularly NHL	Mortality—ICD 7, ICDA 8, ICD 9 (Flood, 1988). Incidence—ICD-O.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Residence in geographical area is a surrogate of undefined exposures; possible exposures are not identified in the paper.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	

>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Record study.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	38 childhood leukemia deaths over a period of 21 yrs. 49 childhood leukemia incident cases over a period of 21 yrs.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and year (1966–1969, 1979–1981, 1982–1986).
Statistical methods	Poisson regression using 1970, 1980, and 1985 population estimates from U.S. Bureau of the Census.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3.8.2. Studies in Tucson, Pima County, Arizona.

B.3.3.8.2.1. Arizona Department of Health Services (1995, 1990).

B.3.3.8.2.1.1. Arizona Department of Health Services (1990) author's summary.

In 1986, responding to community concerns about possible past exposure to low levels of trichloroethylene in drinking water, a committee appointed by the Director of the Arizona Department of health Services recommended that the incidence of childhood leukemia and testicular cancer be studied in the population residing in the Tucson Airport Area (TAA). The study reported here was designed to count all cancer cases occurring in 0-19 year-old Pima County residents, and all testicular cancer cases in Pima County residents of all ages, during the 1970-1986 time period. Based on the incidence rates in the remainder of Pima County, approximately seven cases of childhood leukemia and approximately eight cases of testicular cancer would have been expected in the TAA. Eleven cases of leukemia (SIR = 1.50, 95% C.I. 0.76-2.70) and six cases of testicular cancer (SIR = 0.78, 95% C.I. 0.32-1.59) were observed. Statistical analyses showed that the incidence rates of these cancers were not significantly elevated. Additionally, it was determined that the rates of other childhood cancers in the TAA, grouped as lymphoma, brain/CNS and other, were not significantly elevated. The childhood leukemia, childhood cancer, and testicular cancer rates in Pima County were comparable to rates in other states and cities participating in the National Cancer Institute's Surveillance Epidemiology and End Results Program.

B.3.3.8.2.1.2. Arizona Department of Health Services (1995) author's summary.

In 1986, responding to community concerns about possible past exposure to low levels of trichloroethylene in drinking water, a committee appointed by the Director of the Arizona Department of health Services recommended that the incidence of childhood leukemia and testicular cancer be studied in the population residing in the Tucson Airport Area (TAA). The study reported here was designed to count all cancer cases occurring in 0-19 year-old Pima County residents, and all testicular cancer cases in Pima County residents of all ages, during the 1986-1991 time period. Based on the incidence rates in the remainder of Pima County, approximately 3 cases of childhood leukemia and 4 cases of testicular cancer would have been expected in the TAA. Three cases of leukemia (SIR = .80; 95% C.I. 0.31-2.05) and 4 cases of testicular cancer (SIR = .93; 95% C.I. 0.37-2.35) were observed. Statistical analyses showed that the incidence rates of these cancers were not significantly elevated. Additionally, results indicate no statistically elevated incidence rates of childhood lymphoma, brain/CNS, and other childhood cancers, for ages 0-19, in the TAA. No consistent pattern of disease occurrence was observed when comparing the past incidence and mortality studies conducted by ADHS in the TAA with this present study regarding disease categories.

B.3.3.8.2.1.3. **Study description and comment.**

These reports by staff of AZ DHS of cancer incidence among children ≤19 years old and of testicular cancer incidence among males living at the time a diagnosis in 1970–1986 or 1987–1991 in the Tucson International Airport Area (TAA) of southwest Tucson (ADHS, 1995, 1990) compared to incidence rates for the rest of Pima County were conducted in response to community concerns about cancer and possible past exposure to low levels of TCE in drinking water. In contrast to studies in West Central Phoenix, findings from the 1990 and 1995 AZ DHS studies in Tucson have not been published in the peer-reviewed literature. Childhood cancers included were leukemia, brain/CSN, lymphoma, and a broad category of all other cancers diagnosed in children ≤19 years old. The Arizona Cancer Registry and reviews of medical records of 10 Pima county hospitals served as sources for identifying incident cases. The study area was defined as a geographical area overlaying a plume of contaminated groundwater and was comprised of five census tracts. The approximate areas boundaries are Ajo Way (north), Los Reales Road (south), Country Club Road (east), and the Santa Cruz River (west). Adjacent census tracts in Pima County were aggregated into four separate study areas and incident cancer rates during the 1970–1986 time period (ADHS, 1990) or 1987–1991 (ADHS, 1995) of the aggregated four-area census tract, excluding the TAA area, were used to calculate expected numbers of cancers using the indirect standardization method and population estimates from 1960, 1970, 1975, 1980, and 1985 (ADHS, 1990) or 1990 (ADHS, 1995) of the U.S. Bureau of Census. A secondary analysis of AZ DHS (1990) compared the incidence rate of childhood leukemia and testicular cancer among Pima County residents to that reported to the SEER for a similar time period.

These studies assume residence in the defined geographical area as a surrogate of undefined exposures. The reports do not identify specific exposures for the individual subjects and some information on exposures in the community-at-large may be obtained from Public Health Assessments of the Tucson International Airport Area Superfund Site prepared by the AZ DHS for the ATSDR (2001, 2000). The TAA site includes one main contaminated groundwater plume with smaller areas of groundwater contamination located east of the main plume. Insufficient data existed to evaluate groundwater contamination prior to 1981. Studies conducted by AZ DHS in 1981–1982 showed TCE concentrations of >5 ppb, the maximum contaminant level, in the main groundwater plume with TCE detected in some municipal drinking water wells at concentrations of up to 239 ppb. An ATSDR health assessment conducted in 1988 indicated that soil and groundwater in the Main Plume had been contaminated by chromium and VOCs such as TCE and DCE (ATSDR, 2000). Sampling of private wells from 1981 through 1994 identified both drinking and irrigation private wells in and near the TAA with TCE concentrations ranging from nondetected to 120 ppb. Concentrations of other VOCs and chromium from the 1980s are not presented in the ATSDR reports. Besides groundwater, areas of contaminated soil and sediment have also been identified as part of the site. The “Three

Hangars" area of the airport was found to contain polychlorinated biphenyls in drainage areas with migration off-site into residential neighborhoods (ATSDR, 2001). The exposure assessment in these studies is inadequate to describe exposure to TCE. The studies provide little information on cancer risks and TCE exposure and carry little weight in the overall weight-of-evidence analysis.

AZ DHS (Arizona Department of Health Services). (1990). The incidence of childhood leukemia and testicular cancer in Pima County: 1970–1986. Prepared by the Arizona Department of Health Services, Division of Disease Prevention, Office of Risk Assessment and Investigation, Office of Chronic Disease Epidemiology. September 17, 1990.

AZ DHS (Arizona Department of Health Services). (1995). Update of the incidence of childhood leukemia and testicular cancer in Southwest Tucson, 1987–1991. Prepared by the Arizona Department of Health Services, Office of Risk Assessment and Investigation, Disease Prevention Services. June 6, 1995.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes, from ADHS (1990), "1) To determine whether there was an elevated incidence of leukemia or other cancers among children residing in the Tucson Airport Area (TAA) and 2) To determine whether there was an elevated incidence of testicular cancer in males in the TAA." From ADHS (1995), "The objective of this study is to determine whether the incidence rates of childhood leukemia (ages 0–19) and testicular cancer in males of all ages were significantly elevated in the TAA when compared to the rest of Pima County for the years 1987 through 1991."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cases are identified from the Arizona Cancer Registry and review of medical records at 10 Pima County hospitals. The referent is incidence rates for the remaining population of Pima County, excluding the study area.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly NHL	ICD-O and ICD-9 or equivalent codes from ICDA-8, ICD-7, HICDA, or SNODO.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Residence in geographical area is a surrogate of undefined exposures; possible exposures are not identified in the paper.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Record study.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	ADHS (1990), 31 childhood cancers—11 leukemia cases, 2 lymphoma, 3 CNS/Brain, and 15 other, and 6 testicular cancers. ADHS (1995), 11 childhood cancers—3 leukemia, 1 lymphoma, 2 CNS/Brain, and 5 other, and 4 testicular cancers.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and year.
Statistical methods	SIRs calculated using indirect standardization.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

C. META-ANALYSIS OF CANCER RESULTS FROM EPIDEMIOLOGICAL STUDIES

C.1. METHODOLOGY

An initial review of the epidemiological studies indicated some evidence for associations between TCE exposure and NHL and cancers of the kidney and liver (see Section 4.1). To investigate further these possible associations, we performed meta-analyses of the epidemiological study results for these three cancer types. There was suggestive evidence for some other cancer types, as well; however, fewer TCE studies reported RR estimates for these other site-specific cancers, and meta-analysis was not attempted for these cancer types (see Section 4.1). In addition, at the request of our Science Advisory Board (SAB, 2011), we conducted a meta-analysis of lung cancer in the TCE cohort studies to address the issue of smoking as a possible confounder in the kidney cancer studies (see Section 4.4.2.3).

Meta-analysis provides a systematic way to combine study results for a given effect across multiple (sufficiently similar) studies. The resulting summary (weighted average) estimate is a quantitatively objective way of reflecting results from multiple studies, rather than relying on a single study, for instance. Combining the results of smaller studies to obtain a summary estimate also increases the statistical power to observe an effect, if one exists. Furthermore, meta-analyses typically are accompanied by other analyses of the epidemiological studies, including analyses of publication bias and investigations of possible factors responsible for any heterogeneity across studies.

Given the diverse nature of the epidemiological studies for TCE, random-effects models were used for the primary analyses, and fixed-effect analyses were conducted for comparison. Both approaches combine study results (in this case, RR estimates) weighted by the inverse variance; however, they differ in their underlying assumptions about what the study results represent and how the variances are calculated. For a random-effects model, it is assumed that there is true heterogeneity across studies and that both between-study and within-study components of variation need to be taken into account; this was done using the methodology of DerSimonian and Laird (1986). For a fixed-effect model, it is assumed that the studies are all essentially measuring the same thing and all of the variance is within-study variance; thus, for the fixed-effect model, the RR estimate from each study is simply weighted by the inverse of the (within-study) variance of the estimate.

Studies for the meta-analyses were selected as described in Appendix B, Section B.2.9. Because each of the cancer types being evaluated is considered rare in the populations being studied (all have lifetime risks <10%, and all but lung cancer have lifetime risks <3%), the different measures of RR (e.g., ORs, risk ratios, and rate ratios) are good approximations of each

other (Rothman and Greenland, 1998) and are included together as RR estimates in the meta-analyses. (In addition, the meta-analyses of lung cancer and liver cancer comprised only cohort studies and, thus, no ORs were included in those analyses.) The general approach for selecting RR estimates was to select the reported RR estimate that best reflected an RR for TCE exposure vs. no TCE exposure (overall effect). When multiple estimates were available for the same study based on different subcohorts with different inclusion criteria, the preference for overall exposure was to select the RR estimate that represented the largest population in the study, while trying to minimize the likelihood of TCE exposure misclassification. A subcohort with more restrictive inclusion criteria was selected if the basis was to reduce exposure misclassification (e.g., including only subjects with more probable TCE exposure), but not if the basis was to reflect subjects with greater exposure (e.g., routine vs. any exposure).

When available, RR estimates from internal analyses were selected over standardized incidence or mortality ratios (SIRs, SMRs) and adjusted RR estimates were generally selected over crude estimates. Incidence estimates would normally be preferred to mortality estimates; however, for the two studies providing both incidence and mortality results, incidence ascertainment was for a substantially shorter period of time than mortality follow-up, so the endpoint with the greater number of cases was used to reflect the results that had better case ascertainment. Furthermore, RR estimates based on exposure estimates that discounted an appropriate lag time prior to disease onset were typically preferred over estimates based on unlagged exposures, although few studies reported lagged results.

For separate analyses, an RR estimate for the highest exposure group was selected from studies that presented results for different exposure groups. Exposure groups based on some measure of cumulative exposure were preferred, if available; however, duration was often the sole exposure metric used.

Sensitivity analyses were generally done to investigate the impact of alternate selection choices, as well as to estimate the impact of study findings that were not reported. Specific selection choices are described in the following subsections detailing the actual analyses.

The meta-analysis calculations are based on (natural) logarithm-transformed values. Thus, each RR estimate was transformed to its natural logarithm (referred to here as "log RR," the conventional terminology in epidemiology), and either an estimate of the SE of the log RR was obtained, from which to estimate the variance for the weights, or an estimate of the variance of the log RR was calculated directly. If the reported 95% CI limits were proportionally symmetric about the observed RR estimate (i.e., UCL/RR ≈ RR/LCL), then an estimate of the SE of the log RR estimate was obtained using the formula

$$SE = \frac{\left[\log\left(UCL\right) - \log\left(LCL\right)\right]}{3.92}, \qquad \text{(Eq. C-1)}$$

where UCL is the upper confidence limit and LCL is the lower confidence limit (for 90% CIs, the divisor is 3.29) (Rothman and Greenland, 1998). In all of the TCE cohort studies reporting SMRs or SIRs as the overall RR estimates, reported CIs were calculated assuming the number of deaths (or cases) is approximately Poisson distributed. In such cases, the CIs are not proportionally symmetric about the RR estimate (unless the number of deaths is fairly large), and the SE of the log RR estimate was estimated as the inverse of the square root of the observed number of deaths (or cases) (Breslow and Day, 1987). In some case-control studies, no overall OR was reported, so a crude OR estimate was calculated as OR = (a/b)/(c/d), where a, b, c, and d are the cell frequencies in a 2 × 2 table of cancer cases vs. TCE exposure, and the variance of the log OR was estimated using the formula

$$Var\left[\log\left(OR\right)\right] = \frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}, \qquad \text{(Eq. C-2)}$$

in accordance with the method proposed by Woolf (1955), as described by Breslow and Day (1980).

The analyses that were performed for this assessment include:

- meta-analyses to obtain overall summary estimates of RR (denoted RRm),
- heterogeneity analyses,
- analyses of the influence of single studies on the summary estimates,
- analyses of the sensitivity of the summary estimates to alternate study inclusion selections or to alternate selections of RR estimates from a study,
- publication bias analyses,
- meta-analyses to obtain summary estimates for the highest exposure groups in studies that provide data by exposure group, and
- consideration of some potential sources of heterogeneity across studies.

The analyses were conducted using Microsoft Excel spreadsheets and the software package Comprehensive Meta-Analysis, Version 2 (© 2006, Biostat, Inc.). Funnel plots and cumulative analyses plots were generated using the Comprehensive Meta-Analysis software, and forest plots were created using SAS, Version 9.2 (© 2002–2008, SAS Institute Inc.).

The heterogeneity (or homogeneity) analysis tests the hypothesis that the study results are homogeneous (i.e., that all of the RR estimates are estimating the same population RR and the total variance is no more than would be expected from within-study variance). Heterogeneity was assessed using the statistic *Q* described by DerSimonian and Laird (1986). The *Q*-statistic

represents the sum of the weighted squared differences between the summary RR estimate (obtained under the null hypothesis [i.e., using a fixed-effect model]) and the RR estimate from each study, and, under the null hypothesis, Q approximately follows a χ^2 distribution with degrees of freedom equal to the number of studies minus one. However, this test can be under-powered when the number of studies is small, and it is only a significance test (i.e., it is not very informative about the *extent* of any heterogeneity). Therefore, the I^2 value (Higgins et al., 2003) was also considered. $I^2 = 100\% \times (Q - df)/Q$, where Q is the Q-statistic and df is the degrees of freedom, as described above. This value estimates the percentage of variation that is due to study heterogeneity. Typically, I^2 values of 25, 50, and 75% are considered low, moderate, and high amounts of heterogeneity, respectively. For a negative value of $(Q - df)$, I^2 is set to 0%, indicating no observable heterogeneity.

Subgroup analyses were sometimes conducted to examine whether or not the combined RR estimate varied significantly between different types of studies (e.g., case-control vs. cohort studies). In such subgroup analyses of categorical variables (e.g., study design), ANOVA was used to determine if there was significant heterogeneity between the subgroups. Applying ANOVA to meta-analyses with two subgroups (df = 1), $Q_{\text{between subgroups}} = Q_{\text{overall}} - (Q_{\text{subgroup1}} + Q_{\text{subgroup2}}) = z\text{-value}^2$, where Q_{overall} is the Q-statistic calculated across all of the studies and $Q_{\text{subgroup1}}$ and $Q_{\text{subgroup2}}$ are the Q-statistics calculated within each subgroup.

Publication bias is a systematic error that occurs if statistically significant studies are more likely to be submitted and published than nonsignificant studies. Studies are more likely to be statistically significant if they have large effect sizes (in this case, RR estimates); thus, an upward bias would result in a meta-analysis if the available published studies have higher effect sizes than the full set of studies that were actually conducted. One feature of publication bias is that smaller studies tend to have larger effect sizes than larger studies, since smaller studies need larger effect sizes in order to be statistically significant. Thus, many of the techniques used to analyze publication bias examine whether or not effect size is associated with study size. Methods used to investigate potential publication bias for this assessment included funnel plots, which plot effect size vs. study size (actually, SE vs. log RR here); the "trim and fill" procedure of Duval and Tweedie (2000), which imputes the "missing" studies in a funnel plot (i.e., the studies needed to counterbalance an asymmetry in the funnel plot resulting from an ostensible publication bias) and recalculates a summary effect size with these studies present; forest plots (arrays of RRs and CIs by study) sorted by precision (i.e., SE) to see if effect size shifts with study size; Begg and Mazumdar rank correlation test (Begg and Mazumdar, 1994), which examines the correlation between effect size estimates and their variances after standardizing the effect sizes to stabilize the variances; Egger's linear regression test (Egger et al., 1997), which tests the significance of the bias reflected in the intercept of a regression of effect size/SE on 1/SE; and cumulative meta-analyses after sorting by precision to assess the impact on the summary effect size estimate of progressively adding the smaller studies.

C.2. META-ANALYSIS FOR NHL

C.2.1. Overall Effect of TCE Exposure

C.2.1.1. Selection of RR Estimates

The selected RR estimates for NHL associated with TCE exposure from the selected epidemiological studies are presented in Table C-1 for cohort studies and in Table C-2 for case-control studies. Some of the more recent case-control studies classified NHLs along the lines of the recent World Health Organization/Revised European-American Classification of Lymphoid Neoplasms (WHO/REAL) classification system (Harris et al., 2000), which recognizes lymphocytic leukemias and multiple myelomas (plasma cell myelomas) as (non-Hodgkin) lymphomas; however, most of the available TCE studies reported NHL results according to the International Classification of Diseases (ICD), Revisions 7, 8, and 9, using a traditional definition of NHL that excluded lymphocytic leukemias and multiple myelomas and focused on ICD-7, -8, -9 codes 200 + 202. For consistency of endpoint in the NHL meta-analyses, RR estimates for ICD 200 + 202 were selected, wherever possible; otherwise, estimates for the classification(s) best approximating this traditional definition of NHL were selected. In addition, many of the studies provided RR estimates only for males and females combined, and we are not aware of any basis for a sex difference in the effects of TCE on NHL risk; thus, wherever possible, RR estimates for males and females combined were used. The only study of much size (in terms of number of NHL cancer cases) that provided results separately by sex was Raaschou-Nielsen et al. (2003). This study reports an insignificantly higher SIR for females (1.4, 95% CI: 0.73, 2.34) than for males (1.2, 95% CI: 0.98, 1.52).

Table C-1. Selected RR estimates for NHL associated with TCE exposure (overall effect) from cohort studies

Study	RR	95% LCL	95% UCL	RR type	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Anttila et al. (1995)	1.81	0.78	3.56	SIR	0.593	0.354	None	ICD-7 200 + 202.
Axelson et al. (1994)	1.52	0.49	3.53	SIR	0.419	0.447	1.36 (0.44, 3.18) with estimated female contribution to SIR added (see text)	ICD-7 200 and 202. Results reported separately; combined assuming Poisson distribution. Results reported for males only, but there was a small female component to the cohort.
Boice et al. (1999)	1.19	0.83	1.65	SMR	0.174	0.267	1.19 (0.65, 1.99) for potential routine exposure	ICD-9 200 + 202. For any potential exposure.
Greenland et al. (1994)	0.76	0.24	2.42	Mortality OR	–0.274	0.590	None	ICD-8 200-202. Nested case-control study.
Hansen et al. (2001)	3.1	1.3	6.1	SIR	1.13	0.354	None	ICD-7 200 + 202. Male and female results reported separately; combined assuming Poisson distribution.
Morgan et al. (1998)	1.01	0.46	1.92	SMR	0.00995	0.333	1.36 (0.35, 5.21) unpublished RR for ICD 200 (see text)	ICD 200 + 202. Results reported by Mandel et al. (2006). ICD Revision 7, 8, or 9, depending on year of death.
Raaschou-Nielsen et al. (2003)	1.24	1.01	1.52	SIR	0.215	0.104	1.5 (1.2, 2.0) for subcohort with expected higher exposures	ICD-7 200 + 202.
Radican et al. (2008)	1.36	0.77	2.39	Mortality hazard ratio	0.307	0.289	None	ICD-8,-9 200 + 202; ICD-10 C82-C85. Time variable = age; covariates = sex and race. Referent group is workers with no chemical exposures.
Zhao et al. (2005)	1.44	0.90	2.30	Mortality RR	0.363	0.239	Incidence RR: 0.77 (0.42, 1.39) Boice 2006 SMR for ICD-9 200 + 202: 0.21 (0.01, 1.18)	All lymphohematopoietic cancer (ICD-9 200-208), not just 200 + 202. Males only; adjusted for age, SES, time since first employment. Mortality results reflect more exposed cases (33) than do incidence results (17). Overall RR estimated by combining across exposure groups (see text). Boice et al. (2006b) cohort overlaps Zhao et al. (2005) cohort; just 1 exposed death for ICD 200 + 202; 9 for 200–208 vs. 33 in Zhao et al. (2005).

Table C-2. Selected RR estimates for NHL associated with TCE exposure from case-control studies[a]

Study	RR	95% LCL	95% UCL	log RR	SE (log RR)	NHL type	Comments
Cocco et al. (2010)	0.8	0.5	1.1	-0.223	0.201	NHL	Grouping consistent with traditional NHL definition provided by author (see text). High-confidence subgroup. Adjusted for age, sex, center, and education.
Hardell et al. (1994)	7.2	1.3	42	1.97	0.887	NHL	Rappaport classification system. Males only; controls matched for age, place of residence, vital status.
Miligi et al. (2006)	0.93[b]	0.67[b]	1.29[b]	–0.0726	0.168	NHL + CLL	NCI Working Formulation. Crude OR; overall adjusted OR not presented.
Nordstrom et al. (1998)	1.5	0.7	3.3	0.405	0.396	Hairy cell leukemia	Hairy cell leukemia specifically. Males only; controls matched for age and county; analysis controlled for age.
Persson and Frederikson (1999)	1.2	0.5	2.4	0.182	0.400	NHL	Classification system not specified. Controls selected from same geographic areas; OR stratified on age and sex.
Purdue et al. (2011)	1.4	0.8	2.4	0.336	0.280	NHL	ICD-O-3 codes 967-972. Probable-exposure subgroup. Adjusted for age, sex, SEER center, race, and education.
Siemiatycki (1991)	1.1	0.5	2.5	0.0953	0.424	NHL	ICD-9 200 + 202. SE and 95% CI calculated from reported 90% CIs; males only; adjusted for age, income, and cigarette smoking index.
Wang et al. (2009)	1.2	0.9	1.8	0.182	0.177	NHL	ICD-O M-9590-9595, 9670-9688, 9690-9698, 9700-9723. Females only; adjusted for age, family history of lymphohematopoietic cancers, alcohol consumption, and race.

[a]The RR estimates are all ORs for incident cases.
[b]As calculated by U.S. EPA.

Most of the selections in Tables C-1 and C-2 should be self-evident, but some are discussed in more detail here, in the order the studies are presented in the tables. For Axelson et al. (1994), in which a small subcohort of females was studied but only results for the larger male subcohort were reported, the reported male-only results were used in the primary analysis; however, an attempt was made to estimate the female contribution to an overall RR estimate for both sexes and its impact on the meta-analysis. Axelson et al. (1994) reported that there were no cases of NHL observed in females, but the expected number was not presented. To estimate the expected number, the expected number for males was multiplied by the ratio of female-to-male person-years in the study and by the ratio of female-to-male age-adjusted incidence rates for NHL.[4] The male results and the estimated female contribution were then combined into an RR estimate for both sexes assuming a Poisson distribution, and this alternate RR estimate for the Axelson et al. (1994) study was used in a sensitivity analysis.

For Boice et al. (1999), results for "any potential exposure" were selected for the primary analysis, because this exposure category was considered to best represent overall TCE exposure, and results for "potential routine exposure," which was characterized as reflecting workers assumed to have received more cumulative exposure, were used in a sensitivity analysis.

The Greenland et al. (1994) study is a case-control study nested within a worker cohort, and we treat it here as a cohort study (see Appendix B, Section B.2.9.1). Greenland et al. (1994) report results only for all lymphomas, including Hodgkin lymphoma (ICD-8 201).

For Morgan et al. (1998), the reported results did not allow for the combination of ICD 200 and 202, so the SMR estimate for the combined 200 + 202 grouping was taken from the meta-analysis paper of Mandel et al. (2006), who included one of the investigators from the Morgan et al. (1998) study. RR estimates for overall TCE exposure from internal analyses of the Morgan et al. (1998) cohort data were available from an unpublished report (EHS, 1997) (the published paper only presented the internal analyses results for exposure subgroups), but only for ICD 200; from these, the RR estimate from the Cox model that included age and sex was selected, because those are the variables deemed to be important in the published paper (Morgan et al., 1998). Although the results from internal analyses are generally preferred, in this case, the SMR estimate was used in the primary analysis and the internal analysis RR estimate was used in a sensitivity analysis because the latter estimate represented an appreciably smaller number of deaths (3, based on ICD 200 only) than the SMR estimate (9, based on ICD 200 + 202).

[4]Person-years for men and women $\leq$79 years were obtained from Axelson et al. (1994): 23516.5 and 3691.5, respectively. Lifetime age-adjusted incidence rates for NHL for men and women were obtained from the National Cancer Institute's 2000-2004 SEER-17 (Surveillance Epidemiology and End Results from 17 geographical areas) database (http://seer.cancer.gov/statfacts/html/nhl.html): 23.2/100,000 and 16.3/100,000, respectively. The calculation for estimating the expected number of cases in females in the cohort assumes that the males and females have similar TCE exposures and that the relative distributions of age-related incidence risk for the males and females in the Swedish cohort are adequately represented by the ratios of person-years and U.S. lifetime incidence rates used in the calculation.

For Raaschou-Nielsen et al. (2003), results for the full cohort were used for the primary analysis and results for the subcohort with expected higher exposure levels (≥1-year duration of employment and year of 1st employment before 1980) were used in a sensitivity analysis. Raaschou-Nielsen et al. (2003), in their Table 3, also present overall results for NHL with a lag time of 20 years; however, they use a definition of lag that is different from a lagged exposure in which exposures prior to disease onset are discounted and it is not clear what their lag time actually represents[5], thus these results were not used in any of the meta-analyses for NHL.

For Radican et al. (2008), the Cox model hazard ratio from the 2000 follow-up was used. In the Radican et al. (2008) Cox regressions, age was the time variable, and sex and race were covariates. It should also be noted that the referent group is composed of workers with no chemical exposures, not just no exposure to TCE.

For Zhao et al. (2005), RR estimates were only reported for ICD-9 200–208 (all lymphohematopoietic cancers), and not for 200 + 202 alone. Given that other studies have not reported associations between leukemias and TCE exposure, combining all lymphohematopoietic cancers would dilute any NHL effect, and the Zhao et al. (2005) results are expected to be an underestimate of any TCE effect on NHL alone. Another complication with the Zhao et al. (2005) study is that no results for an overall TCE effect are reported. We were unable to obtain any overall estimates from the study authors, so, as a best estimate, the results across the "medium" and "high" exposure groups were combined, under assumptions of group independence, even though the exposure groups are not independent (the "low" exposure group was the referent group in both cases). Zhao et al. (2005) present RR estimates for both incidence and mortality; however, the time frame for the incidence accrual is smaller than the time frame for mortality accrual and fewer exposed incident cases (17) were obtained than deaths (33). Thus, because better case ascertainment occurred for mortality than for incidence, the mortality results were used for the primary analysis, and the incidence results were used in a sensitivity analysis. A sensitivity analysis was also done using results from Boice et al. (2006b) in place of the Zhao et al. (2005) RR estimate. The cohorts for these studies overlap, so they are not independent studies and should not be included in the meta-analysis concurrently. Boice et al. (2006b) report an RR estimate for an overall TCE effect for NHL alone; however, it is based on far fewer cases (1 death in ICD-9 200 + 202; 9 deaths for 200–208) and is an SMR rather than an internal analysis RR estimate, so the Zhao et al. (2005) estimates are preferred for the primary analysis.

For the case-control studies, the main issue was the NHL classifications. Cocco et al. (2010) present results for NHLs classified according to the WHO/REAL classification system (i.e., including lymphocytic leukemias and multiple myelomas). For this meta-analysis, we were able to obtain results for a grouping of lymphomas generally consistent with the traditional

[5]In their Methods section, Raaschou-Nielsen et al. (2003) define their lag period as the period "from the date of first employment to the start of follow-up for cancer".

definition of NHL (T-cell lymphomas and B-cell lymphomas, excluding Hodgkin lymphomas, CLLs, multiple myelomas, and unspecified lymphomas) from Dr. Cocco (personal communication from Pierluigi Cocco, University of Cagliari, Italy, to Cheryl Scott, U.S. EPA, 19 March 2011; see Section 4.6.1.2). The results used in the meta-analyses are for the high-confidence subgroup, which included workers with jobs with a "certain" probability of exposure and >90% of workers exposed (5.5% of cases).

Hardell et al. (1994) used the Rappaport classification system, which, according to Weisenburger (1992) is consistent with the traditional definition of NHL.

Miligi et al. (2006) include CLLs in their NHL results, consistent with the current WHO/REAL classification. Also, Miligi et al. (2006) do not report an overall adjusted RR estimate, so a crude estimate of the OR was calculated for the two TCE exposure categories together vs. no TCE exposure.

The Nordstrom et al. (1998) study was a case-control study of hairy cell leukemias, so only results for hairy cell leukemia were reported. Hairy cell leukemias are a subgroup of NHLs under current classification systems, but they were not included in the traditional definition of NHL.

Persson and Frederikson (1999) did not report the classification system used.

According to Schenk et al.(2009), Purdue et al. (2011) used ICD-O-3 codes 967-972, which are generally consistent with the traditional definition of NHL. The results used in the meta-analyses are for the probable-exposure subgroup, which includes workers with at least one job assigned an exposure probability of ≥50% (3.8% of cases).

According to Zhang et al. (2004), Wang et al. (2009) used ICD-O-2 codes M-9590-9595, 9670-9688, 9690-9698, 9700-9723, which are consistent with the traditional definition of NHL (i.e., ICD-7, -8, -9 codes 200 + 202).

No alternate RR estimates were considered for any of the case-control studies of NHL. For the Cocco et al. (2010) and Purdue et al. (2011) studies, the RR estimates used are for a higher confidence subgroup. No overall results for the full studies were presented to use as alternative estimates. Results for lower confidence subgroups were presented separately, but no attempt was made to combine the results across confidence groups because these results were not independent, as they relied on the same referent groups.

An alternate analysis was done including only the studies for which RR estimates for the traditional definition of NHL were available. In this analysis, Miligi et al. (2006), Nordstrom et al.(1998), Persson and Frederikson (1999), and Greenland et al. (1994) were omitted and the Boice et al. (2006b) cohort study was used instead of Zhao et al. (2005).

C.2.1.2. Results of Meta-Analyses

Results from some of the meta-analyses that were conducted on the epidemiological studies of TCE and NHL are summarized in Table C-3. The summary estimate (RRm) from the

primary random-effects meta-analysis of the 17 studies was 1.23 (95% CI: 1.07, 1.42) (see Figure C-1). No single study was overly influential; removal of individual studies resulted in RRm estimates that ranged from 1.18 (with the removal of Hansen et al. (2001)) to 1.27 (with the removal of Miligi et al. (2006) or Cocco et al. (2010)) and were all statistically significant (all with $p < 0.02$). Removal of Hardell et al. (1994), whose RR estimate is a relative outlier (see Figure C-1), only decreased the RRm estimate to 1.21 (95% CI: 1.07, 1.38), since this study does not contribute a lot of weight to the meta-analysis. Removal of studies other than Hansen et al. (2001) resulted in RRm estimates that were all >1.20.

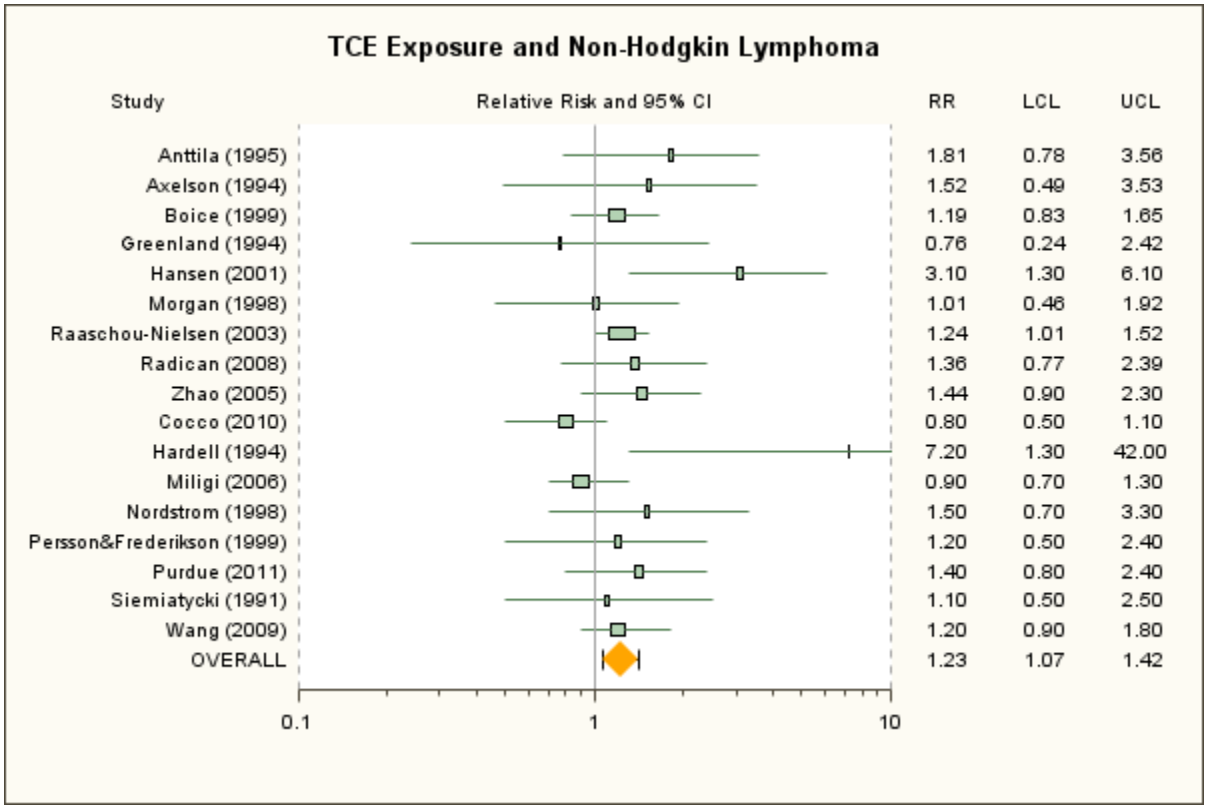


Figure C-1. Meta-analysis of NHL and overall TCE exposure. Rectangle sizes reflect relative weights of the individual studies. The bottom diamond represents the summary RR estimate.

Similarly, the RRm estimate was not highly sensitive to alternate RR estimate selections. Use of the six alternate selections, individually, resulted in RRm estimates that ranged from 1.20 to 1.28 (see Table C-3) and were all statistically significant (all with $p < 0.03$).

Nor was the RRm estimate highly sensitive to restriction of the meta-analysis to only those studies for which RR estimates for the traditional definition of NHL were available. An alternate analysis which omitted Miligi et al. (2006) (which included CLLs), Nordstrom et al. (1998) (which was a study of hairy cell leukemias), Persson and Frederikson (1999) (for which

the classification system not specified), and Greenland et al. (1994) (which included Hodgkin lymphomas) and which included Boice et al. (2006b) instead of Zhao et al. (2005) (which included all lymphohematopoietic cancers) yielded an RRm estimate of 1.27 (95% CI: 1.05, 1.55).

There was some heterogeneity apparent across the 17 studies, although it was not statistically significant ($p = 0.16$). The I^2-value (see Section C.1) was 26%, suggesting low-to-moderate heterogeneity. This small amount of heterogeneity is also indicated by the finding that the RRm estimate from the fixed-effect analysis was slightly different from that of the random-effects model (1.21 vs. 1.23) and had a slightly narrower 95% CI (1.08–1.35 vs. 1.07–1.42). In addition, nonsignificant heterogeneity was apparent in each of the meta-analyses with alternate RR selections—p-values ranged from 0.09 to 0.17 and I^2-values ranged from 25 to 34%.

To investigate the heterogeneity, subgroup analyses were done examining the cohort and case-control studies separately. With the random-effects model (and tau-squared not pooled across subgroups), the resulting RRm estimates were 1.33 (95% CI: 1.13, 1.58) for the cohort studies and 1.11 (95% CI: 0.89, 1.38) for the case-control studies. There was residual heterogeneity in each of the subgroups, but in neither case was it statistically significant. I^2-values were 12% for the cohort studies, suggesting low heterogeneity, and 27% for the case-control studies, suggesting low-to-moderate heterogeneity. The difference between the RRm estimates for the cohort and case-control subgroups was not statistically significant. Some thought was given to further analyses to investigate the source(s) of the heterogeneity, such as qualitative tiering or subgroups based on likelihood for correct exposure classification or on likelihood for higher vs. lower exposures across the studies. Ultimately, these approaches were rejected because in many of the studies, it was difficult to judge (and weight) the extent of exposure misclassification or the degree of TCE exposure with any precision. In other words, there was inadequate information to reliably assess either the extent to which each study accurately classified exposure status or the relative TCE exposure levels and prevalences of exposure to different levels across studies. See Section C.2.3 for a qualitative discussion of some potential sources of heterogeneity.

Table C-3. Summary of some meta-analysis results for TCE (overall) and NHL

Analysis	Number of studies	Model	RRm estimate	95% LCL	95% UCL	Heterogeneity	Comments
All studies	17	Random	1.23	1.07	1.42	Not significant (p = 0.16) I^2 = 26%	Statistical significance of RRm not dependent on individual studies.
		Fixed	1.21	1.08	1.35		
Cohort	9	Random	1.33	1.13	1.58	Not significant (p = 0.34) I^2 = 12%	Not significant difference between CC and cohort studies (p = 0.19).
		Fixed	1.31	1.14	1.51		Not significant difference between CC and cohort studies (p = 0.08).
Case-control	8	Random	1.11	0.89	1.38	Not significant (p = 0.22) I^2 = 27%	
		Fixed	1.07	0.90	1.28		
Alternate RR selections[a]	17	Random	1.20	1.03	1.39	Not significant (p = 0.11) I^2 = 31%	With estimated Zhao et al. (2005) overall RR for incidence rather than mortality.
	17	Random	1.22	1.03	1.43	Not significant (p = 0.09) I^2 = 34%	With Boice et al. (2006b) study rather than Zhao et al. (2005).
	17	Random	1.23	1.07	1.42	Not significant (p = 0.16) I^2 = 25%	With estimated female contribution to Axelson et al. (1994).
	17	Random	1.24	1.07	1.44	Not significant (p = 0.16) I^2 = 26%	With Boice et al. (1999) potential routine exposure SMR.
	17	Random	1.25	1.08	1.44	Not significant (p = 0.17) I^2 = 25%	With Morgan et al. (1998) unpublished RR.
	17	Random	1.28	1.09	1.49	Not significant (p = 0.09) I^2 = 34%	With Raaschou-Nielsen et al. (2003) subgroup expected to have higher exposures
Alternate analysis; traditional definition of NHL only	13	Random	1.27	1.05	1.55	Not significant (p = 0.054) I^2 = 42%	Omitting Miligi et al. (2006), Nordstrom et al. (1998), Persson and Frederikson (1999), and Greenland et al. (1994), and including Boice et al. (2006b) instead of Zhao et al. (2005).

TableC-3. Summary of some meta-analysis results for TCE (overall) and NHL (continued)

Analysis	Number of studies	Model	RRm estimate	95% LCL	95% UCL	Heterogeneity	Comments
Highest exposure groups	13	Random	1.43	1.13	1.82	Not significant (p = 0.30) I^2 = 14%	Statistical significance not dependent on single study. See Table C-5 for results with alternate RR selections.
		Fixed	1.43	1.16	1.75		

[a]Changing the primary analysis by one alternate RR each time; more details on alternate RR estimates in text.

As discussed in Section C.1, publication bias was examined in several different ways. The funnel plot in Figure C-2 suggests some relationship between RR estimate and study size (if there were no relationship, the studies would be symmetrically distributed around the summary RR estimate rather than veering towards higher RR estimates with increasing SEs), although the observed asymmetry is highly influenced by the Hardell et al. (1994) study, which is a relative outlier and which contributes little weight to the overall meta-analysis, as discussed above. The Begg and Mazumdar (1994) rank correlation test and Egger et al.'s (1997) linear regression test were not statistically significant (the one-tailed *p*-values were 0.18 and 0.07, respectively); it should be noted, however, that both of these tests have low power. The trim-and-fill procedure of Duval and Tweedie (2000) yielded a summary RR estimate (under the random-effects model) of 1.15 (95% CI: 0.97, 1.36) when the four studies deemed missing from the funnel plot were filled into the meta-analysis (these studies are filled in so as to counter-balance the apparent asymmetry of the more extreme values in the funnel plot). Eliminating the Hardell et al. (1994) study made little difference to the results of the publication bias analyses. The results of a cumulative meta-analysis, incorporating studies with increasing SE one at a time, are depicted in Figure C-3. This procedure is a transparent way of examining the effects of including studies with increasing SE. The figure shows that the summary RR estimate is 1.16 after inclusion of the four largest (i.e., most precise) studies, which constitute about 50% of the weight. The RRm estimate decreases to 1.10 with the inclusion of the next most precise study, which contributes another 9% of the total weight. The RRm estimate increases to 1.22 with inclusion of the 6 next most precise studies; this summary estimate represents 11 of the 17 studies and about 87% of the weight. Adding in the 6 least precise studies (13% of the weight) barely increases the RRm estimate further. In summary, there is some evidence of potential publication bias in this data set. It is uncertain, however, that this reflects actual publication bias rather than an association between effect size and SE resulting for some other reason, e.g., a difference in study populations or protocols in the smaller studies. Furthermore, if there is publication bias in this data set, it does not appear to account completely for the findings of an increased NHL risk.

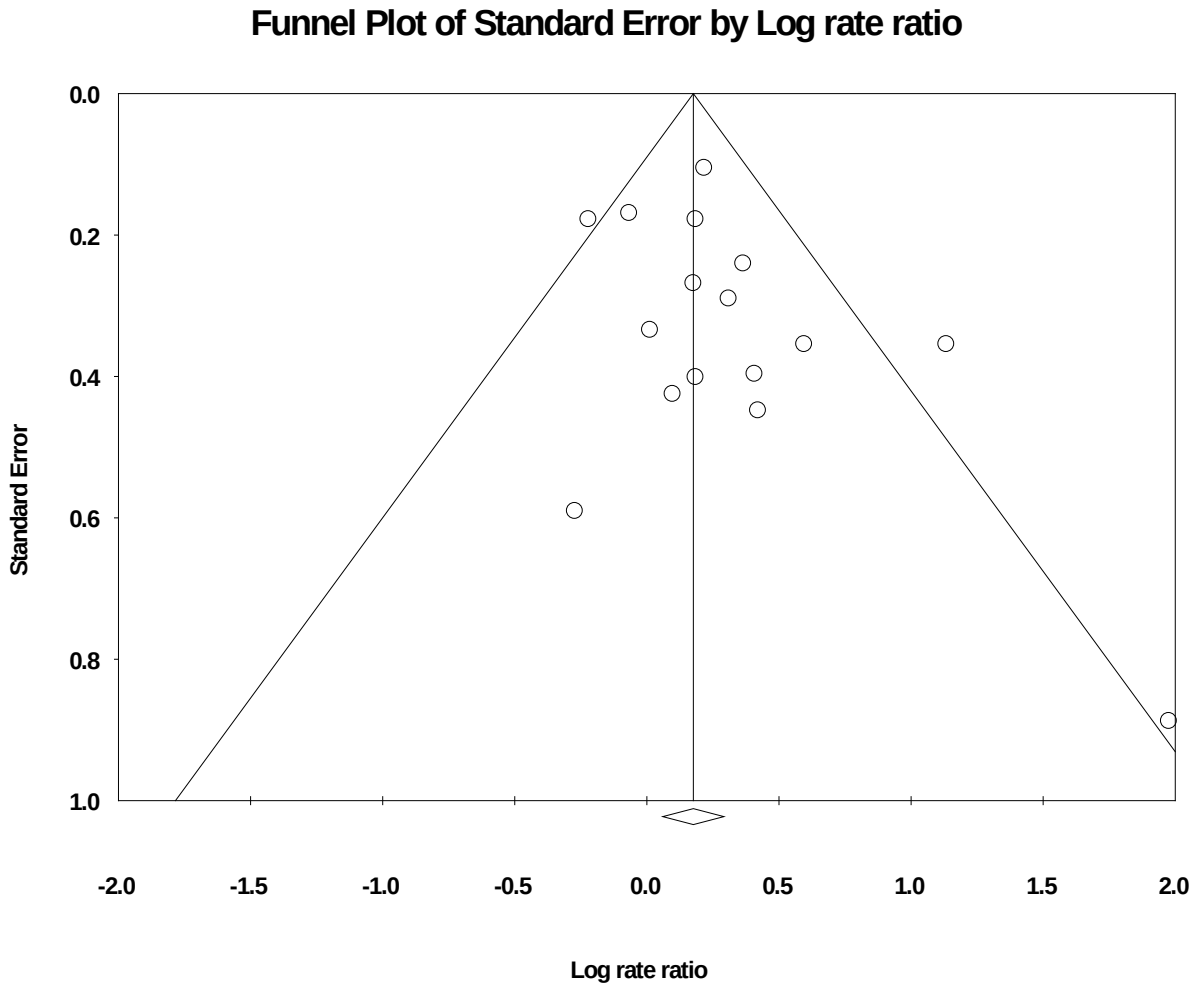


Figure C-2. Funnel plot of SE by log RR estimate for TCE and NHL studies.

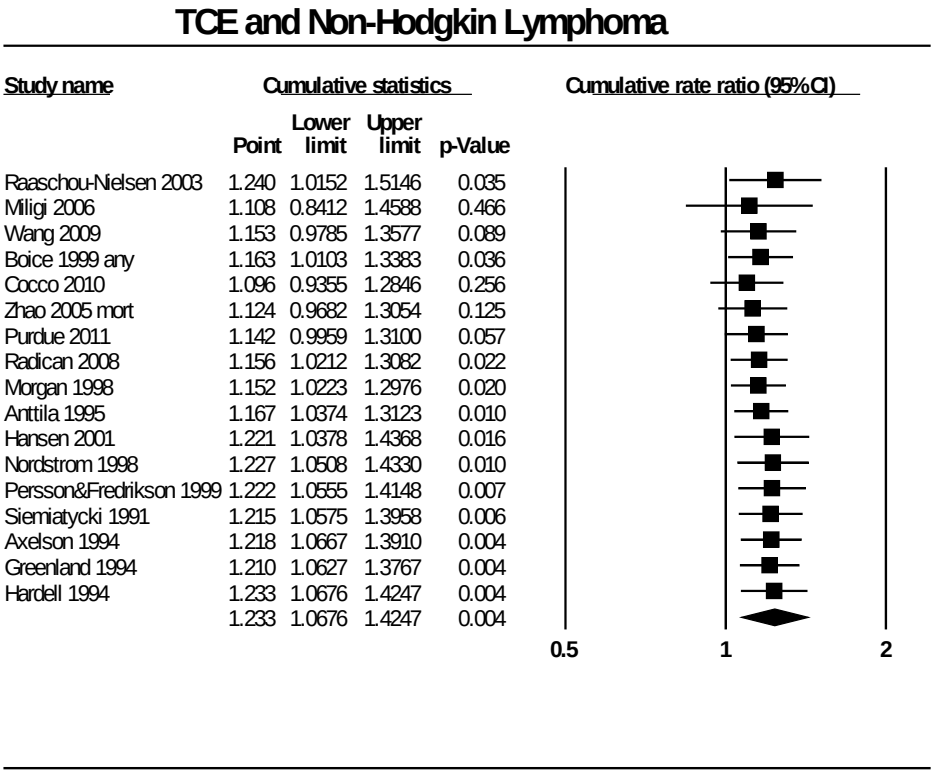


Figure C-3. Cumulative meta-analysis of TCE and NHL studies, progressively including studies with increasing SEs.

C.2.2. NHL Effect in the Highest Exposure Groups

C.2.2.1. Selection of RR Estimates

The selected RR estimates for NHL in the highest TCE exposure categories, for studies that provided such estimates, are presented in Table C-4. All eight cohort studies (but not the nested case-control study of Greenland et al. (1994) and five of the eight case-control studies did report NHL risk estimates categorized by exposure level. As in Section C.2.1.1 for the overall risk estimates, estimates to best correspond to NHL as represented by ICD-7, -8, and -9 200 and 202 were selected, and, wherever possible, RR estimates for males and females combined were used.

Table C-4. Selected RR estimates for NHL risk in highest TCE exposure groups

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Anttila et al. (1995)	1.4	0.17	5.04	100+ µmol/L U-TCA[a]	0.336	0.707	none	SIR. ICD 200 + 202.
Axelson et al. (1994)	6.25	0.16	34.83	≥2 yrs exposure and 100+ mg/L U-TCA	1.83	1.00	5.62 (0.14, 31.3) with estimated female contribution added (see text)	SIR. ICD 200 + 202. Results reported for males only, but there was a small female component to the cohort.
Boice et al. (1999)	1.62	0.82	3.22	≥5 yrs exposure	0.482	0.349	None	Mortality RR. ICD 200 + 202. For potential routine or intermittent exposure. Adjusted for date of birth, dates 1st and last employed, race, and sex. Referent group is workers not exposed to any solvent.
Hansen et al. (2001)	2.7	0.56	8.0	≥1,080 months × mg/m^3	0.993	0.577	3.7 (1.0, 9.5) for ≥75 months exposure duration 2.9 (0.79, 7.5) for ≥19 mg/m^3 mean exposure	SIR. ICD 200 + 202. Exposure-group results presented only for males. Female results estimated and combined with male results assuming Poisson distribution (see text).
Morgan et al. (1998)	0.81	0.1	6.49	High cumulative exposure score	–0.211	1.06	1.31 (0.28, 6.08) for med/high peak vs. low/no	Mortality RR. ICD 200 only. Adjusted for age and sex.
Raaschou-Nielsen et al. (2003)	1.6	1.1	2.2	≥5 yrs in subcohort with expected higher exposure. levels	0.470	0.183	1.45 (0.99, 2.05) for ≥5 yrs in full cohort, both sexes combined	SIR. ICD 200 + 202.

Table C-4. Selected RR estimates for NHL risk in highest TCE exposure groups (continued)

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Radican et al. (2008)	1.41	0.71	2.81	>25 unit-yrs	0.337	0.350	Blair et al. (1998) 0.97 (0.42, 2.2) incidence RR	Mortality hazard ratio. ICD 200 + 202. Male and female results presented separately and combined (see text). Cox regression time variable = age; covariate = race. Referent group is workers with no chemical exposures.
Zhao et al. (2005)	1.30	0.52	3.23	High exposure score	0.262	0.466	Incidence RR: 0.20 (0.03, 1.46)	Mortality RR. Results for all lymphohematopoietic cancer (ICD-9 200–208), not just 200 + 202. Males only; adjusted for age, SES, time since first employment. Mortality results reflect more exposed cases (six in high-exposure group) than do incidence results (one in high-exposure group).
Cocco et al. (2010)	0.7	0.4	1.3	High cumulative exposure	-0.357	0.301	None	Incidence OR. Grouping consistent with traditional NHL definition provided by author (see text). High-confidence subgroup. Adjusted for age, sex, center, and education.
Miligi et al., (2006)	1.2	0.7	2.0	Med/high exposure intensity	0.182	0.268	1.0 (0.5, 2.6) for med/high intensity and >15 yrs	Incidence OR. NHL + CLL (see Section C.2.1.1). Adjusted for age, sex, education, and area.
Purdue et al. (2011)	3.3	1.1	10.1	Cumulative exposure >234,000 ppm × hrs	1.194	0.566	2.3 (1.0, 5.0) for highest exposure tertile (>112,320 ppm × hrs)	Incidence OR. ICD-O-3 codes 967–972. Probable-exposure subgroup. Adjusted for age, sex, SEER center, race, and education.
Siemiatycki (1991)	0.8	0.2	3.3	Substantial	–0.223	0.719	None	Incidence OR. NHL. SE and 95% CI calculated from reported 90% CIs. Males only; adjusted for age, income, and cigarette smoking index.
Wang et al. (2009)	2.2	0.9	5.4	Medium-high intensity	0.788	0.457	None	Incidence OR. NHL. Females only; adjusted for age, family history of lymphohematopoietic cancers, alcohol consumption, and race.

[a]Mean personal TCA in urine. 1 µmol/L = 0.1634 mg/L.

As above for the overall TCE effect, for Axelson et al. (1994), in which a small subcohort of females was studied but only results for the larger male subcohort were reported, the reported male-only high-exposure group results were used in the primary analysis; however, an attempt was made to estimate the female contribution to a high-exposure group RR estimate for both sexes and its impact on the meta-analysis. To estimate the expected number in the highest exposure group for females, the expected number in the highest exposure group for males was multiplied by the ratio of total female-to-male person-years in the study and by the ratio of female-to-male age-adjusted incidence rates for NHL. The RR estimate for both sexes was used as an alternate RR estimate for the Axelson et al. (1994) study in a sensitivity analysis.

For Boice et al. (1999), only results for workers with "any potential exposure" (rather than "potential routine exposure") were presented by exposure category, and the referent group is workers not exposed to any solvent.

For Hansen et al. (2001), exposure group data were presented only for males. To estimate the female contribution to a highest exposure group RR estimate for both sexes, it was assumed that the expected number of cases in females had the same overall-to-highest-exposure-group ratio as in males. The RR estimate for both sexes was then calculated assuming a Poisson distribution, and this estimate was used in the primary analysis. Hansen et al. (2001) present results for three exposure metrics; the cumulative exposure metric was preferred for the primary analysis, and results for the other two metrics were used in sensitivity analyses.

For Morgan et al. (1998), results did not allow for the combination of ICD 200 and 202, so the highest exposure group RR estimate for ICD 200 only was used. The primary analysis used results for the cumulative exposure metric, and a sensitivity analysis was done with the results for the peak exposure metric.

For Radican et al. (2008), it should be noted that the referent group is composed of workers with no chemical exposures, not just no exposure to TCE. In addition, results for exposure groups (based on cumulative exposure scores) were reported separately for males and females and were combined for this assessment using inverse-variance weighting, as in a fixed-effect meta-analysis. Radican et al. (2008) present only mortality hazard ratio estimates by exposure group; however, in an earlier follow-up of this same cohort, Blair et al. (1998) present both incidence and mortality RR estimates by exposure group. The mortality RR estimate based on the more recent follow-up by Radican et al. (2008) (17 deaths in the highest exposure group) was used in the primary analysis, while the incidence RR estimate based on similarly combined results from Blair et al. (1998) (nine cases) was used as an alternate estimate in a sensitivity analysis. Radican et al. (2008) also present results for categories based on frequency and pattern of exposure; however, subjects weren't distributed uniquely across the categories (the numbers of cases across categories exceeded the total number of cases); thus, it was difficult to interpret these results and they were not used in a sensitivity analysis.

For Zhao et al. (2005), RR estimates were only reported for ICD-9 200–208 (all lymphohematopoietic cancers), and not for 200 + 202 alone. Given that other studies have not reported associations between leukemias and TCE exposure, combining all lymphohematopoietic cancers would dilute any NHL effect, and the Zhao et al. (2005) results are expected to be an underestimate of any TCE effect on NHL alone. Zhao et al. (2005) present RR estimates for both incidence and mortality in the highest exposure group; however, the time frame for the incidence accrual is smaller than the time frame for mortality accrual and fewer incident cases (1) were obtained than deaths (6), so the mortality results were used for the primary analysis to reflect the better case ascertainment in the mortality data, and the incidence results were used in a sensitivity analysis.

Cocco et al. (2010) present exposure group results only for their high-confidence subgroup, which included workers with jobs with a "certain" probability of exposure and >90% of workers exposed (5.5% of cases). Results for a grouping of lymphomas generally consistent with the traditional definition of NHL (T-cell lymphomas and B-cell lymphomas, excluding Hodgkin lymphomas, CLLs, multiple myelomas, and unspecified lymphomas) were kindly provided by Dr. Cocco (personal communication from Pierluigi Cocco, University of Cagliari, Italy, to Cheryl Scott, U.S. EPA, 19 March 2011; see Section 4.6.1.2).

Miligi et al. (2006) include CLLs in their NHL results, consistent with the current WHO/REAL classifications. Miligi et al. (2006) report RR estimates for medium and high exposure intensity overall and by duration of exposure; however, there was incomplete information for the duration breakdowns (e.g., a case missing), so the RR estimate for med/high exposure intensity overall was used in the primary analysis, and the RR estimate for med/high exposure for >15 years was used in a sensitivity analysis.

Purdue et al. (2011) used ICD-O-3 codes 967–972, generally consistent with a traditional definition of NHL. These investigators present exposure group results only for their probable-exposure subgroup, which included workers with jobs with an assigned probability of exposure of ≥50% (3.8% of cases). The exposure groups are cumulative exposure tertiles, with cutpoints determined from the exposure distribution in the probably exposed controls. The highest exposure tertile was further subdivided using the intra-category median. The highest exposure group from the subdivided highest exposure tertile was used for the primary analysis (four cases), and the results for the complete highest tertile were used in a sensitivity analysis (nine cases).

Wang et al. (2009) used ICD-O-2 codes (M-9590-9595, 9670-9688, 9690-9698, 9700-9723), consistent with the traditional definition of NHL (i.e., ICD-7, -8, -9 codes 200 + 202). Wang et al. (2009) present exposure-group (low or medium/high intensity) results cross-categorized by exposure probability (low and medium/high). The medium and high exposure-intensity category was used as the highest exposure group, although all of the subjects with medium and high exposure intensity were in the low exposure-probability category.

C.2.2.2. Results of Meta-Analyses

Results from the meta-analyses that were conducted for NHL in the highest exposure groups are summarized at the bottom of Table C-3 and reported in more detail in Table C-5. The summary RR estimate from the primary random-effects meta-analysis of the 13 studies with results presented for exposure groups was 1.43 (95% CI: 1.13, 1.82) (see Figure C-4). No single study was overly influential; removal of individual studies resulted in RRm estimates that were all statistically significant (all with $p \leq 0.025$) and that ranged from 1.38 (with the removal of Purdue et al. [(2011)]) to 1.57 (with the removal of Cocco et al. (2010)). In addition, the RRm estimate was not highly sensitive to alternate RR estimate selections. Use of the nine alternate selections, individually, resulted in RRm estimates that were all statistically significant (all with $p < 0.025$) and all in the narrow range from 1.40 to 1.49 (see Table C-5**).**

There was some heterogeneity apparent across the 13 studies, although it was not statistically significant ($p = 0.30$). The I^2-value was 14%, suggesting low heterogeneity. This small amount of heterogeneity is also indicated by the finding that the RRm estimate from the fixed-effect analysis had a slightly narrower 95% CI (1.16–1.75 vs. 1.13–1.82), although the RRm estimates themselves were essentially identical. In addition, nonsignificant heterogeneity was apparent in each of the meta-analyses with alternate RR selections—*p*-values ranged from 0.12 to 0.37 and I^2-values ranged from 9 to 33%.

Table C-5. Summary of some meta-analysis results for TCE (highest exposure groups) and NHL

Analysis	Model	RRm estimate	95% LCL	95% UCL	Heterogeneity	Comments
All studies (13)	Random	1.43	1.13	1.82	NS (p = 0.30) I^2 = 14%	Statistical significance not dependent on single study.
	Fixed	1.43	1.16	1.75		
Cohort studies (8)	Random	1.60	1.24	2.08	None observable (random = fixed)	Not significant difference between CC and cohort studies (p = 0.47).
	Fixed	1.60	1.24	2.08		Not significant difference between CC and cohort studies (p = 0.15).
Case-control studies (5)	Random	1.29	0.76	2.20	NS (p = 0.08) I^2 = 53%	
	Fixed	1.18	0.84	1.64		
Alternate RR selections[a] (all studies)	Random	1.40	1.11	1.75	NS (p = 0.33) I^2 = 11%	With Raaschou-Nielsen et al. (2003) full cohort instead of subgroup expected to have higher exposures.
	Random	1.40	1.09	1.80	NS (p = 0.25) I^2 = 19%	With Blair et al. (1998) incidence RR instead of Radican et al. (2008) mortality hazard ratio.
	Random	1.41	1.05	1.88	NS (p = 0.12) I^2 = 33%	With Zhao et al. (2005) incidence.
	Random	1.43	1.13	1.80	NS (p = 0.32) I^2 = 13%	With estimated female contribution for Axelson et al. (1994).
	Random	1.43	1.15	1.78	NS (p = 0.37) I^2 = 9%	With Purdue et al. (2011) highest cumulative exposure tertile
	Random	1.44	1.12	1.85	NS (p = 0.29) I^2 = 16%	With Miligi et al. (2006) with >15 yrs.
	Random	1.44	1.14	1.83	NS (p = 0.32) I^2 = 13%	With Morgan et al. (1998) peak.
	Random	1.45	1.14	1.86	NS (p = 0.25) I^2 = 19%	With Hansen et al. (2001) mean exposure.
	Random	1.49	1.14	1.93	NS (p = 0.17) I^2 = 27%	With Hansen et al. (2001) duration.

[a]Changing the primary analysis by one alternate RR estimate each time.

CC: case-control; NS: not statistically significant

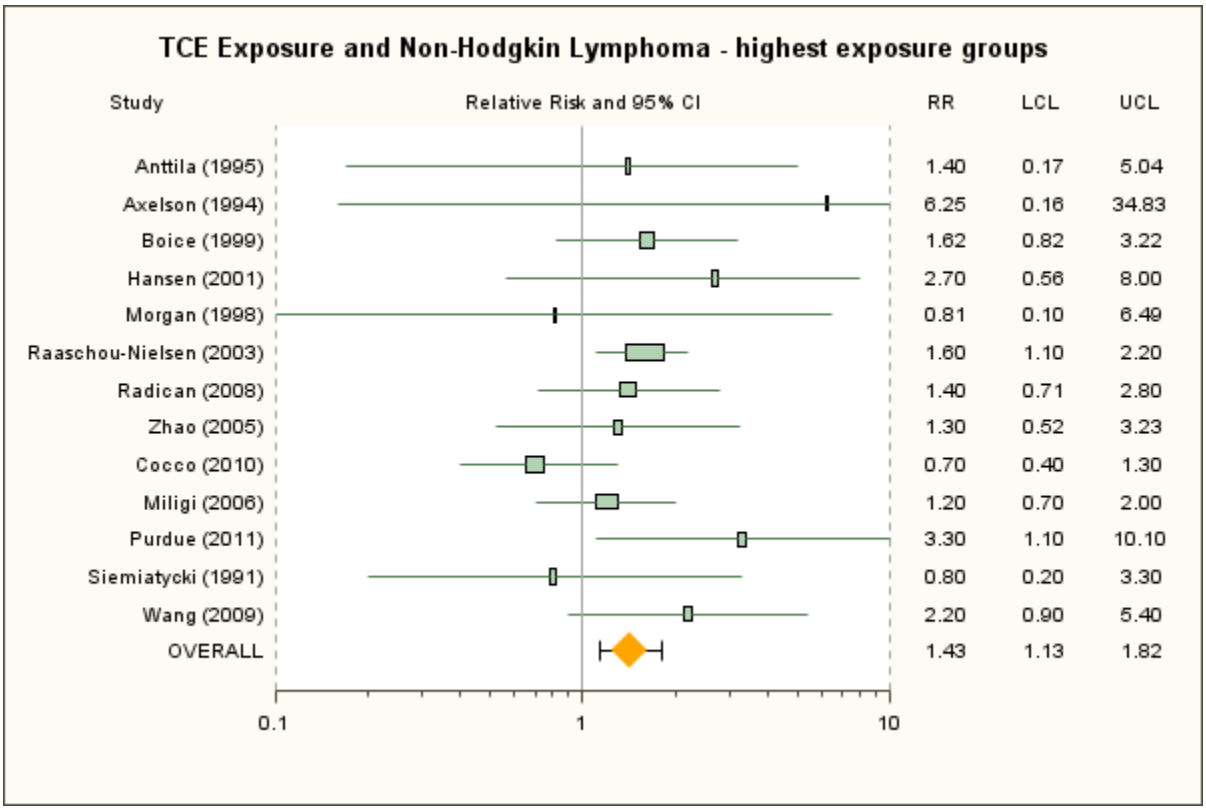


Figure C-4. Meta-analysis of NHL and TCE exposure—highest exposure groups. Rectangle sizes reflect relative weights of the individual studies. The bottom diamond represents the RRm estimate.

To investigate the heterogeneity, subgroup analyses were done examining the cohort and case-control studies separately. With the random-effects model (and tau-squared not pooled across subgroups), the resulting RRm estimates were 1.60 (95% CI: 1.24, 2.08) for the cohort studies and 1.29 (95% CI: 0.76, 2.20) for the case-control studies. There was no residual heterogeneity in the cohort subgroup (I^2 = 0%). Heterogeneity remained in the case-control subgroup, but it was not statistically significant (p = 0.08)—the I^2-value was 53%, suggesting moderate heterogeneity. The difference between the RRm estimates for the cohort and case-control subgroups was not statistically significant. As with the meta-analysis for overall TCE exposure in Section C.2.1.2, no further attempt was made to quantitatively investigate possible sources of heterogeneity; see Section C.2.3 for a qualitative discussion of some potential sources of heterogeneity. It is, however, noted that the RR estimate from Axelson et al. (1994) appears to be a relative outlier at the high end (see Figure C-4). Removal of this study does not eliminate the heterogeneity, however, because the study carries little weight. Similarly, removal of the study with the next largest RR estimate (Purdue et al., 2011), whose removal results in the lowest RRm estimate in the analyses of study influence (see above) does not eliminate the heterogeneity. On the other hand, removal of the study with the lowest RR estimate (Cocco et

al., 2010), which also has a substantial amount of weight and whose removal results in the highest RRm estimate in the analyses of study influence (see above), eliminates all of the heterogeneity. This suggests that the result from Cocco et al. (2010) for the highest exposure group might be an outlier, but it is unclear what about the study might account for this result being inordinately low.

C.2.3. Discussion of NHL Meta-Analysis Results

The meta-analyses of the overall effect of TCE exposure on NHL suggest a small, statistically significant increase in risk. The summary estimate from the primary random-effects meta-analysis of the 17 studies was 1.23 (95% CI: 1.07, 1.42). This result was not overly influenced by any single study, nor was it overly sensitive to individual RR estimate selections or to restricting the analysis to only those studies for which RR estimates based on the traditional definition of NHL were available, and in all of the influence and sensitivity analyses, the RRm estimate was statistically significantly increased. Thus, the finding of an increased risk of NHL associated with TCE exposure, though the increased risk is not large in magnitude, is robust.

There is some evidence of potential publication bias in this data set; however, it is uncertain that this is actually publication bias rather than an association between SE and effect size resulting for some other reason (e.g., a difference in study populations or protocols in the smaller studies). Furthermore, if there is publication bias in this data set, it does not appear to account completely for the finding of an increased NHL risk. For example, using the trim-and-fill procedure of Duval and Tweedie (2000) to impute the values from the four 'missing' studies that would balance the funnel plot yields an RRm estimate of 1.15 (95% CI: 0.97, 1.36).

Although there was some heterogeneity across the 17 studies, it was not statistically significant ($p = 0.16$). The I^2-value was 26%, suggesting low-to-moderate heterogeneity. Similarly, when subgroup analyses were done of cohort and case-control studies separately, there was some observable heterogeneity in each of the subgroups, but it was not statistically significant in either case. I^2-values were 12% for the cohort studies, suggesting low heterogeneity, and 27% for the case-control studies, suggesting low-to-moderate heterogeneity. In the subgroup analyses, the increased risk of NHL was strengthened in the cohort study analysis and nearly eliminated in the case-control study analysis, although the subgroup RRm estimates were not statistically significantly different. Study design itself is unlikely to be an underlying cause of heterogeneity and, to the extent that it may explain some of the differences across studies, is more probably a surrogate for some other difference(s) across studies that may be associated with study design. Furthermore, other potential sources of heterogeneity may be masked by the broad study design subgroupings. The true source(s) of heterogeneity across these studies is an uncertainty. As discussed above, further quantitative investigations of heterogeneity were ruled out because of database limitations. A qualitative discussion of some potential sources of heterogeneity follows.

Study differences in exposure assessment approach, exposure prevalence, average exposure intensity, and NHL classification are possible sources of heterogeneity. Many studies included TCE assignment from information on job and task exposures, e.g., a JEM (Radican et al., 2008; Boice et al., 2006b; Miligi et al., 2006; Zhao et al., 2005; Boice et al., 1999; Morgan et al., 1998; Siemiatycki, 1991); (Purdue et al., 2011; Cocco et al., 2010; Wang et al., 2009), or from an exposure biomarker in either breath or urine (Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994). Three case-control studies relied on self-reported exposure to TCE (Persson and Fredrikson, 1999; Nordström et al., 1998; Hardell et al., 1994). Misclassification is possible with all exposure assessment approaches. No information is available to judge the degree of possible misclassification bias associated with a particular exposure assessment approach; it is quite possible that in some cohort studies, in which past exposure is inferred from various data sources, exposure misclassification may be as great as in population-based or hospital-based case-control studies. Approaches based upon JEMs can provide order-of-magnitude estimates that are useful for distinguishing groups of workers with large differences in exposure; however, smaller differences usually cannot be reliably distinguished (NRC, 2006). Biomonitoring can provide information on potential TCE exposure in an individual, but the biomarkers used aren't necessarily specific for TCE and they reflect only recent exposures.

General population studies have special problems in evaluating exposure, because the subjects could have worked in any job or setting that is present within the population (NRC, 2006; 't Mannetje et al., 2002; McGuire et al., 1998; Nelson et al., 1994; Copeland et al., 1977). Low exposure prevalence in the case-control studies may be another source of heterogeneity. Prevalence of TCE exposure among cases in the case-control studies was low, ranging from 3 in Siemiatycki (1991) to 13% in Wang et al. (2009). However, prevalence of high TCE exposure in these case-control studies was even rarer—3% of all cases in Miligi et al. (2006), 2% in Wang et al. (2009) and Cocco et al. (2010) (high-confidence assessments; personal communication from Pierluigi Cocco, University of Cagliari, Italy, to Cheryl Scott, U.S. EPA, 19 March 2011; see Section 4.6.1.2), 1% (with probable exposure) in Purdue et al. (2011), and <1% in Siemiatycki (1991). Low exposure prevalence may be one of the underlying characteristics differentiating the case-control and cohort studies and explaining some of the heterogeneity across the studies.

Study differences in NHL groupings and in NHL classification schemes are another potential source of heterogeneity in the meta-analysis, although restricting the meta-analysis to only those studies for which RR estimates based on the traditional NHL definition were available did not eliminate all heterogeneity. All studies included a broad but sometimes slightly different group of lymphosarcoma, reticulum-cell sarcoma, and other lymphoid tissue neoplasms, with the exception of the Nordstrom et al. (1998) case-control study, which examined hairy cell leukemia, now considered a (non-Hodgkin) lymphoma, and the Zhao et al. (2005) cohort study, which reported only results for *all* lymphohematopoietic cancers, including nonlymphoid types. Persson and Fredrikson (1999) do not identify the classification system for defining NHL, and

Hardell et al. (1994) define NHL using the Rappaport classification system. Miligi et al. (2006) used the NCI Working Formulation and also considered CLLs as (non-Hodgkin) lymphomas. Cocco et al. (2010) used the WHO/REAL classification system, which reclassifies lymphocytic leukemias and NHLs as lymphomas of B-cell or T-cell origin and considers CLLs and multiple myelomas as (non-Hodgkin) lymphomas; however, results were obtained generally consistent with the traditional NHL definition from Dr. Cocco, although lymphomas not otherwise specified were excluded. Wang et al. (2009) defined NHL using ICD-O-2 codes (M-9590-9595, 9670-9688, 9690-9698, 9700-9723), which is consistent with the traditional definition of NHL (i.e., ICD-7, -8, -9 codes 200 + 202). Purdue et al. (2011) used ICD-O-3 codes 967–972, which is generally consistent with the traditional definition of NHL, although this grouping doesn't include the malignant lymphomas of unspecified type coded as M-9590-9599. The cohort studies [except for Zhao et al. (2005)] and the case-control study of Siemiatycki (1991) have some consistency in coding NHL, with NHL defined as lymphosarcoma and reticulum-cell sarcoma (ICD code 200) and other lymphoid tissue neoplasms (ICD 202) using the ICD Revisions 7, 8, or 9. Revisions 7 and 8 are essentially the same with respect to NHL; under Revision 9, the definition of NHL was broadened to include some neoplasms previously classified as Hodgkin lymphomas (Banks, 1992).

Thirteen of the 17 studies categorized results by exposure level. Different exposure metrics were used, and the purpose of combining results across the different highest exposure groups was not to estimate an RRm associated with some level of exposure, but rather to see the impacts of combining RR estimates that should be less affected by exposure misclassification. In other words, the highest exposure category is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any vs. none) exposure comparison. Thus, if TCE exposure increases the risk of NHL, the effects should be more apparent in the highest exposure groups. Indeed, the RRm estimate from the primary meta-analysis of the highest exposure group results was 1.43 (95% CI: 1.13, 1.82), which is greater than the RRm estimate of 1.23 (95% CI: 1.07, 1.42) from the overall exposure analysis. The statistical significance of the increased RR estimate for the highest exposure groups was not dependent on any single study, nor was it sensitive to individual RR estimate selections. The robustness of this finding lends substantial support to a conclusion that TCE exposure increases the risk of NHL.

Although there was some heterogeneity apparent across the 13 highest-exposure-group studies, it was not statistically significant ($p = 0.30$). The I^2-value was 14%, suggesting low heterogeneity. When subgroup analyses were done examining the cohort and case-control studies separately, there was no residual heterogeneity in the cohort subgroup ($I^2 = 0\%$). Heterogeneity remained in the case-control subgroup, but it was not statistically significant ($p = 0.08$)—the I^2-value was 53%, suggesting moderate heterogeneity. In the subgroup analyses, the increased risk of NHL was strengthened in the cohort study analysis and reduced in the case-

control study analysis, although the subgroup RRm estimates were not statistically significantly different. As with the meta-analysis for overall TCE exposure discussed above, no further attempt was made to quantitatively investigate potential sources of heterogeneity. It is, however, noted that removal of the Cocco et al. (2010) study, whose removal had the greatest impact in the analyses of study influence (RRm = 1.57, 95% CI: 1.27, 1.95), eliminates all of the heterogeneity, suggesting that the RR estimate for the highest exposure group from that study is a relative outlier.

C.3. META-ANALYSIS FOR KIDNEY CANCER

C.3.1. Overall Effect of TCE Exposure

C.3.1.1. Selection of RR Estimates

The selected RR estimates for kidney cancer associated with TCE exposure from the epidemiological studies are presented in Table C-6 for cohort studies and in Table C-7 for case-control studies. The majority of the cohort studies reported results for all kidney cancers, including cancers of the renal pelvis and ureter (i.e., ICD-7 180; ICD-8 and -9 189.0–189.2; ICD-10 C64–C66), whereas the majority of the case-control studies focused on RCC, which comprises roughly 85% of kidney cancers. Where both all kidney cancer and RCC were reported, the primary analysis used the results for RCC, because RCC and the other forms of kidney cancer are very different cancer types and it seemed preferable not to combine them; the results for all kidney cancers were then used in a sensitivity analysis. The preference for the RRC results alone is supported by the results in rodent cancer bioassays, where TCE-associated rat kidney tumors are observed in the renal tubular cells (Section 4.4.5), and in metabolism studies, where the focus of studies for the GSH conjugation pathway (considered the primary metabolic pathway for kidney toxicity) is in renal cortical and tubular cells (Sections 3.3.3.3 and 4.4.6).

Table C-6. Selected RR estimates for kidney cancer associated with TCE exposure (overall effect) from cohort studies

Study	RR	95% LCL	95% UCL	RR type	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Anttila et al. (1995)	0.87	0.32	1.89	SIR	-0.139	0.408	None	ICD-7 180.
Axelson et al. (1994)	1.16	0.42	2.52	SIR	0.148	0.408	1.07 (0.39, 2.33) with estimated female contribution to SIR added (see text)	ICD-7 180. Results reported for males only, but there was a small female component to the cohort.
Boice et al. (1999)	0.99	0.4	2.04	SMR	-0.010	0.378	None	ICD-9 189.0–189.2. For potential routine exposure. Results for any potential exposure not reported.
Greenland et al. (1994)	0.99	0.30	3.32	Mortality OR	-0.010	0.613	None	Nested case-control study. ICD-8 codes not specified, presumably all of 189.
Hansen et al. (2001)	1.1	0.3	2.8	SIR	0.095	0.500	None	ICD-7 180. Male and female results reported separately; combined assuming Poisson distribution.
Morgan et al. (1998)	1.14	0.51	2.58	Mortality RR	0.134	0.415	Published SMR 1.32 (0.57, 2.6)	ICD-9 189.0–189.2. Unpublished RR, adjusted for age and sex (see text).
Raaschou-Nielsen et al. (2003)	1.20	0.94	1.50	SIR	0.182	0.115	1.20 (0.98, 1.46) for ICD-7 180 1.4 (1.0, 1.8) for subcohort with expected higher exposures	RCC.
Radican et al. (2008)	1.18	0.47	2.94	Mortality hazard ratio	0.166	0.468	None	ICD-8, -9 189.0, ICD-10 C64. Time variable = age; covariates = sex and race. Referent group is workers with no chemical exposures.
Zhao et al. (2005)	1.7	0.38	7.9	Mortality RR	0.542	0.775	Incidence RR: 2.0 (0.47, 8.2) Mortality RR no lag: 0.89 (0.22, 3.6) Incidence RR no lag : 2.1 (0.56, 8.1) Boice et al. (2006b) SMR: 2.22 (0.89, 4.57)	ICD-9 189. Males only. Adjusted for age, SES, time since first employment, exposure to other carcinogens. 20-yr lag. Mortality results reflect same number exposed cases (10 with no lag) as do incidence results, so no reason to prefer mortality results, but they are used in primary analysis to avoid appearance of "cherry-picking." Overall RR estimated by combining across exposure groups (see text). Boice et al. (2006b) cohort overlaps Zhao et al. (2005) cohort; just seven exposed deaths.

Table C-7. Selected RR estimates for RCC associated with TCE exposure from case-control studies[a]

Study	RR estimate	95% LCL	95% UCL	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Brüning et al. (2003)	2.47	1.36	4.49	0.904	0.305	1.80 (1.01, 3.20) for longest job held in industry with TCE exposure	Self-assessed exposure. Adjusted for age, sex, and smoking.
Charbotel et al. (2006)	1.88	0.89	3.98	0.631	0.382	1.64 (0.95, 2.84) for full study 1.68 (0.97, 2.91) for full study with 10-yr lag	Subgroup with good level of confidence about exposure assessment. Matched on sex, age. Adjusted for smoking, BMI.
Dosemeci et al. (1999)	1.30	0.9	1.9	0.262	0.191	None	Adjusted for age, sex, smoking, hypertension, and/or use of diuretics and/or anti-hypertension drugs, BMI.
Moore et al. (2010)	2.05	1.13	3.73	0.718	0.305	1.63 (1.04, 2.54) for all subjects	Subgroup with high-confidence assessments. Adjusted for age, sex, and center.
Pesch et al. (2000b)	1.24[b]	1.03[b]	1.49[b]	0.215	0.094	1.13 (0.98,1.30)[b] with German JEM	With JTEM. Crude OR calculated from data provided in personal communication (see text).
Siemiatycki (1991)	0.8	0.3	2.2	-0.223	0.524	None	"Kidney cancer." SE and 95% CI calculated from reported 90% CIs. Males only; adjusted for age, income, and cigarette smoking index.

[a]The RR estimates are all ORs for incident cases.
[b]As calculated by U.S. EPA.

As for NHL, many of the studies provided RR estimates only for males and females combined, and we are not aware of any basis for a sex difference in the effects of TCE on kidney cancer risk; thus, wherever possible, RR estimates for males and females combined were used. Of the three larger (in terms of number of cases) studies that did provide results separately by sex, Dosemeci et al. (1999) suggest that there may be a sex difference for TCE exposure and RCC (OR = 1.04 [95% CI: 0.6, 1.7] in males and 1.96 [95% CI: 1.0, 4.0] in females), while Raaschou-Nielsen et al. (2003) report the same SIR (1.2) for both sexes and crude ORs calculated from data from the Pesch et al. (2000b) study (provided in a personal communication from Beate Pesch, Forschungsinstitut für Arbeitsmedizin [BGFA], to Cheryl Scott, U.S. EPA, 21 February 2008) are 1.28 for males and 1.23 for females. Radican et al. (2008) and Hansen et al. (2001) also present some results by sex, but both of these studies have too few cases to be informative about a sex difference for kidney cancer.

Most of the selections in Tables C-6 and C-7 should be self-evident, but some are discussed in more detail here, in the order the studies are presented in the tables. For Axelson et al. (1994), in which a small subcohort of females was studied but only results for the larger male subcohort were reported, the reported male-only results were used in the primary analysis; however, as for NHL, an attempt was made to estimate the female contribution to an overall RR estimate for both sexes and its impact on the meta-analysis. Axelson et al. (1994) reported neither the observed nor the expected number of kidney cancer cases for females. It was assumed that none was observed. To estimate the expected number, the expected number for males was multiplied by the ratio of female-to-male person-years in the study and by the ratio of female-to-male age-adjusted incidence rates for kidney cancer.[6] The male results and the estimated female contribution were then combined into an RR estimate for both sexes assuming a Poisson distribution, and this alternate RR estimate for the Axelson et al. (1994) study was used in a sensitivity analysis.

For Boice et al. (1999), only results for "potential routine exposure" were reported for kidney cancer. Boice et al. (1999) report in general that the SMRs for workers with any potential exposure "were similar to those for workers with daily potential exposure."

In their published paper, Morgan et al. (1998) present only SMRs for overall TCE exposure, although the results from internal analyses are presented for exposure subgroups. RR estimates for overall TCE exposure from the internal analyses of the Morgan et al. (1998) cohort data were available from an unpublished report (EHS, 1997); from these, the RR estimate from

[6]Person-years for men and women ≤79 years were obtained from Axelson et al. (1994): 23516.5 and 3691.5, respectively. Lifetime age-adjusted incidence rates for cancer of the kidney and renal pelvis for men and women were obtained from the National Cancer Institute's 2000–2004 SEER-17 (Surveillance Epidemiology and End Results from 17 geographical locations) database (http://seer.cancer.gov/statfacts/html/kidrp.html): 17.8/100,000 and 8.8/100,000, respectively. The calculation for estimating the expected number of cases in females in the cohort assumes that the males and females have similar TCE exposures and that the relative distributions of age-related incidence risk for the males and females in the Swedish cohort are adequately represented by the ratios of person-years and U.S. lifetime incidence rates used in the calculation.

the Cox model that included age and sex was selected, because those are the variables deemed to be important in the published paper. The internal analysis RR estimate was preferred for the primary analysis, and the published SMR result was used in a sensitivity analysis.

Raaschou-Nielsen et al. (2003) reported results for RCC and renal pelvis/ureter separately. As discussed above, RCC estimates were used in the primary analysis, and the results for both kidney cancer categories were combined (across sexes as well), assuming a Poisson distribution, and used in a sensitivity analysis. In another sensitivity analysis, results for RCC from the subcohort with expected higher exposure levels (≥1-year duration of employment and year of 1st employment before 1980) were used. Raaschou-Nielsen et al. (2003), in their Table 3, also present the overall results for RCC and for renal pelvis/ureter cancer with a lag time of 20 years; however, they use a definition of lag that is different from a lagged exposure in which exposures prior to disease onset are discounted and it is not clear what their lag time actually represents[7]; thus, as for NHL, these results were not used in any of the meta-analyses for kidney cancer.

For Radican et al. (2008), the Cox model hazard ratio from the 2000 follow-up was used. In the Radican et al. (2008) Cox regressions, age was the time variable, and sex and race were covariates. It should also be noted that the referent group is composed of workers with no chemical exposures, not just no exposure to TCE.

For Zhao et al. (2005), no results for an overall TCE effect are reported. We were unable to obtain any overall estimates from the study authors, so, as a best estimate, as was done for NHL, the results across the "medium" and "high" exposure groups were combined, under assumptions of group independence, even though the exposure groups are not independent (the "low" exposure group was the referent group in both cases). Unlike for NHL, adjustment for exposure to other carcinogens made a considerable difference, so Zhao et al. (2005) also present kidney results with this additional adjustment, with and without a 20-year lag. Estimates of RR with this additional adjustment were selected over those without. In addition, a 20-year lag seemed reasonable for kidney cancer, so the lagged estimates were preferred to the unlagged; unlagged estimates were used in sensitivity analyses. Zhao et al. (2005) present RR estimates for both incidence and mortality. Unlike for NHL, the number of exposed incident cases (10 with no lag) was identical to the number of deaths, so there was no reason to prefer the mortality results over the incidence results. (In fact, there were more exposed incident cases [10 vs. 7] after lagging.) However, the mortality results, which yield a lower RR estimate, were selected for the primary analysis to avoid any appearance of "cherry-picking," and incidence RR estimates were used in sensitivity analyses. A sensitivity analysis was also done using results from Boice et al. (2006b) in place of the Zhao et al. (2005) RR estimate. The cohorts for these studies overlap, so they are not independent studies and should not be included in the meta-analysis concurrently.

[7]In their Methods section, Raaschou-Nielsen et al. (2003) define their lag period as the period "from the date of first employment to the start of follow-up for cancer".

Boice et al. (2006b) report results for an overall TCE effect for kidney cancer; however, the results are SMR estimates rather than internal comparisons and are based on fewer exposed deaths (7), so either Zhao et al. (2005) estimate is preferred over the Boice et al. (2006b) estimate.

Regarding the case-control studies, for Brüning et al. (2003), the results based on self-assessed exposure were preferred because, although TCE exposure was probably under-ascertained with this measure, there were greater concerns about the result based on the alternate measure reported—longest-held job in an industry with TCE exposure. Even though this study was conducted in the Arnsberg region of Germany, an area with high prevalence of exposure to TCE, the exposure prevalence in both cases (87%) and controls (79%) seemed inordinately high, and this for not just any job in an industry with TCE exposure, but for the longest-held job. Furthermore, Table V of Brüning et al. (2003), which presents this result, states that the result is for longest-held job in industries with TCE *or tetrachloroethylene* exposure. Additionally, some of the industries with exposure to TCE presented in Table V have many jobs that would not entail TCE exposure (e.g., white-collar workers), so the assessment based on industry alone likely has substantial misclassification. Both of these—inclusion of tetrachloroethylene and exposure assessment by industry—could result in overstating TCE exposure prevalence. Results based on the longest-held-job measure were used in a sensitivity analysis.

For Charbotel et al. (2006), results from the analysis that considered "only job periods with a good level of confidence for TCE exposure assessment" [Table 7 of Charbotel et al. (2006)] were preferred, as these estimates would presumably be less influenced by exposure misclassification. Estimates from the full study analysis were used in a sensitivity analysis. Results for exposure with a 10-year lag are also provided in an unpublished report (Charbotel et al., 2005); however, lagged results are presented only for the full study and, thus, were similarly used in a sensitivity analysis.

Likewise, for Moore et al. (2010), results from the analysis that considered high-confidence assessments only were preferred. Here, the definition of TCE exposure was restricted to jobs classified as having probable or certain exposure (i.e., at least 40% of workers with that job were expected to be exposed), so these estimates should be less influenced by exposure misclassification. The RR estimate from the analysis of all subjects was used in a sensitivity analysis.

For Pesch et al. (2000b), TCE results were presented for two different exposure assessments. Estimates using the JTEM approach were preferred because they seemed to represent a more comprehensive exposure assessment (see Appendix B, Section B.2.4); estimates based on the JEM approach were used in a sensitivity analysis. Furthermore, results were presented only by exposure category, with no overall RR estimate reported. Case and control numbers for the different exposure categories were kindly provided by Dr. Pesch (personal communication from Beate Pesch, BGFA, to Cheryl Scott, U.S. EPA, 21 February 2008), and we

calculated crude overall ORs for males and females combined for each exposure assessment approach.

C.3.1.2. Results of Meta-Analyses

Results from some of the meta-analyses that were conducted on the epidemiological studies of TCE and kidney cancer are summarized in Table C-8. The summary estimate from the primary random-effects meta-analysis of the 15 studies was 1.27 (95% CI: 1.13, 1.43) (see Figure C-5). As shown in Figure C-5, the analysis was dominated by two (contributing over 65% of the weight) or three (about 75% of the weight) large studies. No single study was overly influential; removal of individual studies resulted in RRm estimates that were all statistically significant (all with $p < 0.005$) and that ranged from 1.24 (with the removal of (Brüning et al., 2003)] to 1.30 (with the removal of Raaschou-Nielsen et al. (2003)).

Similarly, the RRm estimate was not highly sensitive to alternate RR estimate selections. Use of the 13 alternate selections, individually, resulted in RRm estimates that were all statistically significant (all with $p < 0.0005$) and that ranged from 1.21 to 1.32 (see Table C-8). In fact, as can be seen in Table C-8, all but two of the alternates had negligible impact. The Zhao et al. (2005), Axelson et al. (1994), Morgan et al. (1998), Brüning et al. (2003), Charbotel et al. (2006), and Moore et al. (2010) original values and alternate selections were associated with very little weight and, thus, had little influence in the RRm. The Raaschou-Nielsen et al. (2003) all-kidney-cancer value carried more weight, but the alternate RR estimate was identical to the original, although with a narrower CI, and thus did not alter the RRm. Only the Raaschou-Nielsen et al. (2003) high-exposure-subcohort alternate and the Pesch et al. (2000b) alternate (with the JEM exposure assessment approach instead of the JTEM approach) had much impact, resulting in RRm estimates of 1.32 (95% CI: 1.17, 1.49) and 1.21 (95% CI: 1.09, 1.34), respectively. As noted above, the JTEM approach is preferred; thus, the lower RRm estimate obtained with the JEM alternate is considered clearly inferior. The JEM approach takes jobs into account but not tasks; thus, it is expected to have greater potential for exposure misclassification. Indeed, a comparison of exposure prevalences for the two approaches suggests that the JEM approach is less discriminating about exposure; 42% of cases were defined as TCE-exposed under the JEM approach, but only 18% of cases were exposed under the JTEM approach. On the other hand, the higher RRm estimate obtained with the Raaschou-Nielsen et al. (2003) high-exposure-subcohort alternate is consistent with an expectation that the subgroup has higher exposures and less exposure misclassification.

Table C-8. Summary of some meta-analysis results for TCE (overall) and kidney cancer

Analysis	Number of studies	Model	RRm estimate	95% LCL	95% UCL	Heterogeneity	Comments
All studies	15	Random	1.27	1.13	1.43	None observable (fixed = random)	Statistical significance not dependent on single study. No apparent publication bias.
		Fixed	1.27	1.13	1.43		
Cohort	9	Random	1.16	0.96	1.40	None observable	Not significant difference between CC and cohort studies (p = 0.12).
		Fixed	1.16	0.96	1.40		Not significant difference between CC and cohort studies (p = 0.19).
Case-control	6	Random	1.48	1.15	1.91	Not significant (p = 0.14)	
		Fixed	1.36	1.17	1.39		
Alternate RR selections[a]	15	Random	1.27–1.28	1.13–1.14	1.42–1.43	None observable	With 3 different alternates from Zhao et al. (2005) (see Table C-6).
	15	Random	1.29	1.15	1.45	None observable	With Boice et al. (2006b) study rather than Zhao et al. (2005).
	15	Random	1.27	1.13	1.43	None observable	With estimated female contribution to Axelson et al. (1994).
	15	Random	1.28	1.14	1.43	None observable	With Morgan et al. (1998) published SMR.
	15	Random	1.27	1.13	1.42	None observable	With Raaschou-Nielsen et al. (2003) all kidney cancer.
	15	Random	1.32	1.17	1.49	None observable	With Raaschou-Nielsen et al. (2003) high-exposure subcohort.
	15	Random	1.26	1.12	1.41	None observable	With Brüning et al. (2003) longest job held in industry with TCE.
	15	Random	1.28	1.14	1.43	None observable	With Charbotel et al. (2006) full study, with and without 10-yr lag.
	15	Random	1.27	1.13	1.43	None observable	With Moore et al. (2010) full study.
	15	Random	1.21	1.09	1.34	None observable	With Pesch et al. (2000b) JEM.
Highest exposure groups	10	Random	1.64	1.31	2.04	None observable	
	13	Random	1.58	1.28	1.96	None observable	Using RR = 1 for Anttila et al. (1995), Axelson et al. (1994), and Hansen et al. (2001) (see text).
	13	Random	1.47–1.60	1.20–1.29	1.79–1.98	See Table C-10	Using RR = 1 for Anttila et al. (1995), Axelson et al. (1994), and Hansen et al. (2001) and various alternate RR selection results (see Table C-10)[a].

[a]Changing the primary analysis by one alternate RR each time.

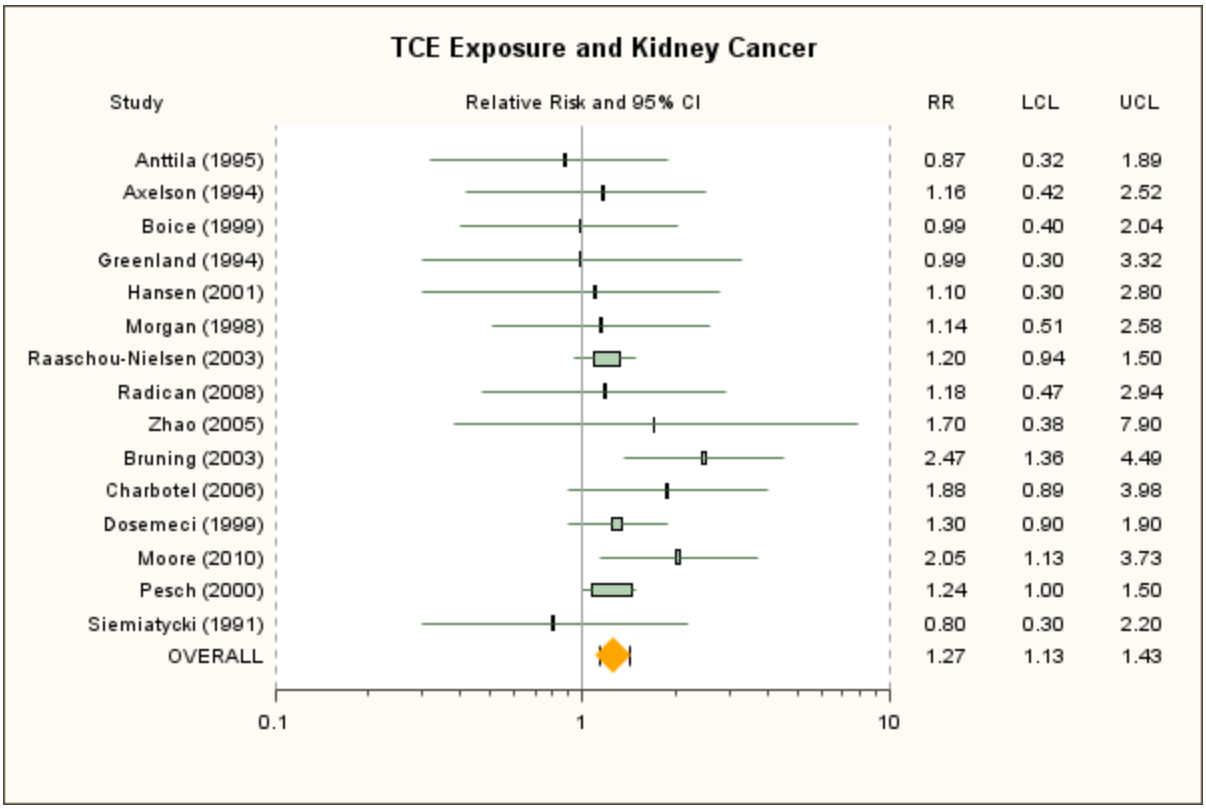


Figure C-5. Meta-analysis of kidney cancer and overall TCE exposure. Random-effects model; fixed-effect model same. Rectangle sizes reflect relative weights of the individual studies. The summary estimate is in the bottom row, represented by the diamond.

There was no apparent heterogeneity across the 15 studies (i.e., the random-effects model and the fixed-effect model gave the same results [p_{hetero} = 0.67; I^2 = 0%]). Nonetheless, subgroup analyses were done examining the cohort and case-control studies separately. With the random-effects model (and tau-squared not pooled across subgroups), the resulting RRm estimates were 1.16 (95% CI: 0.96, 1.40) for the cohort studies and 1.48 (95% CI: 1.15, 1.91) for the case-control studies. There was no heterogeneity in the cohort subgroup (p = 0.998; I^2 = 0%). There was heterogeneity in the case-control subgroup, but it was not statistically significant (p = 0.14) and the I^2-value of 41% suggests that the extent of the heterogeneity in this subgroup was low-to-moderate. Nor was the difference between the RRm estimates for the cohort and case-control subgroups statistically significant under either the random-effects model or the fixed-effect model. Further quantitative investigations of heterogeneity were not pursued because of database limitations and, in any event, there is no evidence for heterogeneity of study results in this database. A qualitative discussion of some potential sources of heterogeneity across studies is nonetheless included in Section C.3.3.

As discussed in Section C.1, publication bias was examined in several different ways. The funnel plot in Figure C-6 shows little relationship between RR estimate and study size, and, indeed, none of the other tests performed found any evidence of publication bias. The trim-and-

fill procedure of Duval and Tweedie (2000), for example, determined that no studies were missing from the funnel plot (i.e., there was no asymmetry to counterbalance). Similarly, the results of a cumulative meta-analysis, incorporating studies with increasing SE one at a time, shows no evidence of a trend of increasing effect size with addition of the less precise studies. Including the three most precise studies, reflecting 75% of the weight, the RRm goes from 1.24 to 1.22 to 1.23. The addition of the Moore et al. (2010) study brings the RRm to 1.26 and the weight to 79% and further addition of the Brüning et al. (2003) study increases the RRm to 1.38 and the weight to 83%. After the addition of the next six studies, the RRm stabilizes at about 1.28, and further addition of the four least precise studies has little impact.

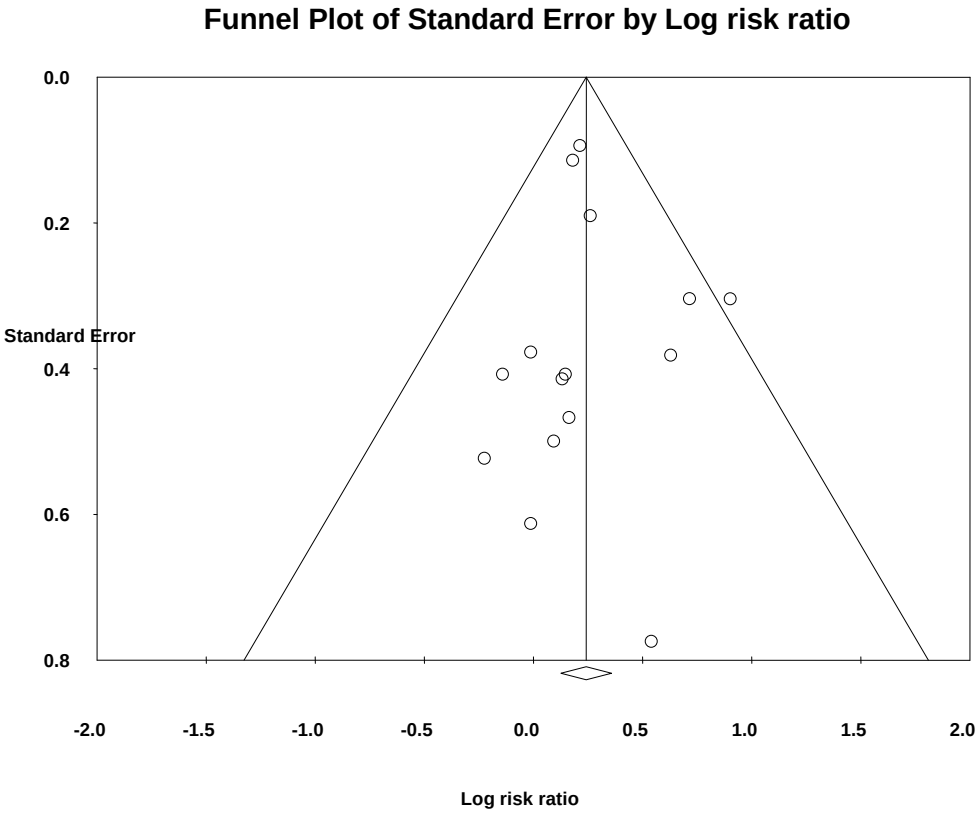


Figure C-6. Funnel plot of SE by log RR estimate for TCE and kidney cancer studies.

C.3.2. Kidney Cancer Effect in the Highest Exposure Groups

C.3.2.1. Selection of RR Estimates

The selected RR estimates for kidney cancer in the highest TCE exposure categories, for studies that provided such estimates, are presented in Table C-9. Five of the nine cohort studies and five of the six case-control studies reported kidney cancer risk estimates categorized by exposure level. As in Section C.3.1.1 for the overall risk estimates, estimates for RCC were preferentially selected when presented, and, wherever possible, RR estimates for males and females combined were used.

Three of the nine cohort studies (Hansen et al., 2001; Anttila et al., 1995; Axelson et al., 1994) did not report kidney cancer risk estimates categorized by exposure level even though these same studies reported such estimates for selected other cancer sites. To address this reporting bias, attempts were made to obtain the results from the primary investigators, and, failing that, an alternate analysis was performed in which null estimates (RR = 1.0) were included for all three studies. This alternate analysis was then used as the main analysis, e.g., the basis of comparison for the sensitivity analyses. For the SE (of the log RR) estimates for these null estimates, SE estimates from other sites for which highest-exposure-group results were available were used. For Anttila et al.(1995), the SE estimate for liver cancer in the highest exposure group was used, because liver cancer and kidney cancer had similar numbers of cases in the overall study (5 and 6, respectively). For Axelson et al. (1994), the SE estimate for NHL in the highest exposure group was used, because NHL and kidney cancer had similar numbers of cases in the overall study (5 and 6, respectively). For Hansen et al. (2001), the SE estimate for NHL in the highest exposure group was used, because NHL was the only cancer site of interest in this assessment for which highest-exposure-group results were available.

For Boice et al. (1999), only results for workers with “any potential exposure” (rather than “potential routine exposure”) were presented by exposure category, and the referent group is workers not exposed to any solvent.

For Morgan et al. (1998), the primary analysis used results for the cumulative exposure metric, and a sensitivity analysis was done with the results for the peak exposure metric.

Table C-9. Selected RR estimates for kidney cancer risk in highest TCE exposure groups

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Anttila et al. (1995)				100+ µmol/L U-TCA [a]			1.0 assumed	Reported high exposure group results for some cancer sites but not kidney.
Axelson et al. (1994)				≥2-yr exposure and 100+ mg/L U-TCA			1.0 assumed	Reported high exposure group results for some cancer sites but not kidney.
Boice et al. (1999)	0.69	0.22	2.12	≥5 yrs exp	–0.371	0.578	None	Mortality RR. ICD-9 189.0–189.2. For potential routine or intermittent exposure. Adjusted for date of birth, dates 1st and last employed, race, and sex. Referent group is workers not exposed to any solvent.
Hansen et al. (2001)				≥1,080 months × mg/m^3			1.0 assumed	Reported high exposure group results for some cancer sites but not kidney.
Morgan et al. (1998)	1.59	0.68	3.71	High cumulative exposure score	0.464	0.433	1.89 (0.85, 4.23) for med/high peak vs. low/no	Mortality RR. ICD-9 189.0–189.2. Adjusted for age and sex.
Raaschou-Nielsen et al. (2003)	1.7	1.1	2.4	≥5 yrs in subcohort with expected higher exposure levels	0.531	0.183	1.6 (1.1, 2.2) for ≥5 yrs in total cohort 1.4 (0.99, 1.9) ICD-7 180 ≥5 yrs in total cohort	SIR. RCC.
Radican et al. (2008)	1.11	0.35	3.49	>25 unit-yrs	0.104	0.582	Blair et al. (1998) incidence RR 0.9 (0.3, 3.2)	Mortality hazard ratio. ICD-8, -9 189.0, ICD-10 C64. Male and female results presented separately and combined (see text). Referent group is workers with no chemical exposures.

Table C-9. Selected RR estimates for kidney cancer risk in highest TCE exposure groups (continued)

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Zhao et al. (2005)	7.40	0.47	116	High exposure score	2.00	1.41	Mortality RR: 1.82 (0.09, 38.6) Incidence RR no lag: 7.71 (0.65, 91.4) Mortality RR no lag: 0.96 (0.09, 9.91) Boice et al. (2006b) mortality RR: 2.12 (0.63, 7.11) for ≥5 yrs as test stand mechanic; 3.13 (0.74,13.2) for ≥4 test-yr engine flush	Incidence RR. ICD-9 189. Males only. Adjusted for age, SES, time since first employment, exposure to other carcinogens. 20-yr lag. Incidence results reflect more exposed cases (4 with no lag) than do mortality results (3), so they are used in primary analysis.
Brüning et al. (2003)	2.69	0.84	8.66	≥20 yrs self-assessed exposure	0.990	0.595	None	Incidence OR. RCC. Adjusted for age, sex, and smoking.
Charbotel et al. (2006)	3.34	1.27	8.74	High cumulative dose	1.21	0.492	3.80 (1.27, 11.40) for high cum + peaks. Full study, high cum: 2.16 (1.02, 4.60) + peaks: 2.73 (1.06, 7.07) Full study with 10-yr lag, high cum: 2.16 (1.01, 4.65) + peaks: 3.15 (1.19, 8.38) Full study, additional adjustment, high cum: 1.96 (0.71, 5.37) + peaks: 2.63 (0.79, 8.83)	Incidence OR. RCC. In subgroup with good level of confidence for TCE exposure. Adjusted for smoking and BMI. Matched on sex and age. Alternate full study estimates (without lag) with additional adjustment were also adjusted for exposure to cutting fluids and other petroleum oils.

Table C-9. Selected RR estimates for kidney cancer risk in highest TCE exposure groups (continued)

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Moore et al. (2010)	2.23	1.07	4.64	≥1.58 ppm × yrs	0.802	0.374	2.02 (1.14, 3.59) for all subjects	Incidence OR. Subgroup with high-confidence assessments. Adjusted for age, sex, and center.
Pesch et al. (2000b)	1.4	0.9	2.1	Substantial	0.336	0.219	1.2 (0.9, 1.7) for JEM	Incidence OR. RCC. JTEM approach. Adjusted for age, study center, and smoking. Sexes combined.
Siemiatycki (1991)	0.8	0.2	3.4	Substantial	-0.233	0.736	None	Incidence OR. Kidney cancer. SE and 95% CI calculated from reported 90% CIs. Males only; adjusted for age, income, and cigarette smoking index.

[a]Mean personal TCA in urine. 1 µmol/L = 0.1634 mg/L.

For Raaschou-Nielsen et al. (2003), results for RCC in the highest duration subgroup from the subcohort with expected higher exposure levels (≥1-year duration of employment and year of 1st employment before 1980) were preferred for the highest-exposure-group analyses. Results for RCC in the highest duration subgroup from the whole cohort were combined across sexes, assuming a Poisson distribution, and used in a sensitivity analysis. Also, for the whole cohort, results for RCC and renal pelvis/ureter cancers in the highest duration group were combined (across sexes as well), assuming a Poisson distribution, and used in an additional sensitivity analysis.

For Radican et al. (2008), it should be noted that the referent group is workers with no chemical exposures, not just no TCE exposure. In addition, results for exposure groups (based on cumulative exposure scores) were reported separately for males and females and were combined for this assessment using inverse-variance weighting, as in a fixed-effect meta-analysis. Radican et al. (2008) present only mortality hazard ratio estimates by exposure group; however, in an earlier follow-up of this same cohort, Blair et al. (1998) present both incidence and mortality RR estimates by exposure group. The mortality RR estimate based on the more recent follow-up by Radican et al. (2008) (six deaths in the highest exposure group) was used in the primary analysis, while the incidence RR estimate based on similarly combined results from Blair et al. (1998) (four cases) was used as an alternate estimate in a sensitivity analysis. Radican et al. (2008) also present results for categories based on frequency and pattern of exposure; however, subjects weren't distributed uniquely across the categories (the numbers of cases across categories exceeded the total number of cases); thus, it was difficult to interpret these results and they were not used in a sensitivity analysis.

Zhao et al. (2005) present kidney cancer RR estimates adjusted for exposure to other carcinogens, because, unlike for NHL, this adjustment made a considerable difference. Estimates of RR with this additional adjustment were selected over those without. Furthermore, the kidney results were presented with and without a 20-year lag. A 20-year lag seemed reasonable for kidney cancer, so the lagged estimates were preferred to the unlagged; unlagged estimates were used in sensitivity analyses. In addition, the incidence results reflect more cases (4 with no lag) in the highest exposure group than do the mortality results (3), so the incidence result (with the 20-year lag) was used for the primary analysis, and the unlagged incidence result and the mortality results were used in a sensitivity analysis. Sensitivity analyses were also done using results from Boice et al. (2006b) in place of the Zhao et al. (2005) RR estimate. The cohorts for these studies overlap, so they are not independent studies. Boice et al. (2006b) report mortality RR estimates for kidney cancer by years worked as a test stand mechanic, a job with potential TCE exposure, and by a measure that weighted years with potential exposure from engine flushing by the number of flushes each year. No results were presented for a third metric, years worked with potential exposure to any TCE, because the Cox proportional hazards model

did not converge. The Boice et al. (2006b) estimates are adjusted for years of birth and hire and for hydrazine exposure.

For Charbotel et al. (2006), results from the analysis that considered "only job periods with a good level of confidence for TCE exposure assessment" [Table 7 of Charbotel et al. (2006)] were preferred, as these estimates would presumably be less influenced by exposure misclassification. Additionally, the high cumulative dose results were preferred, but the results for high cumulative dose + peaks were included in a sensitivity analysis. Exposure group results with a 10-year lag are provided in an unpublished report (Charbotel et al., 2005); however, lagged results are presented only for the full study and, thus, were used in sensitivity analyses. Estimates from the full study analysis (without the lag) that were further adjusted for exposure to cutting fluids and other petroleum oils were also used in sensitivity analyses.

Similarly, for Moore et al. (2010), results from the analysis that considered high-confidence assessments only were preferred. Here the definition of TCE exposure was restricted to jobs classified as having probable or certain exposure (i.e., at least 40% of workers with that job were expected to be exposed), so these estimates should be less influenced by exposure misclassification. Estimates from the analysis of all subjects were used in a sensitivity analysis. The highest exposure group was reported as ≥1.58 ppm × years; however, this value is not based on continuous exposure estimates but rather calculated from midpoints of estimated ranges corresponding to categorical groups, i.e., cumulative exposure = categorical intensity weight (ppm) × categorical frequency weight × duration (years).

For Pesch et al. (2000b), TCE results were presented for two different exposure assessments. As discussed above, estimates using the JTEM approach were preferred because they seemed to represent a more comprehensive exposure assessment; estimates based on the JEM approach were used in a sensitivity analysis.

C.3.2.2. Results of Meta-Analyses

Results from the meta-analyses that were conducted for kidney cancer in the highest exposure groups are summarized at the bottom of Table C-8 and reported in more detail in Table C-10. The RRm estimate from the random-effects meta-analysis of the 10 studies with results presented for exposure groups was 1.64 (95% CI: 1.31, 2.04). The RRm estimate from the primary random-effects meta-analysis with null RR estimates (i.e., 1.0) included for Anttila et al. (1995), Axelson et al. (1994), and Hansen et al. (2001) to address reporting bias (see above) was 1.58 (95% CI: 1.28, 1.96) (see Figure C-7). The inclusion of these three additional studies contributed just over 7% of the total weight. As with the overall kidney cancer meta-analyses, the meta-analyses of the highest exposure groups were dominated by two studies (Raaschou-Nielsen et al., 2003; Pesch et al., 2000b), which provided about 60% of the weight. No single study was overly influential; removal of individual studies resulted in RRm estimates that were

all statistically significant (all with $p < 0.005$) and that ranged from 1.52 [with the removal of Raaschou-Nielsen et al. (2003)] to 1.64 [with the removal of Pesch et al. (2000b)].

Similarly, the RRm estimate was not highly sensitive to alternate RR estimate selections. Use of the 18 alternate selections, individually, resulted in RRm estimates that were all statistically significant (all with $p < 0.0005$) and that ranged from 1.47 to 1.60, with all but two of the alternate selections yielding RRm estimates in the narrow range of 1.54–1.60 (see Table C-10). The lowest RRm estimates, 1.47 in both cases, were obtained when the alternate selections involved the two large studies. One of the alternate selections was for Raaschou-Nielsen et al. (2003), with a highest-exposure-group estimate for all kidney cancer in the total cohort, rather than RCC in the subcohort expected to have higher exposure levels. The latter value is strongly preferred because, as discussed above, the subcohort is likely to have less exposure misclassification. Furthermore, RCC is very different from other types of kidney cancer, and TCE, if an etiological factor, may not be etiologically associated with all kidney cancers, so using the broad category may dilute a true association with RCC, if one exists. The other alternate selection with a considerable impact on the RRm estimate was for Pesch et al. (2000b), with the highest exposure group result based on the JEM exposure assessment approach, rather than the JTEM approach. As discussed above, the JTEM approach is preferred because it seemed to be a more comprehensive and discriminating approach, taking actual job tasks into account, rather than just larger job categories. Thus, although results with these alternate selections are presented for comprehensiveness and transparency, the primary analysis is believed to reflect better the potential association between kidney cancer (in particular, RCC) and TCE exposure.

Other than a negligible amount of heterogeneity observed in the sensitivity analysis with the Pesch et al. (2000b) JEM alternate discussed above ($I^2 = 0.64\%$), there was no observable heterogeneity across the studies for any of the meta-analyses conducted with the highest exposure groups, including those in which RR values for Anttila et al.(1995), Axelson et al. (1994), and Hansen et al. (2001) were assumed. No subgroup analyses (e.g., cohort vs. case-control studies) were done with the highest exposure group results.

Table C-10. Summary of some meta-analysis results for TCE (highest exposure groups) and kidney cancer

Analysis	Model	RRm estimate	95% LCL	95% UCL	Heterogeneity	Comments
Analysis based on reported results	Random	1.64	1.31	2.04	None observable (fixed = random)	
Primary analysis	Random	**1.58**	**1.28**	**1.96**	None observable	Includes assumed values for Anttila et al. (1995), Axelson et al. (1994), and Hansen et al. (2001) (see text). Statistical significance not dependent on single study.
Alternate RR selections[a]	Random	1.57	1.27	1.95	None observable	With Blair et al. (1998) incidence RR instead of Radican et al. (2008) mortality hazard ratio.
	Random	1.60	1.29	1.98	None observable	With Morgan et al. (1998) peak metric.
	Random	1.47, 1.55	1.20, 1.25	1.80, 1.91	None observable	With Raaschou-Nielsen et al. (2003) ≥5 yrs in total cohort for all kidney cancer and for RCC, respectively.
	Random	1.56–1.58	1.26–1.28	1.93–1.96	None observable	With Zhao et al. (2005) incidence unlagged and mortality with and without lag.
	Random	1.58–1.59	1.28–1.29	1.95–1.96	None observable	With Boice et al. (2006b) alternates for Zhao et al. (2005).
	Random	1.59	1.29	1.95	None observable	With Moore et al. (2010) full study.
	Random	1.54–1.58	1.24–1.27	1.90–1.95	None observable	With Charbotel et al. (2006) high cumulative dose + peaks in subgroup; and high cumulative dose and high cumulative dose + peaks in full study with and without 10-yr lag and with and without additional adjustment for exposure to cutting fluids and other petroleum oils.
	Random	1.47	1.20	1.79	Not significant (p = 0.44)	With Pesch et al. (2000b) JEM.

[a]Changing the primary analysis by one alternate RR each time.

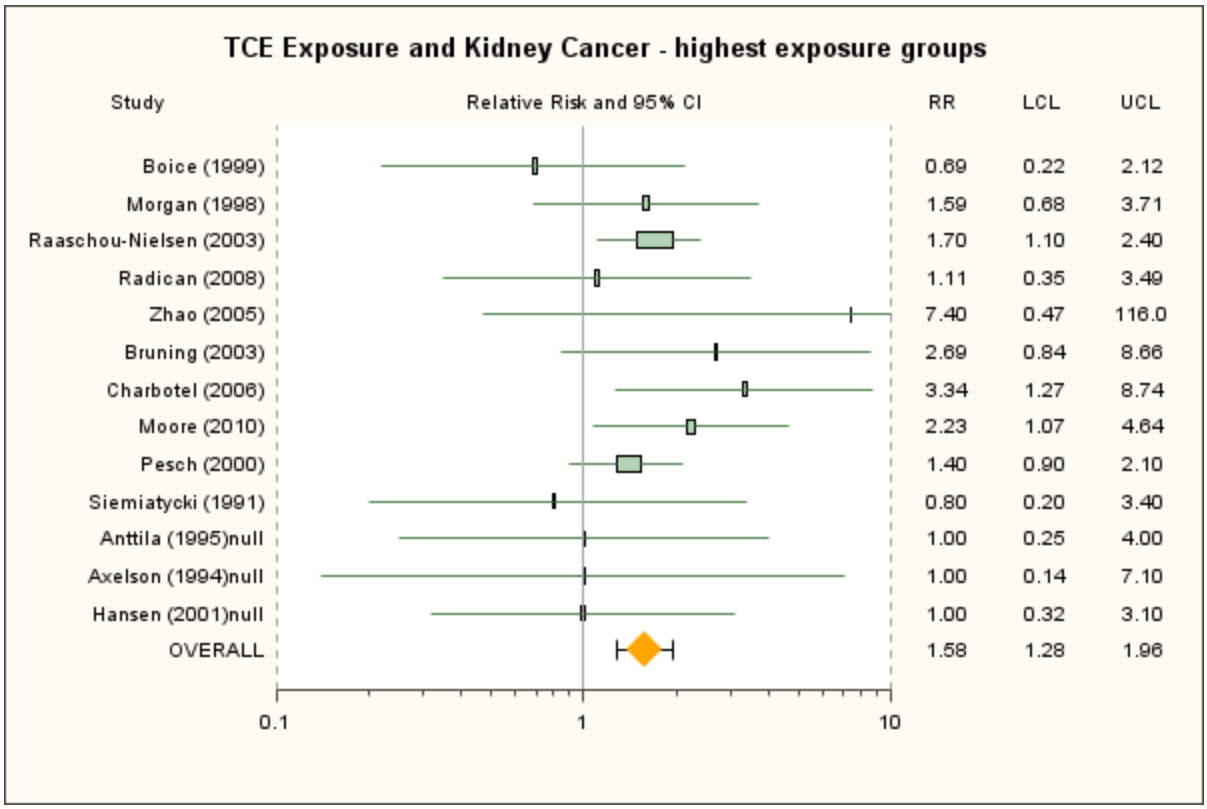


Figure C-7. Meta-analysis of kidney cancer and TCE exposure—highest exposure groups, with assumed null RR estimates for Anttila et al. (1995), Axelson et al. (1994), and Hansen et al. (2001) (see text). Random-effects model; fixed-effect model same. Rectangle sizes reflect relative weights of the individual studies. The summary estimate is in the bottom row, represented by the diamond.

C.3.3. Discussion of Kidney Cancer Meta-Analysis Results

For the most part, the meta-analyses of the overall effect of TCE exposure on kidney cancer suggest a small, statistically significant increase in risk. The summary estimate from the primary random-effects meta-analysis of the 15 studies was 1.27 (95% CI: 1.13, 1.43). Although the analysis was dominated by 2–3 large studies that contribute 65–75% of the weight, the summary estimate was not overly influenced by any single study, nor was it overly sensitive to individual RR estimate selections. The largest downward impacts were from the removal of the Brüning et al. (2003) study, resulting in an RRm estimate of 1.24 (95% CI: 1.10, 1.40), and from the substitution of the Pesch et al. (2000b) JTEM RR estimate with the RR estimate based on the JEM approach, resulting in an RRm estimate of 1.21 (95% CI: 1.09, 1.34). Thus, the finding of an increased risk of kidney cancer associated with TCE exposure is robust. Furthermore, there is no evidence of publication bias in this data set.

In addition, there was no heterogeneity observed across the results of the 15 studies. When subgroup analyses were done of cohort and case-control studies separately, there was

some observable heterogeneity among the case-control studies, but it was not statistically significant ($p = 0.14$) and the I^2-value of 41% suggested the extent of the heterogeneity was low-to-moderate. The increased risk of kidney cancer was strengthened in the case-control study analysis and weakened in the cohort study analysis, but the difference between the two RRm estimates was not statistically significant. One difference between the case-control and cohort studies is that the case-control studies were of RCC and almost all of the cohort studies were of all kidney cancers, including renal pelvis. As discussed above, RCC is very different from other types of kidney cancer, and TCE, if an etiological factor, may not be etiologically associated with all kidney cancers, so using the broad category may dilute a true association with RCC, if one exists.

With respect to the nonsignificant heterogeneity in the six case-control studies, these studies differ in TCE exposure potential to the underlying population from which case and control subjects were identified, and this may be a source of some heterogeneity. Prevalence of exposure to TCE among cases in these studies was 27% in Charbotel et al. (2006) (for high-level-of-confidence jobs), 18% in Brüning et al. (2003) (for self-assessed exposure), 18% in Pesch et al. (2000b), 13% in Dosemeci et al. (1999), 3.6% in Moore et al. (2010) (for high-confidence jobs), and 1% in Siemiatycki (1991). Both Brüning et al. (2003) and Charbotel et al. (2006) are studies designed specifically to assess RCC and TCE exposure. These studies were carried out in geographical areas with both a high prevalence and a high degree of TCE exposure. Some information is provided in these and accompanying papers to describe the nature of exposure, making it possible to estimate the order of magnitude of exposure, even though there were no direct measurements (Fevotte et al., 2006; Brüning et al., 2003; Cherrie et al., 2001). The Charbotel et al. (2006) study was carried out in the Arve Valley region in France, where TCE exposure was through metal-degreasing activity in small shops involved in the manufacturing of screws and precision metal parts (Fevotte et al., 2006). Industrial hygiene data from shops in this area indicated high intensity TCE exposures of ≥100 ppm, particularly from exposures from hot degreasing processes. Considering exposure only from the jobs with a high level of confidence about exposure, 18% of exposed cases were identified with high cumulative exposure to TCE. The source population in the Brüning et al. (2003) study includes the Arnsberg region in Germany, which also has a high prevalence of TCE exposure. A large number of small companies used TCE in metal degreasing in small workrooms. Subjects in this study also described neurological symptoms previously associated with higher TCE intensities. While subjects in the Brüning et al. (2003) study had potential high TCE exposure intensity, average TCE exposure in this study is considered lower than that in the Charbotel et al. (2006) study because the base population was enlarged beyond the Arnsberg region to areas which did not have the same focus of industry.

Siemiatycki (1991), Dosemeci et al. (1999), and Pesch et al. (2000b) are population-based studies. Sources of exposure to TCE and other chlorinated solvents are much less well

defined in these studies, and most subjects identified with TCE exposure probably had minimal contact; estimated average concentrations to exposed subjects were of about 10 ppm or less (NRC, 2006). Pesch et al. (2000b) includes the Arnsberg area and four other regions. Neither Dosemeci et al. (1999) nor Siemiatycki (1991) describe the nature of the TCE exposure. TCE exposure potential in these two studies is likely lower than in the other studies and closer to background. Furthermore, the use of generic job-exposure-matrices for exposure assessment in these studies may result in a greater potential for exposure misclassification bias.

Moore et al. (2010) is a hospital-based study which identified subjects from four Eastern and Central European countries with high kidney cancer rates (Czech Republic, Poland, Russia, and Romania). In their exposure assessment, Moore et al. (2010) accounted for the likelihood of TCE exposure, defined as possible, probable, or definite exposure. This likely increased exposure potential in their subgroup of high-confidence TCE assessments, which was restricted to subjects with probable or definite exposure. Although their semi-quantitative exposure assessment most probably improved exposure rankings, TCE exposure potential is likely lower in their study than in Brüning et al. (2003) and Charbotel et al. (2006), given the many jobs and industries included.

Ten of the 15 studies categorized results by exposure level. Three other studies reported results for other cancer sites by exposure level, but not kidney cancer; thus, to address this reporting bias, null values (i.e., RR estimates of 1.0) were used for these studies. Different exposure metrics were used in the various studies, and the purpose of combining results across the different highest exposure groups was not to estimate an RRm associated with some level of exposure, but rather to see the impacts of combining RR estimates that should be less affected by exposure misclassification. In other words, the highest exposure category is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any vs. none) exposure comparison. Thus, if TCE exposure increases the risk of kidney cancer, the effects should be more apparent in the highest exposure groups. Indeed, the RRm estimate from the primary meta-analysis of the highest exposure group results was 1.58 (95% CI: 1.28, 1.96), which is greater than the RRm estimate of 1.27 (95% CI: 1.13, 1.43) from the overall exposure analysis. This result for the highest exposure groups was not overly influenced by any single study, nor was it overly sensitive to individual RR estimate selections. Heterogeneity was not observed in any of the analyses, with the exception of some negligible heterogeneity (I^2 = 0.64%) in one sensitivity analysis. The robustness of this finding lends substantial support to a conclusion that TCE exposure increases the risk of kidney cancer.

C.4. META-ANALYSIS FOR LIVER CANCER

C.4.1. Overall Effect of TCE Exposure

C.4.1.1. Selection of RR Estimates

The selected RR estimates for liver cancer associated with TCE exposure from the epidemiological studies are presented in Table C-11. There were no case-control studies for liver cancer and TCE exposure that were selected for inclusion in the meta-analysis (see Appendix B, Section B.2.9), so all of the relevant studies are cohort studies. All of the studies reported results for liver cancers plus cancers of the gall bladder and extrahepatic biliary passages (i.e., ICD-7 155.0 + 155.2; ICD-8 and -9 155 + 156). Three of the studies also report results for liver cancer alone (ICD-7 155.0; ICD-8 and -9 155). For the primary analysis, results for cancers of the liver, gall bladder, and biliary passages combined were selected, for the sake of consistency, since these were reported in all of the studies. An alternate analysis was also done using results for liver cancer alone for the three studies that reported them and the combined liver cancer results for the remainder of the studies.

Table C-11. Selected RR estimates for liver cancer associated with TCE exposure (overall effect) from cohort studies

Study	RR	95% LCL	95% UCL	RR type	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Anttila et al. (1995)	1.89	0.86	3.59	SIR	0.637	0.333	2.27 (0.74, 5.29) for 155.0 alone	ICD-7 155.0 + 155.1; combined assuming Poisson distribution.
Axelson et al. (1994)	1.41	0.38	3.60	SIR	0.344	0.5	1.34 (0.36, 3.42) with estimated female contribution to SIR added (see text)	ICD-7 155. Results reported for males only, but there was a small female component to the cohort.
Boice et al. (1999)	0.81	0.45	1.33	SMR	–0.616	0.5	0.54 (0.15, 1.38) for potential routine exposure	ICD-9 155 + 156. For any potential exposure.
Greenland et al. (1994)	0.54	0.11	2.63	Mortality OR	–0.616	0.810	None	ICD-8 155 + 156. Nested case-control study.
Hansen et al. (2001)	2.1	0.7	5.0	SIR	0.742	0.447	None	ICD-7 155. Male and female results reported separately; combined assuming Poisson distribution.
Morgan et al. (1998)	1.48	0.56	3.91	SMR	0.393	0.495	Published SMR 0.98 (0.36, 2.13)	ICD-9 155 + 156. Unpublished RR, adjusted for age and sex (see text).
Raaschou-Nielsen et al. (2003)	1.35	1.03	1.77	SIR	0.300	0.132	1.28 (0.89, 1.80) for ICD-7 155.0	ICD-7 155.0 + 155.1. Results for males and females and different liver cancer types reported separately; combined assuming Poisson distribution.
Radican et al. (2008)	1.12	0.57	2.19	Mortality hazard ratio	0.113	0.343	1.25 (0.31, 4.97) for ICD-8, -9 155.0	ICD-8, -9 155 + 156, ICD-10 C22-C24. Time variable = age; covariates = sex, race. Referent group is workers with no chemical exposures.
Boice et al. (2006b)	1.28	0.35	3.27	SMR	0.247	0.5	1.0 assumed for Zhao et al. (2005)	ICD-9 155 + 156. Boice et al. (2006b) used in lieu of Zhao et al. (2005) because Zhao et al. (2005) do not report liver cancer results. Boice et al. (2006b) cohort overlaps Zhao et al. (2005) cohort.

As for NHL and kidney cancer, many of the studies provided RR estimates only for males and females combined, and we are not aware of any basis for a sex difference in the effects of TCE on liver cancer risk; thus, wherever possible, RR estimates for males and females combined were used. The only study of much size (in terms of number of liver cancer cases) that provided results separately by sex was Raaschou-Nielsen et al. (2003). The results of this study suggest that liver cancer risk in females might be slightly higher than the risk in males, but the number of female cases is small (primary liver cancer SIR: males 1.1 [95% CI: 0.74, 1.64; 27 cases], females 2.8 [95% CI: 1.13, 5.80; 7 cases]; gallbladder and biliary passage cancers SIR: males 1.1 [95% CI: 0.61, 1.87; 14 cases]; females 2.8 [1.28, 5.34; 9 cases]). Radican et al. (2008) report hazard ratios for liver/biliary passage cancers combined of 1.36 (95% CI: 0.59, 3.11; 28 deaths) for males and 0.74 (95% CI: 0.18, 2.97; 3 deaths) for females, but these results are based on fewer cases, especially in females.

Most of the selections in Table C-11 should be self-evident, but some are discussed in more detail here, in the order the studies are presented in the table. For Axelson et al. (1994), in which a small subcohort of females was studied but only results for the larger male subcohort were reported, the reported male-only results were used in the primary analysis; however, as for NHL and kidney cancer, an attempt was made to estimate the female contribution to an overall RR estimate for both sexes and its impact on the meta-analysis. Axelson et al. (1994) reported that there were no cases of liver cancer observed in females, but the expected number was not presented. To estimate the expected number, the expected number for males was multiplied by the ratio of female-to-male person-years in the study and by the ratio of female-to-male age-adjusted incidence rates for liver cancer.[8] The male results and the estimated female contribution were then combined into an RR estimate for both sexes assuming a Poisson distribution, and this alternate RR estimate for the Axelson et al. (1994) study was used in a sensitivity analysis.

For Boice et al. (1999), results for "any potential exposure" were selected for the primary analysis, because this exposure category was considered to best represent overall TCE exposure, and results for "potential routine exposure," which was characterized as reflecting workers assumed to have received more cumulative exposure, were used in a sensitivity analysis. To estimate the SE (log RR) for the primary RR selection, it was assumed that the number of exposed cases (deaths) was 15. The actual number was not presented, but 15 was the number that allowed us to reproduce the reported CIs. The number suggested by exposure level in Boice

[8]Person-years for men and women ≤79 years were obtained from Axelson et al. (1994): 23,516.5 and 3,691.5, respectively. Lifetime age-adjusted incidence rates for liver cancer for men and women were obtained from the National Cancer Institute's 2000-2004 SEER-17 (Surveillance Epidemiology and End Results from 17 geographical areas) database (http://seer.cancer.gov/statfacts/html/livibd.html): 9.5/100,000 and 3.4/100,000, respectively. The calculation for estimating the expected number of cases in females in the cohort assumes that the males and females have similar TCE exposures and that the relative distributions of age-related incidence risk for the males and females in the Swedish cohort are adequately represented by the ratios of person-years and lifetime U.S. incidence rates used in the calculation.

et al. (1999) Table 9 is 13; however, it may be that exposure level data were not available for all of the cases.

In their published paper, Morgan et al. (1998) present only SMRs for overall TCE exposure, although the results from internal analyses are presented for exposure subgroups. RR estimates for overall TCE exposure from the internal analyses of the Morgan et al. (1998) cohort data were available from an unpublished report (EHS, 1997); from these, the RR estimate from the Cox model that included age and sex was selected, because those are the variables deemed to be important in the published paper. The internal analysis RR estimate was preferred for the primary analysis, and the published SMR result was used in a sensitivity analysis.

Raaschou-Nielsen et al. (2003) reported results for primary liver cancer (ICD-7 155.0), gallbladder and biliary passage cancers (ICD-7 155.1), and unspecified liver cancers (ICD-7 156) separately. As discussed above, RR estimates for cancers of the liver, gall bladder, and biliary passages combined were preferred for the primary analysis; thus, the results for primary liver cancer and gallbladder/biliary passage cancers were combined (across sexes as well), assuming a Poisson distribution. The results for primary liver cancer only (similarly combined across sexes) were used in an alternate analysis. The results for unspecified liver cancers (ICD-7 156) were not included in any analyses because, under the ICD-7 coding, 156 can include secondary liver cancers. Raaschou-Nielsen et al. (2003), in their Table 3, also present overall results for primary liver cancer and gallbladder/biliary passage cancers with a lag time of 20 years; however, they use a definition of lag that is different from a lagged exposure in which exposures prior to disease onset are discounted and it is not clear what their lag time actually represents[9], thus, as for NHL and kidney cancer, these results were not used in any of the meta-analyses for liver cancer. In addition, results for the subcohort with expected higher exposure levels were not provided for liver cancer, so no alternate analysis was done based on the subcohort.

For Radican et al. (2008), the Cox model hazard ratio from the 2000 follow-up was used. In the Radican et al. (2008) Cox regressions, age was the time variable, and sex and race were covariates. It should also be noted that the referent group is composed of workers with no chemical exposures, not just no exposure to TCE.

Zhao et al. (2005) did not present RR estimates for liver cancer; thus, results from Boice et al. (2006b) were used in the primary analysis. The cohorts for these studies overlap, so they are not independent studies. Zhao et al. (2005), however, was our preferred study for NHL and kidney cancer results; thus, in a sensitivity analysis, a null value (RR = 1.0) was assumed for Zhao et al. (2005) to address the potential reporting bias. The SE estimate for kidney cancer (incidence with 0 lag) was used as the SE for the liver cancer. (It is not certain that there was a reporting bias in this case. In the "Methods" section of their paper, Zhao et al. [(2005) list the

[9]In their Methods section, Raaschou-Nielsen et al. (2003) define their lag period as the period "from the date of first employment to the start of follow-up for cancer".

cancer sites examined in the cohort, and liver was not listed; it is not clear if the list of sites was determined a priori or post hoc.)

Also, on the issue of potential reporting bias, the Siemiatycki (1991) study should be mentioned. This study was a case-control study for multiple cancer sites, but only the more common sites, in order to have greater statistical power. Thus, NHL and kidney cancer results were available, but not liver cancer results. Because no liver results were presented for any of the chemicals, this is not a case of reporting bias.

C.4.1.2. Results of Meta-Analyses

Results from some of the meta-analyses that were conducted on the epidemiological studies of TCE and liver cancer are summarized in Table C-12. The RRm from the primary random-effects meta-analysis of the nine studies was 1.29 (95% CI: 1.07, 1.56) (see Figure C-8). As shown in Figure C-8, the analysis was dominated by one large study (contributing about 53% of the weight). That large study was critical in terms of the statistical significance of the RRm estimate. Without the large Raaschou-Nielsen et al. (2003) study, the RRm estimate decreases somewhat and is no longer statistically significant (RRm = 1.22; 95% CI: 0.93, 1.61). No other single study was overly influential; removal of any of the other individual studies resulted in RRm estimates that were all statistically significant (all with $p \leq 0.03$) and that ranged from 1.24 [with the removal of Anttila et al. (1995)] to 1.39 [with the removal of Boice et al. (1999)].

As discussed in Section C.4.1.1, all of the nine studies presented results for liver and gall bladder/biliary passage cancers combined, and these results were the basis for the primary analysis discussed above. An alternate analysis was performed substituting, simultaneously, results for liver cancer alone for the three studies for which these were available. The RRm estimate from this analysis was slightly lower than the one based entirely on results from the combined cancer categories and was just short of statistical significance (1.25; 95% CI: 0.99, 1.57). This result was driven by the fact that the RR estimate from the large Raaschou-Nielsen et al. (2003) study decreased from 1.35 for liver and gall bladder/biliary passage cancers combined to 1.28 for liver cancer alone.

Table C-12. Summary of some meta-analysis results for TCE and liver cancer

Analysis	Number of studies	Model	RRm estimate	95% LCL	95% UCL	Heterogeneity	Comments
All studies (all cohort studies)	9	Random	1.29	1.07	1.56	None observable (fixed = random)	Statistical significance not dependent on single study, except for Raaschou-Nielsen et al. (2003), without which $p = 0.15$. No apparent publication bias.
		Fixed	1.29	1.07	1.56		
All studies; liver cancer only, when available	9	Random	1.25	0.99	1.57	None observable	Used RR estimates for liver cancer alone for the three studies that presented these; remaining RR estimates are for liver and gall bladder/biliary passage cancers.
Alternate RR selections[a]	9	Random	1.28	1.06	1.55	None observable	With RR = 1 assumed for Zhao et al. (2005) in lieu of Boice et al. (2006b) (see text).
	9	Random	1.34	1.09	1.63	None observable	With Boice et al. (1999) potential routine exposure rather than any potential exposure.
	9	Random	1.29	1.07	1.55	None observable	With estimated female contribution to Axelson et al. (1994).
	9	Random	1.26	1.05	1.52	None observable	With Morgan et al. (1998) published SMR.
Highest exposure groups	6	Random	1.32	0.93	1.86	None observable	
	8	Random	1.28	0.93	1.77	None observable	Primary analysis. Using RR = 1 for Hansen et al. (2001) and Zhao et al. (2005) (see text).
	7–8	Random	1.24–1.26	0.88–0.91	1.73–1.82	None observable	Using alternate selections for Morgan et al. (1998) and Raaschou-Nielsen et al. (2003) and excluding Axelson et al. (1994) (see text).[a]

[a]Changing the primary analysis by one alternate RR each time.

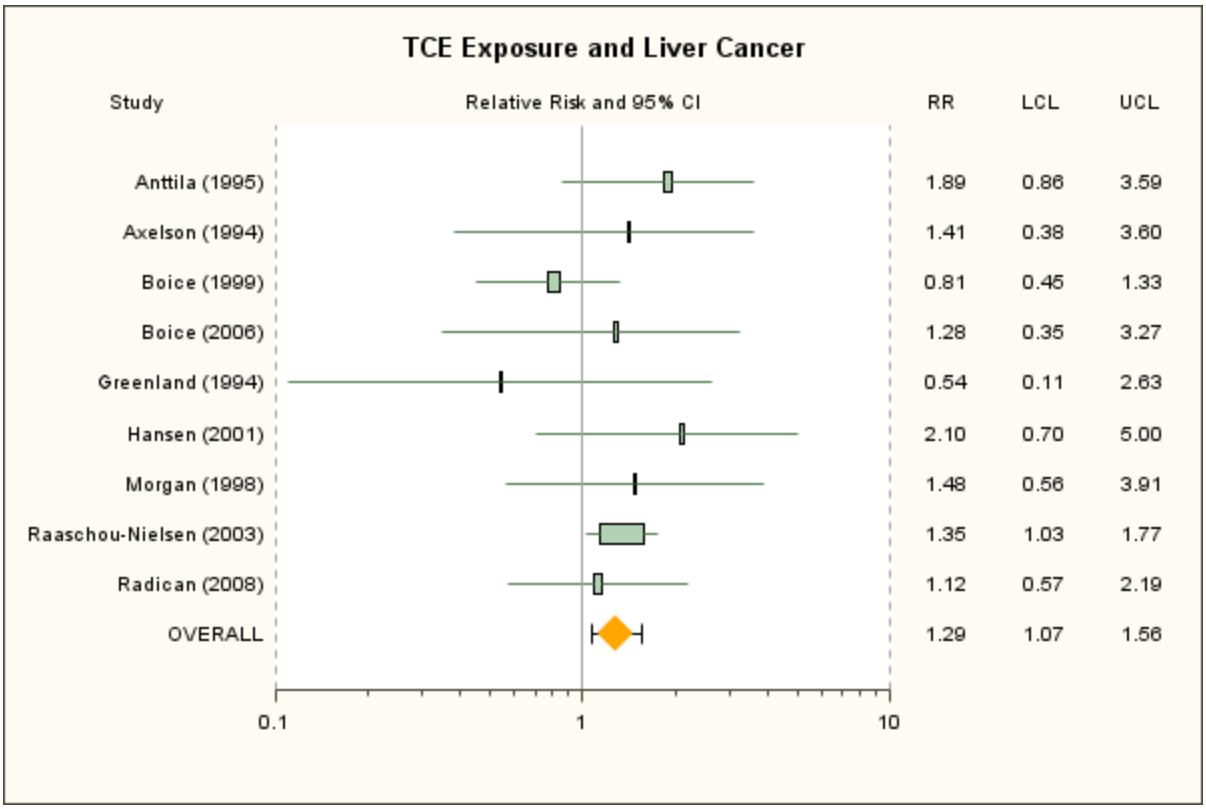


Figure C-8. Meta-analysis of liver cancer and TCE exposure. Random-effects model; fixed-effect model same. Rectangle sizes reflect relative weights of the individual studies. The summary estimate is in the bottom row, represented by the diamond.

Similarly, the RRm estimate was not highly sensitive to other alternate RR estimate selections. Use of the 4 other alternate selections, individually, resulted in RRm estimates that were all statistically significant (all with $p < 0.02$) and that ranged from 1.26 to 1.34 (see Table C-12). In fact, as can be seen in Table C-12, only one of the alternates had notable impact. The Boice et al. (2006b), Morgan et al. (1998), and Axelson et al. (1994) original values and alternate selections were associated with very little weight and, thus, have little influence in the RRm. Using the Boice et al. (1999) alternate RR estimate based on potential routine exposure rather than any potential exposure increased the RRm slightly from 1.29 to 1.34. The alternate Boice et al. (1999) RR estimate is actually smaller than the original value (0.54 vs. 0.81); however, use of the more restrictive exposure metric captures fewer liver cancer deaths, causing the weight of that study to decrease from almost 14% to about 4.1%.

There was no apparent heterogeneity across the nine studies (i.e., the random-effects model and the fixed-effect model gave the same results [$I^2 = 0\%$]). Furthermore, all of the liver cancer studies were cohort studies, so no subgroup analyses examining cohort and case-control studies separately, as was done for NHL and kidney cancer, were conducted. No alternate

quantitative investigations of heterogeneity were pursued because of database limitations and, in any event, there is no evidence of heterogeneity of study results in this database.

As discussed in Section C.1, publication bias was examined in several different ways. The funnel plot in Figure C-9 shows little relationship between RR estimate and study size, and, indeed, none of the other tests performed found any evidence of publication bias. The trim-and-fill procedure of Duval and Tweedie (2000), for example, suggested that no studies were missing from the funnel plot (i.e., there was no asymmetry to counterbalance). Similarly, the results of a cumulative meta-analysis, incorporating studies with increasing SE one at a time, shows no evidence of a trend of increasing effect size with addition of the less precise studies. The Raaschou-Nielsen et al. (2003) study contributes about 53% of the weight. Including the two next most precise studies, the RRm goes from 1.35 to 1.10 to 1.25 and the weight to 75%. With the addition of the next two most precise studies, the RRm estimate goes to 1.23 and then 1.29. Further addition of the four least precise studies leaves the RRm essentially unchanged.

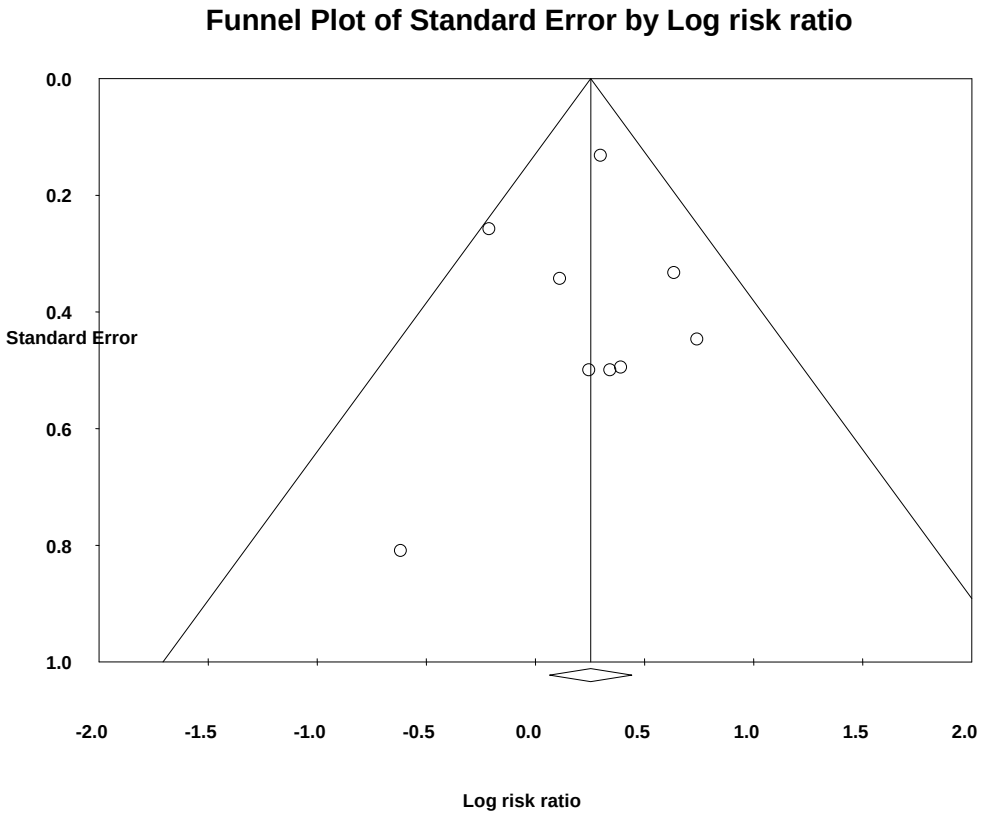


Figure C-9. Funnel plot of SE by log RR estimate for TCE and liver cancer studies.

C.4.2. Liver Cancer Effect in the Highest Exposure Groups

C.4.2.1. Selection of RR Estimates

The selected RR estimates for liver cancer in the highest TCE exposure categories, for studies that provided such estimates, are presented in Table C-13. Six of the nine cohort studies reported liver cancer risk estimates categorized by exposure level. As in Section C.4.1.1 for the overall risk estimates, estimates for cancers of the liver and gall bladder/biliary passages combined were preferentially selected, when presented, for the sake of consistency, and, wherever possible, RR estimates for males and females combined were used.

Two of the nine cohort studies (Zhao et al., 2005; Hansen et al., 2001) did not report liver cancer risk estimates categorized by exposure level, even though these same studies reported such estimates for selected other cancer sites. To address this reporting bias (as discussed above, Zhao et al. (2005) did not present any liver results, and it is not clear if this was actual reporting bias or an a priori decision not to examine liver cancer in the cohort), attempts were made to obtain the results from the primary investigators, and, failing that, alternate analyses were performed in which null estimates (RR = 1.0) were included for both studies. This alternate analysis was then used as the main analysis, e.g., the basis of comparison for the sensitivity analyses. For the SE (of the log RR) estimates for the null estimates, SE estimates from other sites for which highest-exposure-group results were available were used. For Hansen et al. (2001), the SE estimate for NHL in the highest exposure group was used, because NHL was the only cancer site of interest in this assessment for which highest-exposure-group results were available. For Zhao et al. (2005), the SE estimate for kidney cancer in the highest exposure group (incidence with 0 lag) was used. (Note that Boice et al. (2006b), who studied a cohort that overlapped that of Zhao et al. (2005), also did not present liver cancer results by exposure level.)

Table C-13. Selected RR estimates for liver cancer risk in highest TCE exposure groups

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Anttila et al. (1995)	2.74	0.33	9.88	100+ µmol/L U-TCA [a]	1.008	0.707	None	SIR. ICD-7 155.0 (liver only).
Axelson et al. (1994)	3.7	0.09	21	100+ mg/L U-TCA	1.308	1.000	Exclude study	SIR. ICD-7 155. 0 cases observed in highest exposure group (i.e., ≥2 yrs and 100+ U-TCA), so combined with <2 yrs and 100+ subgroup and females, estimating the expected numbers (see text).
Boice et al. (1999)	0.94	0.36	2.46	≥5 yrs exposure	–0.062	0.490	None	Mortality RR. ICD-9 155 + 156. For potential routine or intermittent exposure. Adjusted for date of birth, dates 1st and last employed, race, and sex. Referent group is workers not exposed to any solvent.
Hansen et al. (2001)				≥1,080 months × mg/m^3			1.0 assumed	Reported high exposure group results for some cancer sites but not liver.
Morgan et al. (1998)	1.19	0.34	4.16	High cumulative exposure score	0.174	0.639	0.98 (0.29, 3.35) for med/high peak vs. low/no	Mortality RR. ICD-9 155 + 156. Adjusted for age and sex.
Raaschou-Nielsen et al. (2003)	1.2	0.7	1.9	≥5 yrs	0.182	0.243	1.1 (0.5, 2.1) ICD-7 155.0 (liver only)	SIR. ICD-7 155.0 + 155.1. Male and female results presented separately and combined assuming a Poisson distribution.
Radican et al. (2008)	1.49	0.67	3.34	>25 unit-yrs	0.399	0.411	None (see text)	Mortality hazard ratio. ICD-8, -9 155 + 156, ICD-10 C22-C24. Male and female results presented separately and combined (see text). Time variable = age, covariate = race. Referent group is workers with no chemical exposures.
Zhao et al. (2005)				High exposure score			1.0 assumed	No liver results reported.

[a]Mean personal TCA in urine. 1 µmol/L = 0.1634 mg/L.

For Axelson et al. (1994), there were no liver cancer cases in the highest exposure group (≥2 years and 100+ mean U-TCA level), so no log RR and SE (log RR) estimates were available for the meta-analysis. Instead, the <2 and ≥2 years results were combined, assuming expected numbers of cases were proportional to person-years, and 100+ U-TCA (with any exposure duration) was used as the highest exposure category. The female contribution to the expected number was also estimated, again assuming proportionality to person-years, and adjusting for the difference between female and male age-adjusted liver cancer incidence rates. The estimated RR and SE values for the combined exposure times and sexes were used in the primary analysis. In an alternate analysis, the Axelson et al. (1994) study was excluded altogether, because we estimated that <0.2 cases were expected in the highest exposure category, suggesting that the study had low power to detect an effect in the highest exposure group and would contribute little weight to the meta-analysis.

For Boice et al. (1999), only results for workers with "any potential exposure" were presented by exposure category, and the referent group is workers not exposed to any solvent. For Morgan et al. (1998), the primary analysis used results for the cumulative exposure metric, and a sensitivity analysis was done with the results for the peak exposure metric. For Raaschou-Nielsen et al. (2003), unlike for NHL and RCC, liver cancer results for the subcohort with expected higher exposure levels were not presented, so the only highest-exposure-group results were for duration of employment in the total cohort. Results for cancers of the liver and gall bladder/biliary passages combined were used for the primary analysis and results for liver cancer alone in a sensitivity analysis.

For Radican et al. (2008), it should be noted that the referent group is workers with no chemical exposures, not just no TCE exposure. Furthermore, results for exposure groups (based on cumulative exposure scores) were reported separately for males and females and were combined for this assessment using inverse-variance weighting, as in a fixed-effect meta-analysis. In addition to results for biliary passage and liver cancer combined, Radican et al. (2008) present results for liver only by exposure group; however, there were no liver cancer deaths in females and the number expected was not reported, so no alternate analysis for the highest exposure groups with an RR estimate from Radican et al. (2008) for liver cancer only was conducted. Radican et al. (2008) present only mortality hazard ratio estimates by exposure group; however, in an earlier follow-up of this same cohort, Blair et al. (1998) present both incidence and mortality RR estimates by exposure group. As with the Radican et al. (2008) liver cancer only results, however, there were no incident cases for females in the highest exposure group in Blair et al. (1998) (and the expected number was not reported). Additionally, there were more biliary passage/liver cancer deaths (31) in Radican et al. (2008) than incident cases (13) in Blair et al. (1998) overall and in the highest exposure group (14 vs. 4). Thus, we elected to use only the Radican et al. (2008) mortality results from this cohort and not to include an alternate analysis based on incidence results from the earlier follow-up. Radican et al. (2008)

also present results for categories based on frequency and pattern of exposure; however, subjects weren't distributed uniquely across the categories (the numbers of cases across categories exceeded the total number of cases), thus it was difficult to interpret these results and they were not used in a sensitivity analysis.

C.4.2.2. Results of Meta-Analyses

Results from the meta-analyses that were conducted for liver cancer in the highest exposure groups are summarized at the bottom of Table C-12. The RRm estimate from the random-effects meta-analysis of the six studies with results presented for exposure groups was 1.32 (95% CI: 0.93, 1.86). As with the overall liver cancer meta-analyses, the meta-analyses of the highest exposure groups were dominated by one study (Raaschou-Nielsen et al., 2003), which provided about 52% of the weight. The RRm estimate from the primary random-effects meta-analysis with null RR estimates (i.e., 1.0) included for Hansen et al. (2001) and Zhao et al. (2005) to address (potential) reporting bias (see above) was 1.28 (95% CI: 0.93, 1.77) (see Figure C-10). The inclusion of these two additional studies contributed about 10% of the total weight. No single study was overly influential (removal of individual studies resulted in nonsignificant RRm estimates that ranged from 1.23 to 1.36), and the RRm estimate was not highly sensitive to alternate RR estimate selections (RRm estimates with alternate selections ranged from 1.24 to 1.26, all nonsignificant; see Table C-12). In addition, there was no observable heterogeneity across the studies for any of the meta-analyses conducted with the highest exposure groups (I^2 = 0%). However, none of the RRm estimates was statistically significant.

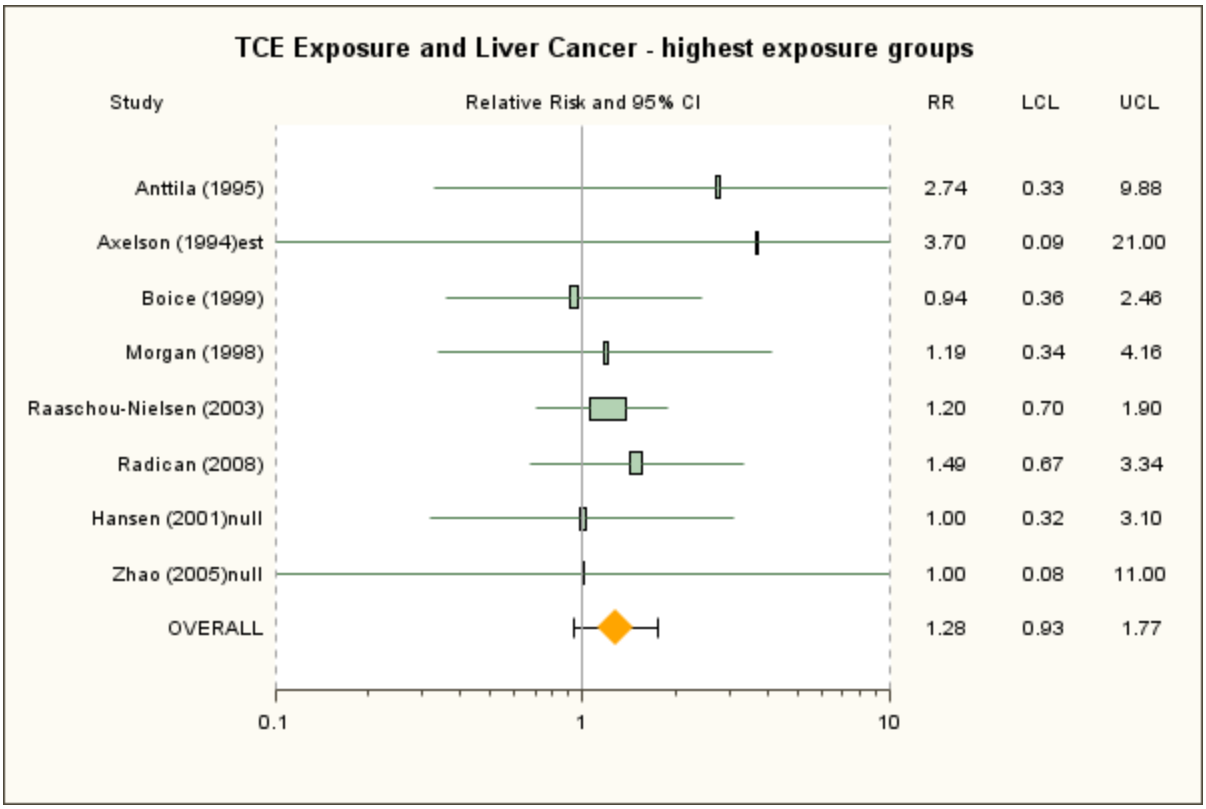


Figure C-10. Meta-analysis of liver cancer and TCE exposure—highest exposure groups, with assumed null RR estimates for Hansen et al. (2001) and Zhao et al. (2005) (see text). Random-effects model; fixed-effect model same. Rectangle sizes reflect relative weights of the individual studies. The summary estimate is in the bottom row, represented by the diamond.

Furthermore, most of the RRm estimates for the highest exposure groups were less than the significant RRm estimate for an overall effect on liver cancer (1.29; 95% CI: 1.07, 1.56; see Section C.4.2.2 and Table C-12). This contradictory result is driven by the fact that the RR estimate for the highest exposure group was less than the overall RR estimate for Raaschou-Nielsen et al. (2003), which contributes the majority of the weight to the meta-analyses. The liver cancer results are relatively underpowered with respect to numbers of studies and number of cases, and the Raaschou-Nielsen et al. (2003) study, which dominates the analysis, uses duration of employment as an exposure-level surrogate for liver cancer, and duration of employment is a notoriously weak exposure metric[10]. Thus, the contradictory finding that most of the RRm estimates for the highest exposure groups were less than the RRm estimate for an

[10]Moreover, this study is prone to misclassifying some of the subjects with longer durations of employment as having lesser durations of employment due to the fact that employment information prior to 1964 was not available and, thus, employment prior to 1964 was not included in the calculations of duration of employment. For example, 17 of the 27 primary liver cancer cases in men were observed in men first employed before 1970 and some of these might have occurred in men first employed before 1964. Thus, some of the 18 cases with durations of employment reported as < 5 years may actually have had durations ≥5 years and hence may have belonged in the highest exposure group.

overall effect does not rule out an effect of TCE on liver cancer; however, it certainly does not provide additional support for such an effect.

C.4.3. Discussion of Liver Cancer Meta-Analysis Results

For the most part, the meta-analyses of the overall effect of TCE exposure on liver (and gall bladder/biliary passages) cancer suggest a small, statistically significant increase in risk. The summary estimate from the primary random-effects meta-analysis of the nine (all cohort) studies was 1.29 (95% CI: 1.07, 1.56). The analysis was dominated by one large study that contributed about 53% of the weight. When this study was removed, the RRm estimate decreased somewhat and was no longer statistically significant (RRm = 1.22; 95% CI: 0.93, 1.61). The summary estimate was not overly influenced by any other single study, nor was it overly sensitive to individual RR estimate selections. The next largest downward impacts were from the removal of the Anttila et al. (1995) study, resulting in an RRm estimate of 1.24 (95% CI: 1.02, 1.51), and from the substitution of the Morgan et al. (1998) unpublished RR estimate (EHS, 1997) with the published SMR estimate (Morgan et al., 1998), resulting in an RRm estimate of 1.26 (95% CI: 1.05, 1.52). Substituting the RR estimates for liver/gall bladder/biliary passage cancers with those of liver cancer alone for the three studies that provided these results yielded an RRm estimate of 1.25 (95% CI: 0.99, 1.57). There was no evidence of publication bias in this data set, and there was no observable heterogeneity across the study results.

Six of the nine studies provided liver cancer results by exposure level. Two other studies reported results for other cancer sites by exposure level, but not liver cancer; thus, to address this reporting bias, null values (i.e., RR estimates of 1.0) were used for these studies. Different exposure metrics were used in the various studies, and the purpose of combining results across the different highest exposure groups was not to estimate an RRm associated with some level of exposure, but rather to see the impacts of combining RR estimates that should be less affected by exposure misclassification. In other words, the highest exposure category is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any vs. none) exposure comparison. Thus, if TCE exposure increases the risk of liver cancer, the effects should be more apparent in the highest exposure groups. However, the RRm estimate from the primary meta-analysis of the highest exposure group results (and most of the RRm estimates from the sensitivity analyses) was less than the RRm estimate from the overall exposure analysis. This anomalous result is driven by the fact that for Raaschou-Nielsen et al. (2003), which contributes the majority of the weight to the meta-analyses, the RR estimate for the highest exposure group, although >1, was less than the overall RR estimate.

Thus, while there is the suggestion of an increased risk for liver cancer associated with TCE exposure, the statistical significance of the overall summary estimate is dependent on one

study, which provides the majority of the weight in the meta-analyses. Removal of this study yields an RRm estimate that is decreased somewhat but is still >1; however, it becomes nonsignificant (p = 0.15). Furthermore, meta-analysis results for the highest exposure groups yielded generally *lower* RRm estimates than for an overall effect. These results do not rule out an effect of TCE on liver cancer, because the liver cancer results are relatively underpowered with respect to numbers of studies and number of cases and the overwhelming study in terms of weight uses the weak exposure surrogate of duration of employment for categorizing exposure level; however, at present, there is only modest support for such an effect.

C.5. META-ANALYSIS FOR LUNG CANCER

C.5.1. Overall Effect of TCE Exposure

C.5.1.1. Selection of RR Estimates

Although there was no general indication of an increased risk of lung cancer associated with TCE exposure in the epidemiologic literature, the Science Advisory Board recommended a meta-analysis for lung cancer to more exhaustively examine the issue of smoking as a possible confounder in the kidney cancer studies (SAB, 2011). Only the cohort studies were considered for the meta-analysis because these provide a consistent group of studies to compare RRm estimates for kidney cancer to those for lung cancer and the cohort studies are the studies of concern for potential confounding since the kidney cancer results from these studies were not adjusted for smoking. The selected RR estimates for lung cancer from the nine cohort studies are presented in Table C-14. All of the studies, with the possible exception of Greenland et al. (1994), reported cancers of the lung and bronchus combined. Some also included cancer of the trachea; however, this is a rare tumor (<0.1% of tumors) (Macchiarini, 2006) and so its inclusion is negligible.

As for NHL and kidney and liver cancer, many of the studies provided RR estimates only for males and females combined, and we are not aware of any basis for a sex difference in the effects of TCE on lung cancer risk; thus, wherever possible, RR estimates for males and females combined were used. The only two studies of much size (in terms of number of lung cancer cases) that provided results separately by sex were Raaschou-Nielsen et al. (2003) and Radican et al. (2008). The results from Raaschou-Nielsen et al. (2003) suggest that lung cancer RR in females might be slightly higher than the RR in males (SIR: males 1.4 [95% CI: 1.3, 1.5; 559 cases], females 1.9 [95% CI: 1.5, 2.4; 73 cases]), but the difference narrows when a 20-year lag is taken into account (males 1.4 [95% CI: 1.2, 1.6; 202 cases], females 1.6 [95% CI: 1.0, 2.3; 26 cases]). Radican et al. (2008) report hazard ratios for lung cancer of 0.91 (95% CI: 0.67, 1.24; 155 deaths) for males and 0.53 (95% CI: 0.27, 1.07; 11 deaths) for females, but these results are based on fewer cases, especially in females.

Table C-14. Selected RR estimates for lung (& bronchus) cancer associated with TCE exposure (overall effect) from cohort studies

Study	RR	95% LCL	95% UCL	RR type	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Anttila et al. (1995)	0.92	0.59	1.35	SIR	-0.0834	0.2	None	
Axelson et al. (1994)	0.69	0.31	1.30	SIR	-0.371	0.333	None	Results reported for males only, but there was a small female component to the cohort.
Boice et al. (1999)	0.76	0.66	0.87	SMR	-0.274	0.0705	0.76 (0.60, 0.95) for potential routine exposure	For any potential exposure.
Greenland et al. (1994)	1.01	0.69	1.47	OR	0.00995	0.193	None	Nested case-control study.
Hansen et al. (2001)	0.8	0.5	1.3	SIR	-0.223	0.243	None	Male and female results reported separately; combined assuming Poisson distribution.
Morgan et al. (1998)	1.14	0.90	1.44	SMR	0.133	0.119	Published SMR 1.10 (0.89, 1.34)	Unpublished RR, adjusted for age and sex (see text).
Raaschou-Nielsen et al. (2003)	1.43	1.32	1.55	SIR	0.358	0.0398	None	
Radican et al. (2008)	0.83	0.63	1.08	Mortality hazard ratio	-0.186	0.138	None	Time variable = age; covariates = sex, race. Referent group is workers with no chemical exposures.
Zhao et al. (2005)	1.04	0.81	1.34	RR	0.0392	0.128	1.27 (0.88, 1.83) for incidence. 1.24 (0.92, 1.63) for Boice et al. (2006b) mortality.	Mortality

Most of the selections in Table C-14 should be self-evident, but some are discussed in more detail here, in the order the studies are presented in the table. For Axelson et al. (1994), in which a small subcohort of females was studied but only results for the larger male subcohort were reported, only the reported male results were used. Unlike for NHL and kidney and liver cancer, no attempt was made to estimate the female contribution to an overall RR estimate for both sexes and its impact on the meta-analysis because, unlike for those other cancer types, the meta-analysis for lung cancer was not done to test a null hypothesis of no effect, but rather to investigate whether or not smoking might be confounding the kidney cancer results. An association of TCE exposure and lung cancer might indicate a confounding effect of smoking (or a causal association with lung cancer), but a finding of no association would essentially rule out a confounding effect of smoking, since smoking is such a strong risk factor for lung cancer. Axelson et al. (1994) reported neither the number of lung cancers observed in females nor the number expected. To test a null hypothesis of no effect, one might conservatively assume none was observed and estimate the number expected, as was done for kidney cancer; however, since that is not the hypothesis here, we chose not to make any assumptions or estimates for the female component of the cohort.

For Boice et al. (1999), results for "any potential exposure" were selected for the primary analysis, because this exposure category was considered to best represent overall TCE exposure, and results for "potential routine exposure," which was characterized as reflecting workers assumed to have received more cumulative exposure, were used in a sensitivity analysis. The number of cases (deaths) with "any potential exposure" was not presented, but a value of 200 allowed us to reproduce the reported CIs. The number suggested by exposure level in Boice et al. (1999) Table 9 is 173; however, it may be that exposure level data were not available for all of the cases. Because the exact number is unknown but is a large number, consistent with CIs that are proportionally symmetric, the SE (log RR) was calculated as from symmetric CIs (see Section C.1).

In their published paper, Morgan et al. (1998) present only SMRs for overall TCE exposure, although the results from internal analyses are presented for exposure subgroups. RR estimates for overall TCE exposure from the internal analyses of the Morgan et al. (1998) cohort data were available from an unpublished report (EHS, 1997); from these, the RR estimate from the Cox model that included age and sex was selected, because those are the variables deemed to be important in the published paper. The internal analysis RR estimate was preferred for the primary analysis, and the published SMR result was used in a sensitivity analysis.

Raaschou-Nielsen et al. (2003) reported results for lung cancer for both sexes combined in the text. In their Table 3, Raaschou-Nielsen et al. (2003) also present overall results for lung cancer with a lag time of 20 years; however, they use a definition of lag that is different from a lagged exposure in which exposures prior to disease onset are discounted and it is not clear what

their lag time actually represents[11], thus, these results were not used in any of the meta-analyses for lung cancer. In addition, results for the subcohort with expected higher exposure levels were not provided for lung cancer, so no alternate analysis was done based on the subcohort.

For Radican et al. (2008), the Cox model hazard ratio from the 2000 follow-up was used. In the Radican et al. (2008) Cox regressions, age was the time variable, and sex and race were covariates. It should also be noted that the referent group is composed of workers with no chemical exposures, not just no exposure to TCE.

Zhao et al. (2005) do not report results for an overall TCE effect. Therefore, as for NHL and kidney cancer, the results across the "medium" and "high" exposure groups were combined, under assumptions of group independence, even though the exposure groups are not independent (the "low" exposure group was the referent group in both cases). Zhao et al. (2005) present RR estimates for both incidence and mortality; however, the time frame for the incidence accrual is smaller than the time frame for mortality accrual and fewer exposed incident cases (49) were obtained than deaths (95). Thus, because better case ascertainment occurred for mortality than for incidence, the mortality results were used for the primary analysis, and the incidence results were used in a sensitivity analysis. A sensitivity analysis was also done using results from Boice et al. (2006b) in place of the Zhao et al. (2005) RR estimate. The cohorts for these studies overlap, so they are not independent studies and should not be included in the meta-analysis concurrently. Boice et al. (2006b) report an RR estimate for an overall TCE effect for lung cancer mortality; however, it is based on fewer deaths (51) and is an SMR rather than an internal analysis RR estimate, so the Zhao et al. (2005) mortality estimate is preferred for the primary analysis.

C.5.1.2. Results of Meta-Analyses

Results from some of the meta-analyses that were conducted on the epidemiological studies of TCE and lung cancer are summarized in Table C-15. The RRm from the fixed-effect meta-analysis of the nine studies was 1.16 (95% CI: 1.09, 1.23) (see Figure C-11). As shown in Figure C-11, the analysis was dominated by one large study [Raaschou-Nielsen et al. (2003), contributing about 58% of the weight]. The RR estimate from that large study was higher than the RR estimates from all of the other studies and, with its relatively narrow CI, was largely inconsistent with the results of the other studies, in particular that of the next largest study (Boice (1999), contributing about 18% of the weight). While the RR estimate of Raaschou-Nielsen et al. (2003) was statistically significantly elevated, that of Boice et al. (1999) was statistically significantly decreased. This heterogeneity of study results is corroborated by a statistically significant p-value for the test of heterogeneity ($p < 10^{-8}$) and an I^2-value of 90%, indicating a high amount of heterogeneity. Because of this heterogeneity, the appropriateness of conducting

[11]In their Methods section, Raaschou-Nielsen et al. (2003) define their lag period as the period "from the date of first employment to the start of follow-up for cancer".

any meta-analysis without attempting to explain the heterogeneity is arguable, but a fixed-effect meta-analysis is clearly improper (see Section C.1).

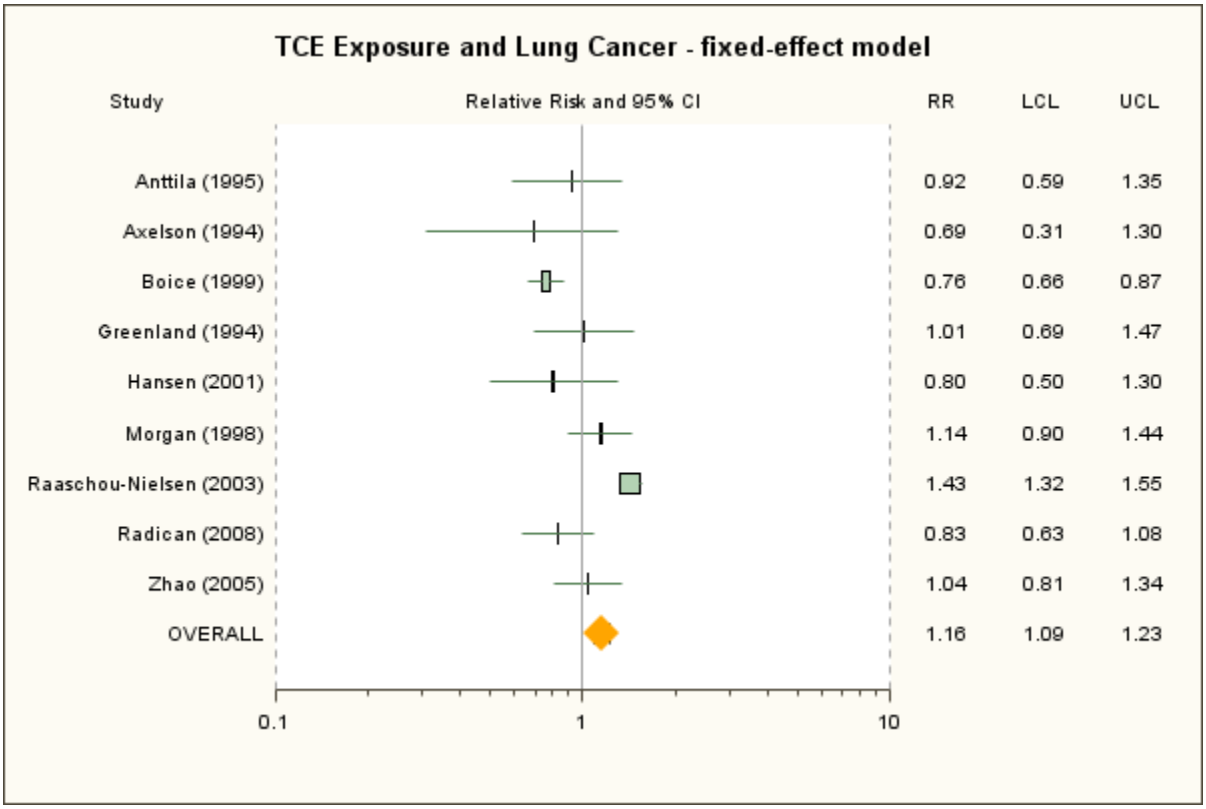


Figure C-11. Meta-analysis of lung cancer and TCE exposure—fixed-effect model. Rectangle sizes reflect relative weights of the individual studies. The summary estimate is in the bottom row, represented by the diamond.

The RRm from the primary random-effects meta-analysis of the nine studies was 0.96 (95% CI: 0.76, 1.21) (see Figure C-12). As shown in Figure C-12, because the random-effects model takes both between-study and within-study variation into account in the study weight, and because the between-study variation is fairly substantial for these studies, study size has minimal impact on study weight. The relative weights for the nine studies range from 6.7 to 13.9% in the random-effects meta-analysis; thus, no single study dominates the analysis in terms of weight. The most influential single study is nonetheless the largest study, Raaschou-Nielsen et al. (2003) (2003), because it also has an RR estimate well above the others, and its removal from the analysis reduces the RRm estimate to 0.90 (95% CI: 0.79, 1.04). In contrast, removal of Boice et al. (1999), the study with the lowest RR estimate, increases the RRm estimate to 1.01 (95% CI: 0.82, 1.24). Removal of any of the other individual studies resulted in RRm estimates that were all nonsignificantly decreased and that ranged from 0.93 [with the removal of Morgan et al. (1998)] to 0.98 [with the removal of Axelson et al. (1994), Hansen et al. (2001), or Radican et al.

(2008)]. Use of the four alternate selections, individually, resulted in RRm estimates that were all nonsignificant and that fell in a narrower range—0.96 to 0.98 (see Table C-15).

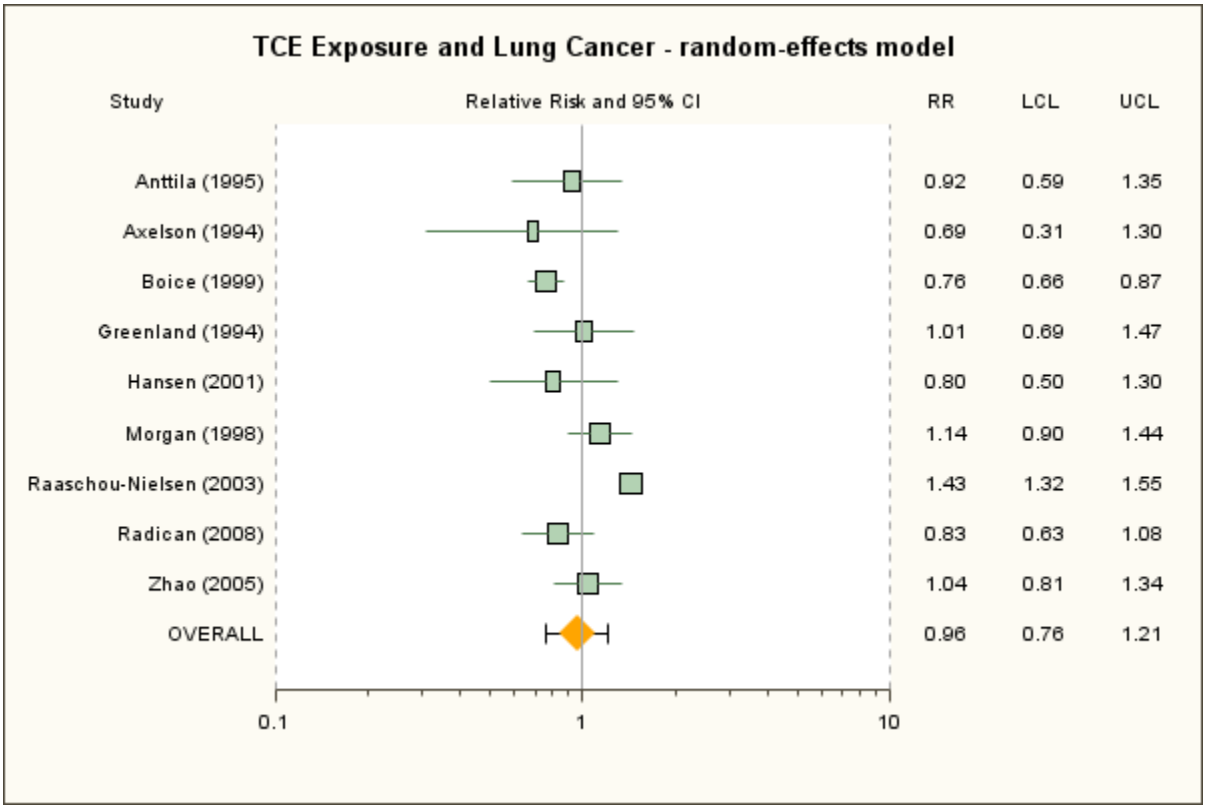


Figure C-12. Meta-analysis of lung cancer and TCE exposure—random-effects model. Rectangle sizes reflect relative weights of the individual studies. The summary estimate is in the bottom row, represented by the diamond.

Table C-15. Summary of some meta-analysis results for TCE and lung cancer

Analysis	Number of studies	Model	RRm estimate	95% LCL	95% UCL	Heterogeneity	Comments
All studies (all cohort studies)	9	Random	0.96	0.76	1.21	Significant ($p < 10^{-8}$) $I^2 = 90\%$	Nonsignificance of RRm not dependent on any single study. No apparent publication bias.
		Fixed	1.16	1.09	1.23	Because of significant heterogeneity, fixed-effect model not appropriate	Significant elevation in RRm dependent on single study, Raaschou-Nielsen et al. (2003), without which the RRm would be significantly *decreased* (RRm = 0.87, $p = 0.004$).
Alternate RR selections[a]	9	Random	0.98	0.78	1.25	Significant ($p < 10^{-8}$) $I^2 = 90\%$	With Zhao et al. (2005) incidence instead of mortality.
	9	Random	0.98	0.77	1.24	Significant ($p < 10^{-8}$) $I^2 = 90\%$	With Boice et al. (2006b) instead of Zhao et al. (2005).
	9	Random	0.97	0.78	1.20	Significant ($p < 10^{-7}$) $I^2 = 85\%$	With Boice et al. (1999) potential routine exposure rather than any potential exposure.
	9	Random	0.96	0.76	1.20	Significant ($p < 10^{-8}$) $I^2 = 90\%$	With Morgan et al. (1998) published SMR.
Highest exposure groups	6	Random	0.96	0.72	1.27	Significant	See Table C-17 for details.
	6	Random	0.92–0.98	0.67–0.75	1.25–1.30		Using alternate selections (see text).[a]

[a]Changing the primary analysis by one alternate RR each time.

As discussed above, there was significant heterogeneity across the nine studies. All of the lung cancer studies were cohort studies, so no subgroup analyses examining cohort and case-control studies separately, as was done for NHL and kidney cancer, were conducted. In addition, no alternate quantitative investigations of heterogeneity were pursued because our goal here was to investigate lung cancer risks as an indication of possible confounding of the kidney cancer results by smoking, not to do an all-encompassing meta-analysis of lung cancer. The majority of the studies have nonsignificant RR estimates for lung cancer that fall near or <1. The relative outliers are the significantly increased RR estimate from Raaschou-Nielsen et al. (2003) and the significantly decreased RR estimate from Boice et al. (1999). The Raaschou-Nielsen et al. (2003) study considered a lot of different job titles and the RR estimate could reflect a TCE effect or exposure to other chemicals that are lung carcinogens. Alternatively, because the study is an SMR study of largely blue-collar workers and the comparison population is the general Danish population, the elevated RR estimate could reflect small differences in smoking rates between those two populations. However, if the observed increase is attributable to smoking, it's not enough of an effect to explain the increased RR estimate for RCC in the same study because smoking is a much stronger risk factor for lung cancer than for RCC, whereas the increased RR estimate for lung cancer in the study was relatively small (Raaschou-Nielsen et al., 2003); see also Section 4.4.2.3). It is unclear why the Boice et al. (1999) study reports a significantly decreased RR estimate. In any event, there is no increase in the RRm estimate for all nine studies from the random-effects model, suggesting that there is no confounding of the overall RRm for kidney cancer by smoking, in particular for the cohort studies.

As discussed in Section C.1, publication bias was examined in several different ways, and there is no indication of publication bias for these lung cancer studies (results not shown). If anything, the relationship between study size and RR estimate is the opposite of what would be expected if publication bias were occurring because the one large study is the only study with a significantly increased RR estimate and incorporating studies with increasing SE one at a time, generally shows a *decrease* in effect size with addition of the less precise studies.

C.5.2. Lung Cancer Effect in the Highest Exposure Groups

C.5.2.1. Selection of RR Estimates

The selected RR estimates for lung cancer in the highest TCE exposure categories, for studies that provided such estimates, are presented in Table C-16. Six of the nine cohort studies reported lung cancer risk estimates categorized by exposure level. As in Section C.5.1.1 for the overall risk estimates, RR estimates for males and females combined were used, wherever possible.

Three of the nine cohort studies (Axelson et al., 1994); (Hansen et al., 2001); (Zhao et al., 2005) did not report lung cancer risk estimates categorized by exposure level, even though these same studies reported such estimates for selected other cancer sites. Unlike for the other cancer

types, we did not attempt to address the issue of unreported results by including RR estimates of 1 for the missing estimates. This is because, as discussed in Section C.5.1.1 above with respect to estimate a female contribution to the Axelson et al. (1994) study, unlike for the other cancer types, we are not testing a null hypothesis of no effect for lung cancer but rather investigating whether smoking might be a confounder in the kidney cancer studies. Thus, we would not want to bias the RRm estimate toward 1 in this case by including estimates of 1 for missing RR values.

For Boice et al. (1999), only results for workers with "any potential exposure" were presented by exposure category, and the referent group is workers not exposed to any solvent.

For Morgan et al. (1998), the primary analysis used results for the cumulative exposure metric, and a sensitivity analysis was done with the results for the peak exposure metric.

For Raaschou-Nielsen et al. (2003), unlike for NHL and RCC, lung cancer results for the subcohort with expected higher exposure levels were not presented, so the only highest-exposure-group results were for duration of employment in the total cohort. Results for males and females combined were estimated assuming a Poisson distribution.

Table C-16. Selected RR estimates for lung cancer risk in highest TCE exposure groups

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE (log RR)	Alternate RR estimates (95% CI)	Comments
Anttila et al. (1995)	0.83	0.33	1.71	100+ µmol/L U-TCA [a]	-0.186	0.378	None	SIR.
Boice et al. (1999)	0.64	0.46	0.89	≥5 yrs exposure	-0.446	0.168	None	Mortality RR. For any potential exposure. Adjusted for date of birth, dates 1st and last employed, race, and sex. Referent group is workers not exposed to any solvent.
Morgan et al. (1998)	0.96	0.72	1.29	High cumulative exposure score	-0.041	0.149	1.07 (0.82, 1.40) for medium/high peak vs. low/no	Mortality RR. Adjusted for age and sex.
Raaschou-Nielsen et al. (2003)	1.4	1.2	1.6	≥5 yrs	0.336	0.070	None	SIR. Male and female results presented separately and combined assuming a Poisson distribution.
Radican et al. (2008)	0.90	0.63	1.27	>25 unit-yrs	-0.105	0.179	0.8 (0.4, 1.7) for Blair et al. (1998) incidence	Mortality hazard ratio. Male and female results presented separately and combined (see text). Time variable = age, covariate = race. Referent group is workers with no chemical exposures.
Zhao et al. (2005)	1.0	0.68	1.53	High exposure score	0.020	0.207	1.1 (0.60, 2.06) for Zhao et al. (2005) incidence. Boice et al. (2006b): 0.80 (0.46, 1.41) for ≥4 yrs with any potential exp; 0.86 (0.56, 1.33) for ≥5 yrs test stand mechanic, 0.76 (0.42, 1.36) for ≥4 test-yrs.	Mortality RR. Males only. Adjusted for time since 1st employment, SES, age.

[a]Mean personal TCA in urine. 1 µmol/L = 0.1634 mg/L.

For Radican et al. (2008), it should be noted that the referent group is workers with no chemical exposures, not just no TCE exposure. Furthermore, results for exposure groups (based on cumulative exposure scores) were reported separately for males and females and were combined for this assessment using inverse-variance weighting, as in a fixed-effect meta-analysis. Radican et al. (2008) present only mortality hazard ratio estimates by exposure group; however, in an earlier follow-up of this same cohort, Blair et al. (1998) present both incidence and mortality RR estimates by exposure group. There were no incident cases for females in the highest exposure group in Blair et al. (1998) (and the expected number was not reported); thus, for the same reasons we didn't use RR estimates of 1 for unreported RR estimates in the Axelson et al. (1994), Hansen et al. (2001), and Zhao et al. (2005) studies discussed above, the male-only results were used for the RR estimate without attempting to approximate a contribution to the RR estimate from the females in the cohort. Radican et al. (2008) also present results for categories based on frequency and pattern of exposure; however, subjects weren't distributed uniquely across the categories (the numbers of cases across categories exceeded the total number of cases); thus, it was difficult to interpret these results and they were not used in a sensitivity analysis.

Unlike for kidney cancer, Zhao et al. (2005) present lung cancer RR estimates only for unlagged exposures. The mortality results reflect more cases (33) in the highest exposure group than do the incidence results (14), so the mortality RR estimate was used for the primary analysis, and the incidence estimate was used in a sensitivity analysis. Sensitivity analyses were also done using results from Boice et al. (2006b) in place of the Zhao et al. (2005) RR estimate. The cohorts for these studies overlap, so they are not independent studies. Boice et al. (2006b) report mortality RR estimates for lung cancer by years worked with any potential exposure, years worked as a test stand mechanic, a job with potential TCE exposure, and by a measure that weighted years with potential exposure from engine flushing by the number of flushes each year. The Boice et al. (2006b) estimates are adjusted for years of birth and hire and for hydrazine exposure.

C.5.2.2. Results of Meta-Analyses

Results from the meta-analyses that were conducted for lung cancer in the highest exposure groups are summarized at the bottom of Table C-15 and reported in more detail in Table C-17. The RRm estimate from the random-effects meta-analysis of the six studies with results presented for exposure groups was 0.96 (95% CI: 0.72, 1.27). As with the overall results for lung cancer, the highest-exposure-group results exhibited significant heterogeneity, with the largest study (Raaschou-Nielsen et al., 2003) having a statistically significantly increased RR estimate and the next largest (Boice et al., 1999) having a statistically significantly decreased RR estimate (see Figure C-13). The remaining four studies all had nonsignificant RR estimates closer to 1. Nonsignificance of the RRm estimate was not dependent on any single study,

although removing Raaschou-Nielsen et al. (2003) decreased the RRm estimate to 0.86 and removing Boice et al. (1999) increased the RRm estimate to 1.07. The RRm estimate was not highly sensitive to alternate RR estimate selections. Use of the six alternate selections, individually, resulted in RRm estimates that were all nonsignificant and that ranged from 0.92 to 0.98 (see Table C-17). As with the primary analysis, significant heterogeneity was observed for all of the meta-analyses with alternate selections (see Table C-17).

The RRm estimate from the primary analysis of the highest exposure groups was the same as that for the overall TCE analysis (0.96), indicating no evidence of an exposure-response relationship and confirming the absence of evidence of an increased risk of lung cancer associated with TCE exposure from these studies as a whole.

C.5.3. Discussion of Lung Cancer Meta-Analysis Results

Significant heterogeneity was observed in the lung cancer results (for both overall TCE exposure and for the highest exposure groups) from the different studies, and there was no clear explanation for the source(s) of the heterogeneity, as discussed in Section C.5.1.2. Nonetheless, we conducted (random-effects) meta-analyses of the lung cancer results with the goal of addressing the question of whether or not there was evidence of an association between TCE exposure and lung cancer that might suggest that smoking could be confounding the kidney cancer results, in particular in the cohort studies, which did not adjust for smoking.

Both the overall and highest-exposure-group analyses yielded nonsignificant RRm estimates of 0.96 for lung cancer. Influence analyses and sensitivity analyses using alternate RR estimate selection for various studies similarly found no evidence of an association between TCE exposure and lung cancer from these studies as a whole. This finding suggests that there is no confounding of the overall RRm for kidney cancer by smoking, in particular from the cohort studies (see Section 4.4.2.3 for a more comprehensive discussion of the issue of potential confounding of the kidney cancer results by smoking).

Table C-17. Summary of some meta-analysis results for TCE (highest exposure groups) and lung cancer

Analysis	Model	RRm estimate	95% LCL	95% UCL	Heterogeneity	Comments
Primary analysis	Random	0.96	0.72	1.27	Significant ($p < 0.0002$) $I^2 = 80\%$	Nonsignificance of RRm not dependent on any single study.
	Fixed	1.15	1.03	1.27	Because of significant heterogeneity, fixed-effect model not appropriate	Significant elevation in RRm dependent on single study, Raaschou-Nielsen et al. (2003), without which the RRm would be nonsignificantly *decreased* (RRm = 0.86, $p = 0.07$).
Alternate RR selections[a]	Random	0.95	0.70	1.29	Significant ($p < 0.0003$) $I^2 = 79\%$	With Blair et al. (1998) incidence RR instead of Radican et al. (2008) mortality hazard ratio.
	Random	0.98	0.75	1.29	Significant ($p = 0.0003$) $I^2 = 79\%$	With Morgan et al. (1998) peak metric.
	Random	0.96	0.71	1.30	Significant ($p = 0.0002$) $I^2 = 79\%$	With Zhao et al. (2005) incidence.
	Random	0.92–0.93	0.67–0.69	1.25	Significant ($p < 0.0002$) $I^2 = 81\%$	With Boice et al. (2006b) alternates for Zhao et al. (2005) (see text).

[a]Changing the primary analysis by one alternate RR each time.

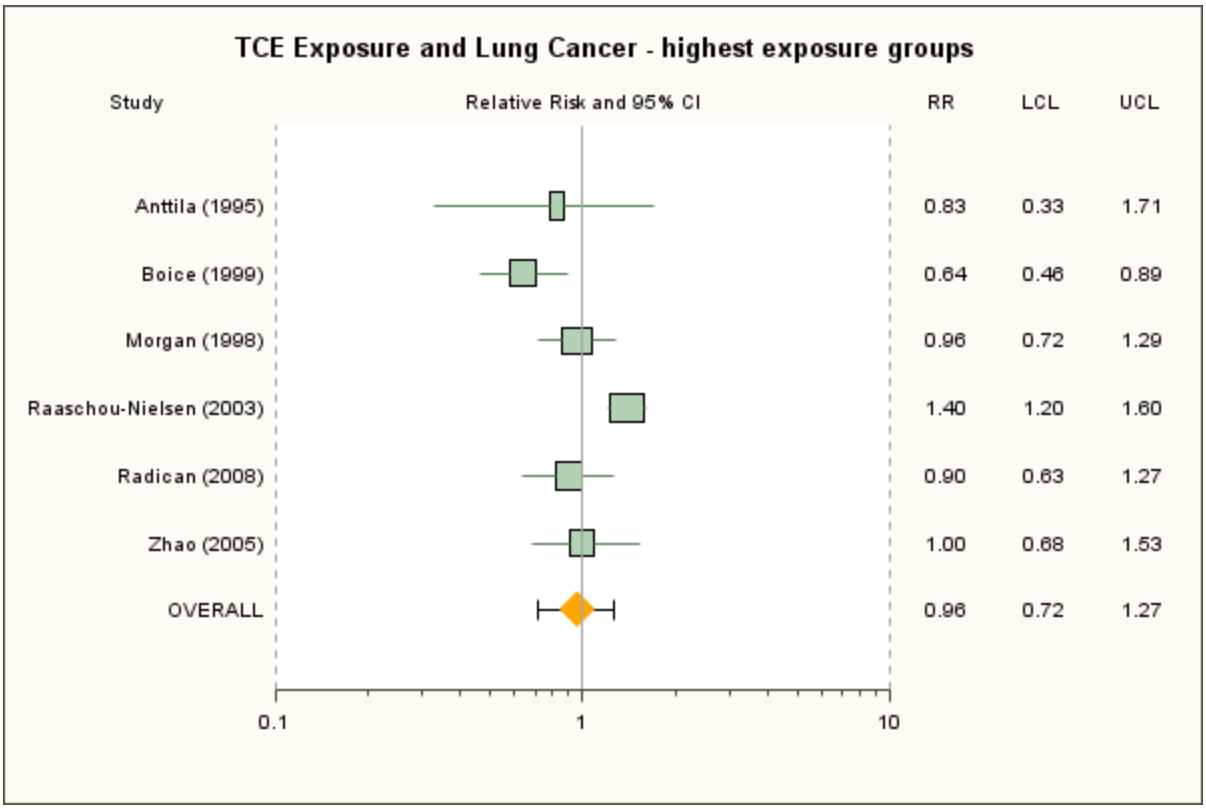


Figure C-13. Meta-analysis of lung cancer and TCE exposure—highest exposure groups. Random-effects model. Rectangle sizes reflect relative weights of the individual studies. The summary estimate is in the bottom row, represented by the diamond.

C.6. DISCUSSION OF STRENGTHS, LIMITATIONS, AND UNCERTAINTIES IN THE META-ANALYSES

Meta-analysis provides a systematic way of objectively and quantitatively combining the results of multiple studies to obtain a summary effect estimate. Use of meta-analysis can help risk assessors avoid some of the potential pitfalls in overly relying on a single study or in making more subjective qualitative judgments about the apparent weight of evidence across studies. Combining the results of smaller studies also increases the statistical power to observe an effect, if one exists. In addition, meta-analysis techniques assist in systematically investigating issues such as potential publication bias and heterogeneity in a database.

While meta-analysis can be a useful tool for analyzing a database of epidemiological studies, the analysis is limited by the quality of the input data. If the individual studies are deficient in their abilities to observe an effect (in ways other than low statistical power, which meta-analysis can help ameliorate), the meta-analysis will be similarly deficient. A critical step in the conduct of a meta-analysis is to establish eligibility criteria and clearly and transparently identify all relevant studies for inclusion in the meta-analysis. For the TCE database, a

comprehensive qualitative review of available studies was conducted and eligible studies were identified, as described in Appendix B, Section B.2.9.

Identifying all relevant studies may be hampered if publication bias has occurred. Publication bias is a systematic error that can arise if statistically significant studies are more likely to be published than nonsignificant studies. This can result in an upward bias on the effect size measure (i.e., the RR estimate). To address this concern, potential publication bias was investigated for the databases for which meta-analyses were undertaken. For the studies of kidney cancer and liver cancer, there was no evidence of publication bias. For the studies of NHL, there was some evidence of potential publication bias. It is uncertain whether this reflects actual publication bias or rather an association between SE and effect size (as discussed in Section C.1, a feature of publication bias is that smaller studies tend to have larger effect sizes) resulting for some other reason, e.g., a difference in study populations or protocols in the smaller studies. Furthermore, if there is publication bias in this data set, it may be creating an upward bias on the RR estimate, but this bias does not appear to account completely for the finding of an increased NHL risk (see Section C.2.1.2).

Another concern in meta-analyses is heterogeneity across studies. Random-effects models were used for the primary meta-analyses in this assessment because of the diverse nature of the individual studies. When there is no heterogeneity across the study results, the random-effects model will give the same result as a fixed-effect model. When there is heterogeneity, the random-effects model estimates the between-study variance. Thus, when there is heterogeneity, the random-effects model will generate wider CIs and be more "conservative" than a fixed-effect model. However, if there is substantial heterogeneity, it may be inappropriate to combine the studies at all. In cases of significant heterogeneity, it is important to try to investigate the potential sources of the heterogeneity.

For the studies of kidney and liver cancer, there was no apparent heterogeneity across the study results (i.e., random- and fixed-effects models gave identical summary estimates). For the NHL studies, there was heterogeneity, but it was not statistically significant ($p = 0.16$). The I^2-value was 26%, suggesting low-to-moderate heterogeneity. When subgroup analyses were done for the cohort and case-control studies separately, there was some heterogeneity in both groups, but in neither case was it statistically significant. Further attempts to quantitatively investigate the heterogeneity were not pursued because of limitations in the database. The sources of heterogeneity are an uncertainty in the database of studies of TCE and NHL. Some potential sources of heterogeneity, which are discussed qualitatively in Section C.2.3, include differences in exposure assessment or in the intensity or prevalence of TCE exposures in the study population and differences in NHL classification.

The joint occurrence of heterogeneity and potential publication bias in the database of studies of TCE and NHL raises special concerns. Because of the heterogeneity, a random-effects model should be used if these studies are to be combined; yet, the random-effects model gives

relatively large weight to small studies, which could exacerbate the potential impacts of publication bias. For the NHL studies, the summary RR estimates from the random-effects and fixed-effect models are not very different (RRm = 1.23 [95% CI: 1.07, 1.42] and 1.21 [95% CI: 1.08, 1.35], respectively); however, the CI for the fixed-effect estimate does not reflect the between-study variance and is, thus, overly narrow.

Heterogeneity was statistically significant for the lung cancer studies ($p < 10^{-8}$) and the I^2-value was 90%, indicating that the amount of heterogeneity was high. Nonetheless, (random-effects) meta-analyses were conducted for the purpose of investigating the potential for smoking to be confounding the kidney cancer results (see Sections C.5 and 4.4.2.3).

C.7. CONCLUSIONS

The strongest finding from the meta-analyses was for TCE and kidney cancer. The summary estimate from the primary random-effects meta-analysis of the 15 studies was RRm = 1.27 (95% CI: 1.13, 1.43). There was no apparent heterogeneity across the study results (i.e., fixed-effect model gave same summary estimate), and there was no evidence of potential publication bias. The summary estimate was robust across influence and sensitivity analyses; the estimate was not markedly influenced by any single study, nor was it overly sensitive to individual RR estimate selections. The findings from the meta-analyses of the highest exposure groups for the studies that provided kidney cancer results categorized by exposure level were similarly robust. The summary estimate was RRm = 1.58 (95% CI: 1.28, 1.96) for the 13 studies included in the analysis. There was no apparent heterogeneity in the highest-exposure-group results, and the estimate was not markedly influenced by any single study, nor was it overly sensitive to individual RR estimate selections. In sum, these robust results support a conclusion that TCE exposure increases the risk of kidney cancer.

The meta-analyses of the overall effect of TCE exposure on NHL also suggest a small, statistically significant increase in risk. The summary estimate from the primary random-effects meta-analysis of the 17 studies was 1.23 (95% CI: 1.07, 1.42). This result was not overly influenced by any single study, nor was it overly sensitive to individual RR estimate selections. There is some evidence of potential publication bias in the NHL study data set; however, it is uncertain that this is actually publication bias rather than an association between SE and effect size resulting for some other reason, e.g., a difference in study populations or protocols in the smaller studies. Furthermore, if there is publication bias, it does not appear to account completely for the findings of an increased NHL risk. There was some heterogeneity across the results of the 17 studies, but it was not statistically significant ($p = 0.16$). The I^2-value was 26%, suggesting low-to-moderate heterogeneity. The source(s) of this heterogeneity remains an uncertainty. The summary estimate from the meta-analysis of the highest exposure groups for the 13 studies which provided NHL results categorized by exposure level was RRm = 1.43 (95% CI: 1.13, 1.82). The statistical significance of the increased RR estimate for the highest

exposure groups was not dependent on any single study, nor was it sensitive to individual RR estimate selections. Although there was some heterogeneity across the 13 highest-exposure-group studies, it was not statistically significant (p = 0.30) and the I^2-value was 14%, suggesting that the amount of heterogeneity was low. Furthermore, the heterogeneity is dependent on a single study, Cocco et al. (2010), suggesting that the RR estimate for the highest exposure group from that study is a relative outlier. Overall, the robustness of the finding of an increased NHL risk for the highest exposure groups strengthens the more moderate evidence from the meta-analyses for overall effect.

The meta-analyses of the overall effect of TCE exposure on liver (and gall bladder/biliary passages) cancer also suggest a small, statistically significant increase in risk, but the study database is more limited. The summary estimate from the primary random-effects meta-analysis of the nine (all cohort) studies was 1.29 (95% CI: 1.07, 1.56). The analysis was dominated by one large study that contributed about 53% of the weight. When this study was removed, the RRm estimate decreased somewhat and was less precise (RRm = 1.22; 95% CI: 0.93, 1.61). The summary estimate was not overly influenced by any other single study, nor was it overly sensitive to individual RR estimate selections. There was no evidence of publication bias in this data set, and there was no observable heterogeneity across the study results. However, the findings from the meta-analyses of the highest exposure groups for the studies that provided liver cancer results categorized by exposure level do not add support to the overall effect findings. The summary estimate was RRm = 1.28 (95% CI: 0.93, 1.77) for the eight studies included in the analysis, which is slightly *lower* than the summary estimate for the overall effect. This contradictory result is driven by the fact that the RR estimate for the highest exposure group in the individual study which contributes the majority of the weight to the meta-analyses, although >1, was less than the overall RR estimate for the same study. In sum, these results do not rule out an effect of TCE on liver cancer, because the liver cancer results are relatively underpowered with respect to numbers of studies and number of cases and the overwhelming study in terms of weight uses the weak exposure surrogate of duration of employment for categorizing exposure level; however, at present, there is only modest support for an increased risk of liver cancer. Meta-analyses were also conducted for lung cancer with the goal of addressing the question of whether or not there was evidence of an association between TCE exposure and lung cancer that might suggest that smoking could be confounding the kidney cancer results, in particular in the cohort studies, which did not adjust for smoking. Both the overall and highest-exposure-group random-effects meta-analyses yielded a nonsignificant RRm estimate of 0.96 for lung cancer. Influence analyses and sensitivity analyses using alternate RR estimate selection for various studies similarly found no evidence of an association between TCE exposure and lung cancer from these studies as a whole. This finding suggests that there is no confounding of the overall RRm for kidney cancer by smoking (see Section 4.4.2.3 for a more comprehensive discussion of the issue of potential confounding of the kidney cancer results by smoking).

D. NEUROLOGICAL EFFECTS OF TCE

D.1. HUMAN STUDIES ON THE NEUROLOGICAL EFFECTS OF TCE

There is an extensive body of evidence in the literature on the neurological effects caused by exposure to TCE in humans. The primary functional domains that have been studied and reported are trigeminal nerve function and nerve conductivity (latency), psychomotor effects, including RTs (simple and choice), visual and auditory effects, cognition, memory, and subjective neurological symptoms, such as headache and dizziness. This section discusses the primary studies presented for each of these effects. Summary tables for all of the human TCE studies are at the end of this section.

D.1.1. Changes in Nerve Conduction

There is strong evidence in the literature that exposure to TCE results in impairment of trigeminal nerve function in humans exposed occupationally, by inhalation, or environmentally, by ingestion. Functional measures such as the blink reflex and masseter reflex tests were used to determine if physiological functions mediated by the trigeminal nerve were significantly impacted. Additionally, TSEPs were also measured in some studies to ascertain if nerve activity was directly affected by TCE exposure.

D.1.1.1. Blink Reflex and Masseter Reflex Studies—Trigeminal Nerve

Barret et al. (1984) conducted a study on 188 workers exposed to TCE occupationally from small and large factories in France (type of factories not disclosed). The average age of the workers was 41 (SD not provided, but authors noted 14% <30 years and 25% >50 years) and the average exposure duration was 7 hours/day for 7 years. The 188 workers were divided into high- and low-exposure groups for both TCE exposure measured using detector tubes and TCA levels measured in urine. There was no unexposed control population, but responses in the high-exposure group were compared response in the low-exposure group. TCE exposure groups were divided into a low-exposure group (<150 ppm; n = 134) and a high-exposure group (>150 ppm; n = 54). The same workers (n = 188) were also grouped by TCA urine measurements such that a high exposure was ≥100 mg TCA/g creatinine. Personal factors including age, tobacco use, and alcohol intake were also analyzed. No mention was made regarding whether or not the examiners were blind to the subjects' exposure status. Complete physical examination including testing visual performance (acuity and color perception), evoked trigeminal potential latencies and audiometry, facial sensitivity, reflexes, and motoricity of the masseter muscles. χ^2 analysis was used to examine distribution of the different groups for comparing high and low exposed workers followed by one way ANOVA. Overall, 22/188 workers (11.7%) experienced trigeminal nerve impairment ($p < 0.01$) as measured by facial sensitivity, reflexes (e.g., jaw,

corneal, blink) and movement of the masseter muscles. When grouped by TCE exposure, 12/54 workers (22.2%) in the high-exposure group (≥150 ppm) and 10/134 workers (7.4%) in the low-exposure group had impaired trigeminal nerve mediated responses. When grouped by the presence of TCA in the urine, 41 workers were now in the high TCA group and 10/41 workers (24.4%) experienced trigeminal nerve impairment in comparison to the 12/147 (8.2%) in the low TCA (<100 mg TCA/g creatinine) group. Statistically significant results were also presented for the following symptoms based on TCE and TCA levels: trigeminal nerve impairment ($p < 0.01$), asthenia ($p < 0.01$), optic nerve impairment ($p < 0.001$), and dizziness ($0.05 < p < 0.06$). Statistically significant results were also presented for the following symptoms based on TCA levels: trigeminal nerve impairment ($p < 0.01$), asthenia ($p < 0.01$), optic nerve impairment ($p < 0.001$), headache ($p < 0.05$), and dizziness ($0.05 < p < 0.06$). Symptoms for which there is a synergistic toxic role for TCE and alcohol ($p < 0.05$) were liver impairment and degreaser flush. This study presents a good statistically significant dose-response relationship between TCE/TCA exposure and trigeminal nerve impairment. TCE concentrations are not available for individual subjects, but exposure assessment was inferred based on occupational standards at the time of the study.

Feldman et al. (1988) conducted an environmental study on 21 Woburn, Massachusetts residents with alleged chronic exposure to TCE in drinking water, resulting from an environmental spill by a local industry. These were from eight families whose drinking water wells were found to be contaminated with TCE and other solvents. The subjects were self selected, having been referred for clinical evaluation due to suspected neurotoxicity, and were involved in litigation. The control group was 27 unexposed residents from a nearby community with TCE concentrations in drinking water below state standards. TCE in residential well water was measured over a prior 2-year period (1979–1981); the maximum reported concentration for the study population was 267 ppb. The residents' water supply came from two different TCE-contaminated wells that had an average measured concentration of 256 ppb (labeled "Well G" based on six samples) and 111 ppb (labeled "Well H;" based on four samples). The residents' exposure ranged from 1 to 12 years and was dependent on the length of residence and the age of the subject. There were other solvents found to be present in the well water, and TCE data were not available for the entire exposure period. TCE concentrations for the control population were less than the maximum contaminant level (5 ppb). The blink reflex was used to measure the neurotoxic effects of TCE. The blink reflex was measured using an electrode to stimulate the supraorbital nerve (above the eyelid) with a shock (0.05 ms in duration) resulting in a response and the response was measured using a recording electrode over the orbicularis oculi muscle (the muscle responsible for closing the eyelid and innervated by the trigeminal nerve). The blink reflex generated an R1 and an R2 component from each individual. Blink reflexs were recorded and the supraorbital nerve was stimulated with single electrical shocks of increasing intensity until nearly stable R1 and R2 ipsilateral and R2 contralateral responses were obtained. The

student's t-test was used for testing the difference between the group means for the blink reflex component latencies. Because of the variability of R2 responses, this study focused primarily on the R1 response latencies. Highly significant differences in the conduction latency means of the blink reflex components for the TCE exposed population vs. control population were observed when comparing means for the right and left side R1 to the controls. The mean R1 blink reflex component latency for the exposed group was 11.35 ms, SD = 0.74 ms, 95% CI: 11.03–11.66. The mean for the controls was 10.21 ms, SD = 0.78 ms, 95% CI: 9.92–10.51; ($p < 0.001$). The study was well conducted with consistency of methods, and statistically significant findings for trigeminal nerve function impairment resulting from environmental exposures to TCE. However, the presence of other solvents in the well water, self selection of subjects involved in litigation, and incomplete characterization of exposure present problems in drawing a clear conclusion of TCE causality or dose-response relationship.

Kilburn and Warshaw (1993a) conducted an environmental study on 544 Arizona residents exposed to TCE in well water. TCE concentrations were 6–500 ppb and exposure ranged from 1 to 25 years. Subjects were recruited and categorized in three groups. Exposed group 1 consisted of 196 family members with cancer or birth defects. Exposed group 2 consisted of 178 individuals from families without cancer or birth defects; and exposed group 3 included 170 parents whose children had birth defects and rheumatic disorders. Well water was measured from 1957 to 1981 by several governmental agencies and average annual TCE exposures were calculated and then multiplied by each individual's years of residence for 170 subjects. A referent group of histology technicians (n = 113) was used as a comparison for the blink reflex test. For this test, recording electrodes were placed over the orbicularis oculi muscles (upper and lower) and the blink reflex was elicited by gently tapping the glabeela (located on the mid-frontal bone at the space between the eyebrows and above the nose). A two-sided Student's t-test and linear regression were used for statistical analysis. Significant increases in the R1 component of the blink reflex response was observed in the exposed population as compared to the referent group. The R1 component measured from the right eye appeared within 10.9 ms in TCE-exposed subjects, whereas in referents, this component appeared 10.2 ms after the stimulus was elicited, indicating a significant delay ($p < 0.008$) in the reflex response. Similarly, delays in the latency of appearance for the R1 component were also noted for the left eye but the effect was not statistically significant ($p = 0.0754$). This study shows statistically significant differences in trigeminal nerve function between subjects environmentally exposed and nonexposed to TCE. This is an ecological study with TCE exposure inferred to subjects by residence in a geographic area. Estimates of TCE concentrations in drinking water to individual subjects are lacking. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Kilburn (2000a, 2002b) studied 236 residents (age range: 18–83 years old) lived nearby manufacturing plants (e.g., microchip plants) in Phoenix, Arizona. Analysis of the groundwater

in the residential area revealed contamination with many VOCs including TCE. Concentrations of TCE in the well water ranged from 0.2 to >10,000 ppb and the exposure duration varied between 2 and 37 years. Additional associated solvents included dichloroethane (DCE), perchloroethylene, and vinyl chloride. A group-match design was used to compare the 236 TCE-exposed residents to 161 unexposed regional referents and 67 referents in Northeastern Phoenix in the blink reflex test. The blink reflex response was recorded from surface electrodes placed over the location of the orbicularis oculi muscles. The reflex response was elicited by gently tapping the left and right supraorbital notches with a small hammer. The R1 component of the blink reflex response was measured for both the left and right eye. Statistically significant increases in latency time for the R1 component was observed for residents exposed to TCE in comparison to the control groups. In unexposed individuals, the R1 component occurred within 13.4 ms from the right eye and 13.5 ms from the left eye. In comparison, the residents near the manufacturing plant had latency times of 14.2 ms ($p < 0.0001$) for the right eye and 13.9 ms ($p < 0.008$) for the left eye. This study shows statistically significant differences between environmentally exposed and unexposed populations for trigeminal nerve function, as a result of exposures to TCE. This is an ecological study with TCE exposure potential to subjects inferred by residence in a geographic area. Estimates of TCE concentrations in drinking water to individuals are lacking. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Feldman et al. (1992) evaluated the blink reflex in 18 subjects occupationally exposed to neurotoxic chemicals (e.g., degreasers, mechanics, and pesticide sprayers among many others). Eight of the subjects were either extensively (n = 4) or occupationally (n = 4) exposed to TCE. The remaining subjects (n = 10) were exposed to other neurotoxic chemicals, but not TCE. Quantitative exposure concentration data were not reported in the study, but TCE exposure was characterized as either “extensive” or “occupational.” Subjects in the “extensive” exposure group were chronically exposed (≥1 year) to TCE at least 5 days/week and for at >50% of the workday (n = 3) or experienced a direct, acute exposure to TCE for >15 minutes (n = 1). Subjects in the “occupational” group were chronically exposed (≥1 year) to TCE for 1–3 days/week and for >50% of the workday. The blink reflex responses from the TCE-exposed subjects were compared to a control group consisting of 30 nonexposed subjects with no noted neurological disorders. Blink reflex responses were measured using surface electrodes over the lower lateral portion of the orbicularis oculi muscle. Electrical shocks with durations of 0.05 ms were applied to the supraorbital nerve to generate the R1 and R2 responses. All of the subjects that were extensively exposed to TCE had significantly increased latency times in the appearance of the R1 component (no *p*-value listed) and for three subjects, this increased latency time persisted for at least 1 month and up to 20 years postexposure. However, none of the subjects occupationally exposed to TCE had changes in the blink reflex response in comparison to the control group. In comparing the remaining neurotoxicant-exposed subjects to the TCE-exposed

individuals, the sensitivity, or the ability of a positive blink reflex test to identify correctly those who had TCE exposure was 50%. However, in workers with no exposure to TCE, 90% demonstrated a normal R1 latency.

Mixed results were obtained in a study by Ruijten et al. (1991) on 31 male printing workers exposed to TCE. The mean age was 44; mean exposure duration was 16 years and had at least 6 years of TCE exposure. The control group consisted of 28 workers with a mean age 45 years. Workers in the control group were employed at least 6 years in print factories (similar to TCE-exposed), had no exposure to TCE, but were exposed to "turpentine-like organic solvents." TCE exposure potential was inferred from historical monitoring of TCE at the plant using gas detection tubes. These data indicated TCE concentrations in the 1960s of around 80 ppm, mean concentration of 70 ppm in the next decade, with measurements from 1976 and 1981 showing a mean concentration of 35 ppm. The most recent estimate of TCE concentrations in the factory was 17 ppm (stable for 3 years) at the time of the report. The authors calculated that mean cumulative TCE exposure would be 704 ppm × years worked in factory. The masseter and blink reflexes were measured to evaluate trigeminal nerve function in TCE-exposed and control workers. For measurement of the masseter reflex, surface electrodes were attached over the right masseter muscle (over the cheek area). A gentle tap on a roller placed under the subject's chin was used to elicit the masseter reflex. For measurement of the blink reflex, surface electrodes were placed on the muscle near the upper eyelid. Electrical stimulation of the right supraorbital nerve was used to generate the blink reflex. There was a significant increase in the latency of the masseter reflex to appear for the TCE-exposed workers ($p < 0.05$). However, there was no significant change in the blink reflex measure between TCE-exposed workers and control. Although no change in the blink reflex measures were observed between the two groups, it should be noted that the control group was exposed to other volatile organic solvents (not specified) and this VOC exposure could be a possible confounder for determination of TCE-induced effects.

There are two studies that reported no effect of TCE exposure on trigeminal nerve function (Rasmussen et al., 1993a; El Ghawabi et al., 1973). El Ghawabi et al. (1973) conducted a study on 30 money printing shop workers occupationally exposed to TCE. Metabolites of total TCA and TCOH were found to be proportional to TCE concentrations up to 100 ppm (550 mg/m^3). Controls were 20 age- and SES-matched nonexposed males and 10 control workers not exposed to TCE. Trigeminal nerve involvement was not detected, but the authors failed to provide details as to how this assessment was made. It is mentioned that each subject was clinically evaluated and trigeminal nerve involvement may have been assessed through a clinical evaluation. As a result, the conclusions of this study are tempered since the authors did not provide details as to how trigeminal nerve function was evaluated in this study.

Rasmussen et al. (1993a) conducted an historical cohort study on 99 metal degreasers. Subjects were selected from a population of 240 workers from 72 factories in Denmark. The

participants were divided into three groups based on solvent exposure durations where low exposure was up to 0.5 years, medium was 2.1 years and high was 11.0 years (mean exposure duration). Most of the workers (70/99) were primarily exposed to TCE with an average exposure duration of 7.1 years for 35 hours/week. TCA and TCOH levels were measured in the urine samples provided by the workers and mean TCA levels in the high group was 7.7 mg/L and was as high as 26.1 mg/L. Experimental details of trigeminal nerve evaluation were not provided by the authors. It was reported that 1/21 people (5%) in the low-exposure group, 2/37 (5%) in the medium-exposure group, and 4/41 (10%) in the high-exposure group experienced abnormalities in trigeminal nerve sensory function. No linear association was seen on trigeminal nerve function (Mantel-Haenzel test for linear association, $p = 0.42$). However, the trigeminal nerve function findings were not compared to a control (no TCE exposure) group and it should be noted that some of the workers (29/99) were not exposed to TCE.

D.1.1.2. TSEP Studies—Trigeminal Nerve

In a preliminary study, Barret et al. (1982) measured TSEPs) in 11 workers that were chronically exposed to TCE. Nine of these workers were suffering effects from TCE intoxication (changes in facial sensitivity and clinical changes in trigeminal nerve reflexes), and two were TCE-exposed without exhibiting any clinical manifestations from exposure. A control group of 20 nonexposed subjects of varying ages were used to establish the normal response curve for the trigeminal nerve function. In order to generate a TSEP, a surface electrode was placed over the lip and a voltage of 0.05 ms in duration was applied. The area was stimulated 500 times at a rate of 2 times/second. TSEPs were recorded from a subcutaneous electrode placed between the international CZ point (central midline portion of the head) and the ear. In 8 of the 11 workers, an increased voltage ranging from a 25 to a 45 volt increase was needed to generate a normal TSEP. Two of the 11 workers had an increased latency of appearance for the TSEP and 3 workers had increases in TSEP amplitudes. The preliminary findings indicate that TCE exposure results in abnormalities in trigeminal nerve function. However, the study does not provide any exposure data and lacks information with regards to the statistical treatment of the observations.

Barret et al. (1987) conducted a study on 104 degreaser machine operators in France (average age = 41.6 years; range = 18–62 years) who were highly exposed to TCE with an average exposure of 7 hours/day for 8.23 years. Although TCE exposure concentrations were not available, urinary concentrations of TCOH and TCA were measured for each worker. A control group consisting of 52 subjects without any previous solvent exposure and neurological deficits was included in the study. Trigeminal nerve symptoms and TSEPs were collected for each worker. Trigeminal nerve symptoms were clinically assessed by examining facial sensitivity and reflexes dependent on this nerve such as the jaw and blink reflex. TSEPs were elicited by electrical stimulation (70–75 V for 0.05 ms) of the nerve using an electrode on the lip

commissure. Eighteen out of 104 TCE-exposed machine operators (17.3%) had trigeminal nerve symptoms. The subjects that experienced trigeminal nerve symptoms were significantly older (47.8 years vs. 40.5; $p < 0.001$). Both groups had a similar duration of exposure with a mean of 9.2 years in the sensitive group and 7.8 years in the nonsensitive group. Urinary concentrations of TCOH and TCA were also statistically similar although the levels were slightly higher in the sensitive group (245 vs. 162 mg/g creatinine for TCOH; 131 vs. 93 mg/g creatinine for TCA). However, in the same group, 40/104 subjects (38.4%) had an abnormal TSEP. Abnormal TSEPs were characterized as potentials that exhibited changes in latency and/or amplitude that were at least 2.5 times the SD of the normal TSEPs obtained from the control group. Individuals with abnormal TSEP were significantly older (45 vs. 40.1 years; $p < 0.05$) and were exposed to TCE longer (9.9 vs. 5.6 years; $p < 0.01$). Urinary concentrations TCOH and TCA were similar between the groups with sensitive individuals having average metabolite levels of 195 mg TCOH/g creatinine and 98.3 mg TCA/g creatinine in comparison to 170 mg TCOH/g creatinine and 96 mg TCA/g creatinine in nonsensitive individuals. When a comparison was made between workers that had normal TSEP and no trigeminal symptoms and workers that had an abnormal TSEP and experienced trigeminal symptoms, it was found that in the sensitive individuals (abnormal TSEP and trigeminal symptoms) there was a significant increase in age (48.5 vs. 39.5 years old, $p < 0.01$), duration of exposure (11 vs. 7.5 years, $p < 0.05$) and an increase in urinary TCA (313 vs. 181 mg TCA/g creatinine). No significant changes were noted in urinary TCOH, but the levels were slightly higher in sensitive individuals (167 vs. 109 mg TCOH/g creatinine). Overall, it was concluded that abnormal TSEPs were recorded in workers who were exposed to TCE for a longer period (average duration 9.9 years). This appears to be a well-designed study with statistically significant results reported for abnormal trigeminal nerve response in TCE exposed workers. Exposure assessment to TCE is by exposure duration and mean urinary TCOH and TCA concentrations. TCE concentrations to exposed subjects as measured by atmospheric or personal monitoring are lacking.

Mhiri et al. (2004) measured TSEPs from 23 phosphate industry workers exposed to TCE for 6 hours/day for at least 2 years while cleaning tanks. Exposure assessment was based on measurement of urinary metabolites of TCE, which were performed 3 times/worker, and air measurements. Blood tests and hepatic enzymes were also collected. The mean exposure duration was 12.4 ± 8.3 years (exposure duration range = 2–27 years). Although TCE exposures were not provided, mean urinary concentrations of TCOH, TCA, and total trichlorides were 79.3 ± 42, 32.6 ± 22, and 111.9 ± 55 mg/g urinary creatinine, respectively. The control group consisted of 23 unexposed workers who worked in the same factory without being exposed to any solvents. TSEPs were generated from a square wave pulses (0.1 ms in duration) delivered through a surface electrode that was placed 1 cm under the corner of the mouth. The responses to the stimuli (TSEPs) were recorded from another surface electrode that was placed over the contralateral parietal area of the brain. The measured TSEP was divided into several

components and labeled according to whether it was: (1) a positive (P) or negative (N) potential and (2) the placement of the potential in reference to the entire TSEP (e.g., P1 is the first positive potential in the TSEP). TSEPs generated from the phosphate workers that were ± 2.5 times the SD from the TSEPs obtained from the control group were considered abnormal. Abnormal TSEP were observed in six workers with clinical evidence of trigeminal involvement and in nine asymptomatic workers. Significant increases in latency were noted for all TSEP potentials (N1, P1, N2, P2, N3, $p < 0.01$) measured from the phosphate workers. Additionally, significant decreases in the P1 ($p < 0.02$) and N2 ($p < 0.05$) amplitudes were observed. A significant positive correlation was demonstrated between duration of exposure and the N2 latency ($p < 0.01$) and P2 latency ($p < 0.02$). Only one subject had urinary TCE metabolite levels over tolerated limits. TCE air contents were over tolerated levels, ranging from 50 to 150 ppm (275–825 mg/m^3). The study is well presented with statistically significant results for trigeminal nerve impairment resulting from occupational exposures to TCE. Exposure potential to TCE is defined by urinary biomarkers, TCA, TTCs, and TCOH. The study lacks information on atmospheric monitoring of TCE in this occupational setting.

D.1.1.3. Nerve Conduction Velocity Studies

Nerve conduction latencies were also studied in two occupational studies by Triebig et al. (1983; 1982) using methods for measurement of nerve conduction that differ from most published studies, but the results indicate a potential impact on nerve conduction following occupational TCE exposure. There was no impact seen on latencies in the 1982 study, but a statistically significant response was observed in the latter study. The latter study, however, is confounded by multiple solvent exposures.

In Triebig et al. (1982), 24 healthy workers (20 males, 4 females) were exposed to TCE occupationally at three different plants. The ages ranged from 17 to 56 years, and length of exposure ranged from 1 to 258 months (mean 83 months). TCE concentrations measured in air at work places ranged from 5 to 70 ppm (27–385 mg/m^3). A control group of 144 healthy, complaint-free individuals were used to establish 'normal' responses on the nerve conduction studies. The matched control group consisted of 24 healthy nonexposed individuals (20 males, 4 females), chosen to match the subjects for age and sex. TCA, TCE, and TCOH were measured in blood, and TCE and TCA were measured in urine. Nerve conduction velocities were measured for sensory and motor nerve fibers using the following tests: MCV_{MAX} (U): Maximum NLG of the motor fibers of the N. ulnaris between the wrist joint and the elbow; dSCV (U): Distal NLG of mixed fibers of the N. ulnaris between finger V and the wrist joint; pSCV (U): Proximal NLG of sensory fibers of the N. medianus between finger V and Sulcus ulnaris; and dSCV (M): Distal NLG of sensory fibers of the N. medianus between finger III and the wrist joint. Data were analyzed using parametric and nonparametric tests, rank correlation, linear regression, with 5% error probability. Results show no statistically significant difference in

nerve conduction velocities between the exposed and unexposed groups. This study has measured exposure data, but exposures/responses are not reported by dose levels.

Triebig et al. (1983) has a similar study design to the previous study (Triebig et al., 1982) in the tests used for measurement of nerve conduction velocities, and in the analysis of blood and urinary metabolites of TCE. However, in this study, subjects were exposed to a mixture of solvents, including TCE, specifically "ethanol, ethyl acetate, aliphatic hydrocarbons (gasoline), methyl ethyl ketone (MEK), toluene, and trichloroethene." The exposed group consists of 66 healthy workers selected from a population of 112 workers. Workers were excluded based on polyneuropathy (n = 46) and alcohol consumption (n = 28). The control group consisted of 66 healthy workers with no exposures to solvents. Subjects were divided into three exposure groups based on length of exposure, as follows: 20 employees with "short-term exposure" (7–24 months); 24 employees with "medium-term exposure" (25–60 months); and 22 employees with "long-term exposure" (>60 months). TCA, TCE, and TCOH were measured in blood, and TCE and TCA were measured in urine. Subjects were divided into exposure groups based on length of exposures, and results were compared for each exposure group to the control group. In this study, there was a dose-response relationship observed between length of exposure to mixed solvents and statistically significant reduction in nerve conduction velocities observed for the medium and long-term exposure groups for the ulnar nerve (NCV). Interpretation of this study is limited by the mixture of solvent exposure, with no results reported for TCE alone.

D.1.2. Auditory Effects

There are three large environmental studies reported that assessed the potential impact of TCE exposures through groundwater ingestion on auditory functioning. They present mixed results. All three studies were conducted on the population in the TCE Subregistry from the National Exposure Registry (NER) developed by the ATSDR. The two studies conducted by Burg et al. (1999; 1995) report an increase in auditory effects associated with TCE exposure, but the auditory endpoints were self reported by the population, as opposed to testing of measurable auditory effects in the subject population. The third of these studies, reported by ATSDR (2002), conducted measurements of auditory function on the subject population, but failed to demonstrate a positive relationship between TCE exposure and auditory effects. Results from these studies strongly suggest that children ≤9 years old are more susceptible to hearing impairments from TCE exposure than the rest of the general population. These studies are described below.

Burg et al. (1995) conducted a study on registrants in the National Health Interview Survey (NHIS) TCE subregistry of 4,281 (4,041 living and 240 deceased) residents environmentally exposed to TCE via well water in Indiana, Illinois, and Michigan. Morbidity baseline data were examined from the TCE Subregistry from the NER developed by the ATSDR. Participants were interviewed in the NHIS, which consists of 25 questions about health

conditions. Data were self reported via face-to-face interviews. Neurological endpoints were hearing and speech impairments. This study assessed the long-term health consequences of long-term, low-level exposures to TCE in the environment. The collected data were compared to the NHIS, and the National Household Survey on Drug Abuse. Poisson Regression analysis model was used for registrants ≥19 years old. The statistical analyses performed treated the NHIS population as a standard population and applied the age- and sex-specific period prevalence and prevalence rates obtained from the NHIS data to the corresponding age- and sex-specific denominators in the TCE Subregistry. This one-sample approach ignored sampling variability in the NHIS data because of the large size of the NHIS database when compared to the TCE Subregistry data file. A binomial distribution was assumed in estimating SEs for the TCE Subregistry data. Weighted age- and sex-specific period prevalence and prevalence rates by using the person-weights were derived for the TCE subregistry. These "standard" rates were applied to the corresponding TCE Subregistry denominators to obtain expected counts in each age and sex combination. In the NHIS sample, 18% of the subjects were nonwhite. In the TCE Subregistry sample, 3% of the subjects were nonwhite. Given this discrepancy in the proportion of nonwhites and the diversity of races reported among the nonwhites in the TCE Subregistry, the statistical analyses included 3,914 exposed white TCE registrants who were alive at baseline. TCE registrants that were ≤9 years old had a statistically significant increase in hearing impairment as reported by the subjects. The RR in this age group for hearing impairments was 2.13. The RR decreased to 1.12 for registrants aged 10–17 years and to ≤0.32 for all other age groups. As a result, the effect magnitude was lower for children 10–17 years and for all other age groups. The study reports a dose-response relationship, but the hearing effects are self-reported, and exposure data are modeled estimates.

Burg and Gist (1999) reported a study conducted on the same subregistry population described for Burg et al. (1995). It investigated intrasubregistry differences among 3,915 living members of the National Exposure Registry's Trichloroethylene Subregistry (4,041 total living members). The participants' mean age was 34 years (SD = 19.9 years), and included children in the registry. All registrants had been exposed to TCE through domestic use of contaminated well water. All were Caucasian. All registrants had been exposed to TCE though domestic use of contaminated well water; there were four exposure subgroups, each divided into quartiles: (1) maximum TCE measured in well water, exposure subgroups include 2–12, 12–60, and 60–800 ppb; (2)cumulative TCE exposure subgroups include <50, 50–500, 500–5,000, and >5,000 ppb; (3) cumulative chemical exposure subgroups include TCA, DCE, DCA, in conjunction with TCE, with the same exposure Categories as in # 2; and (4) duration of exposure subgroups include <2, 2–5, 5–10, and >10 years; 2,867 had TCE exposure of ≤50 ppb; 870 had TCE exposure of 51–500 ppb; 190 had TCE exposure of 501–5,000 ppb; and 35 had TCE exposure >5,000 ppb. The lowest quartile was used as a control group. Interviews included occupational, environmental, demographic, and health information. A large number of health

outcomes were analyzed, including speech impairment and hearing impairment. Statistical methods used include Logistic Regression and ORs. The primary purpose was to evaluate the rate of reporting health-outcome variables across exposure categories. The data were evaluated for an elevation of the risk estimates across the highest exposure categories or for a dose-response effect, while controlling for potential confounders. Estimated prevalence ORs for the health outcomes, adjusted for the potential confounders, were calculated by exponentiating the β-coefficients from the exposure variables in the regression equations. The SE of the estimate was used to calculate 95% CIs. The referent group used in the logistic regression models was the lowest exposure group. The results variables were modeled as dichotomous, binary dependent variables in the regression models. Nominal, independent variables were modeled, using dummy variables. The covariables used were sex, age, occupational exposure, education level, smoking history, and the sets of environmental subgroups. The analyses were restricted to persons ≥19 years old when the variables of occupational history, smoking history, and education level were included. When the registrants were grouped by duration of exposure to TCE, a statistically significant association (adjusted for age and sex) between duration of exposure and reported hearing impairment was found. The prevalence ORs were 2.32 (95% Cl: 1.18, 4.56) (>2–<5 years); 1.17 (95% Cl: 0.55, 2.49) (>5–<10 years); and 2.46 (95% Cl = 1.30, 5.02) (>10 years). Higher rates of speech impairment (although not statistically significant) were associated with maximum and cumulative TCE exposure, and duration of exposure. The study reports dose-response relationships, but the effects are self reported, and exposure data are estimates. No information was reported on presence or absence of additional solvents in drinking water.

ATSDR (2002) conducted a follow-up study to the TCE subregistry findings (Burg and Gist, 1999; Burg et al., 1995) and focused on the subregistry children. Of the 390 subregistry children (≤10 years old at time of original study), 116 agreed to participate. TCE exposure ranged from 0.4 to 5,000 ppb from the drinking water. The median TCE exposure for this subgroup was estimated to be 23 ppb per year of exposure. To further the hearing impairments reported in Burg et al. (1999; 1995), comprehensive auditory tests were conducted with the 116 children and compared to a control group of 182 children that was age-matched. The auditory tests consisted of a hearing screening (typanometry, pure tone and distortion product otoacoustic emissions [DPOAE]) and a more in-depth hearing evaluation for children that failed the initial screening. Ninety percent of the TCE-exposed children passed the typanometry and pure tone tests, and there were no significant differences between control and TCE-exposed groups. Central auditory processing tests were also conducted and consisted of a test for acoustic reflexes and a screening test for auditory processing disorders (SCAN). The acoustic reflex tested the ipsilateral and contralateral auditory pathway at 1,000 Hz for each ear. In this test, each subject hears the sound frequency and determines if the sound causes the stapedius muscle to tighten the stapes (normal reflex to noise). Approximately 20% of the children in the TCE subregistry and 5–7% in the controls exhibited an abnormal acoustic reflex, and this increased abnormality in the

test was a significant effect ($p = 0.003$). No significant effects were noted in the SCAN tests. The authors concluded that the significant decrease in the acoustic reflex for the TCE subregistry children is reflective of potential abnormalities in the middle ear, which may reflect abnormalities in lower brainstem auditory pathway function. Lack of effects with the pure tone and typanometry tests suggests that the cochlea is not affected by TCE exposure.

Although auditory function was not directly measured, Rasmussen et al. (1993c) used a psychometric test to measure potential auditory effects of TCE exposure in an environmental study. Results from 96 workers exposed to TCE and other solvents were presented in this study. The workers were divided into three exposure groups: low, medium, and high. Details of the exposure groups and exposure levels are provided in Table 4-22 [under study description of Rasmussen et al. (1993c)]. Three auditory-containing tasks were included in this study, but only the acoustic motor function test could be used for evaluation of auditory function. In the acoustic motor function test, high and low frequency tones were generated and heard through a set of earphones. Each individual then had to imitate the tones by knocking on the table using the flat hand for a low frequency and using a fist for a high frequency. A maximal score of 8 could be achieved through this test. The tones were provided in either a set of one or three groups. In the one group acoustic motor function test, the average score for the low-exposure group was 4.8 in comparison to 2.3 in the high-exposure group. Similar decrements were noted in the 3-group acoustic motor function test. A significant association was reported for TCE exposure and performance on the one group acoustic motor function test ($p < 0.05$) after controlling for confounding variables.

D.1.3. Vestibular Effects

The data linking acute TCE exposure with transient impairment of vestibular function are quite strong based on human chamber studies, occupational exposure studies, and laboratory animal investigations. It is clear from the human literature that these effects can be caused by exposures to TCE, as they have been reported extensively in the literature.

The earliest reports of neurological effects resulting from TCE exposures focused on subjective symptoms, such as headaches, dizziness, and nausea. These symptoms are subjective and self-reported, and, therefore, offer no quantitative measurement of cause and effect. However, there is little doubt that these effects can be caused by exposures to TCE, as they have been reported extensively in the literature, resulting from occupational exposures (Liu et al., 1988; Rasmussen and Sabroe, 1986; Smith, 1970; Grandjean et al., 1955), environmental exposures (Hirsch et al., 1996), and in chamber studies (Stewart et al., 1970; Kylin et al., 1967). These studies are described below in more detail.

Grandjean et al. (1955) reported on 80 workers exposed to TCE from 10 different factories of the Swiss mechanical engineering industry. TCE air concentrations varied from 6 to 1,120 ppm (33–6,200 mg/m^3) depending on time of day and proximity to tanks, but mainly

averaged between 20 and 40 ppm (100–200 mg/m^3). Urinalysis (TCA) varied from 30 mg/L to 300 mg/L. This study does not include an unexposed referent group, although prevalences of self-reported symptoms or neurological changes among the higher-exposure group are compared to the lower-exposure group. Workers were classified based on their exposures to TCE and there were significant differences (p = 0.05) in the incidence of neurological disorder between Groups I (10–20 ppm), II (20–40 ppm; 110–220 mg/m^3), and III (>40 ppm; 220 mg/m^3). Thirty-four percent of the workers had slight or moderate psycho-organic syndrome; 28% had neurological changes. Approximately 50% of the workers reported incidences of vertigo and 30% reported headaches (primarily an occasional and/or minimal disorder). Based on TCA eliminated in the urine, results show that subjective, vegetative, and neurological disorders were more frequent in Groups II (40–100 mg/L) and III (101–250 mg/L) than in Group I (10–39 mg/L). Statistics do support a dose-effect relationship between neurological effects and TCE exposure, but exposure data are questionable.

Liu et al. (1988) evaluated the effects of occupational TCE exposure on 103 factory workers in Northern China. The workers (79 men, 24 women) were exposed to TCE during vapor degreasing production or operation. An unexposed control group of 85 men and 26 women was included for comparison. Average TCE exposure was mostly at <50 ppm (275 mg/m^3). The concentration of breathing zone air during entire shift was measured by diffusive samplers placed on the chest of each worker. Subjects were divided into three exposure groups; 1–10 ppm (5.5–55 mg/m^3), 11–50 ppm (60–275 mg/m^3), and 51–100 ppm (280–550 mg/m^3). Results were based on a self-reported subjective symptom questionnaire. The frequency of subjective symptoms, such as nausea, drunken feeling, light-headedness, floating sensation, heavy feeling of the head, forgetfulness, tremors and/or cramps in extremities, body weight loss, changes in perspiration pattern, joint pain, and dry mouth (all ≥3 times more common in exposed workers); reported as 'prevalence of affirmative answers', was significantly greater in exposed workers than in unexposed (p < 0.01). *"Bloody strawberry jam-like feces"* was borderline significant in the exposed group and "*frequent flatus"* was statistically significant. Dose-response relationships were established (but not statistically significant) for symptoms. Most workers were exposed at <10 ppm, and some at 11–50 ppm. The differences in exposure intensity between men and women was of borderline significance (0.05 < p < 0.10). The study appears to be well done, although the self reporting of symptoms and the 'prevalence of affirmative answers' metric is not standard practice.

Rasmussen et al. (1986) conducted a cross-sectional study on 368 metal degreasers working in various factories in Denmark (industries not specified) with chlorinated solvents. The control group consisted of 94 randomly selected semiskilled metal workers from same area. The mean age was 37.7 years (range: 17–65+ years). Neurological symptoms of the subjects were assessed by questionnaire. The workers were categorized into four groups as follows: (1) currently working with chlorinated solvents (n = 171; average duration: 7.3 years, 16.5

hours/week; 57% TCE and 37% 1,1,1-trichloroethane); (2) currently working with other solvents (n = 131; petroleum, gasoline, toluene, xylene); (3) previously (1–5 years.) worked with chlorinated or other solvents (n = 66); and (4) never worked with organic solvents (n = 94). A dose-response relationship was observed between exposure to chlorinated solvents and chronic neuropsychological symptoms including vestibular system effects such as dizziness ($p < 0.005$), and headache ($p < 0.01$). The authors indicated that TCE exposure resulted in the most overall symptoms. Significant associations were seen between previous exposure and consumption of alcohol with chronic neuropsychological symptoms. Results are confounded by exposures to additional solvents.

Smith (1970) conducted an occupational study on 130 workers (108 males, 22 females) exposed to TCE (industry not reported). The control group consisted of 63 unexposed men working at the same factories matched by age, marital status, and other nonspecified criteria. A referent group was included and consisted of 112 men and women exposed to low concentration of lead and matched to the TCE exposed group in age and sex distribution. Seventy-three out of 130 workers (56.2%) reported dizziness and 23 workers reported having headaches (17.7%). The number of complaints reported by subjects was greater for those with ≥60 mg/L TCA than for those with <60 mg/L TCA. There was no difference in the number of symptoms reported between those with shorter durations of exposure and those with longer durations of exposure. No statistics were reported.

Hirsch et al. (1996) evaluated the vestibular effects of an environmental exposure to TCE in Roscoe, Illinois residents. A medical questionnaire was mailed to 103 residents of Roscoe with 100% response. These 103 and an additional 15 residents, not previously surveyed, brought the subject population to 118 residents. During the course of testing, 12 subjects (young children and uncooperative patients) were excluded bringing the total number of subjects to 106, all of whom were in the process of taking legal action against the company whose industrial waste was assumed to be the source of the polluting TCE. This was a case series report with no controls. Random testing of the wells between 1983 and 1984 revealed groundwater in wells to have levels of TCE between 0 and 2,441 ppb. The distance of residence from contaminated well was used to estimate exposure level. Sixty-six subjects (62%) complained of headaches at the time of evaluation. Diagnosis of TCE-induced cephalagia was considered credible for 57 patients (54%). Forty-seven of these had a family history of headaches. Retrospective TCE level of well water or well's distance from the industrial site analysis did not correlate with the occurrence of possibly-TCE induced headaches. This study shows a general association between headaches and exposure to TCE in drinking water wells. There were no statistics to support a dose-response relationship. All subjects were involved in litigation.

Stewart et al. (1970) evaluated vestibular effects in 13 subjects who were exposed to TCE vapor 100 ppm (550 mg/m^3) and 200 ppm (1,100 mg/m^3) for periods of 1 hour to a 5-day work week. Experiments 1–7 were for a duration of 7 hours with a mean TCE concentration of 198–

200 ppm (1,090–1,100 mg/m^3). Experiments 8 and 9 exposed subjects to 190–202 ppm (1,045–1,110 mg/m^3) TCE for a duration of 3.5 and 1 hour, respectively. Experiment 10 exposed subjects to 100 ppm (550 mg/m^3) TCE for 4 hours. Experiments 2–6 were carried out with the same subjects over 5 consecutive days. Gas chromatography of expired air was measured. There were no self controls. Subjects reported symptoms of lightheadedness, headache, eye, nose, and throat irritation. Prominent fatigue and sleepiness by all were reported >200 ppm (1,100 mg/m^3). There were no quantitative data or statistics presented regarding dose and effects of neurological symptoms.

Kylin et al. (1967) exposed 12 volunteers to 1,000 ppm (5,500 mg/m^3) TCE for 2 hours in a 1.5 × 2 × 2 meters chamber. Volunteers served as their own controls since 7 of the 12 were pretested prior to exposure and the remaining 5 were post-tested days after exposure. Subjects were tested for optokinetic nystagmus, which was recorded by electronystogmography, that is, "the potential difference produced by eye movements between electrodes placed in lateral angles between the eyes." Venous blood was also taken from the volunteers to measure blood TCE levels during the vestibular task. The authors concluded that there was an overall reduction in the limit ("fusion limit") to reach optokinetic nystagmus when individuals were exposed to TCE. Reduction of the "fusion limit" persisted for up to 2 hours after the TCE exposure was stopped and the blood TCE concentration was 0.2 mg/100 mL.

D.1.4. Visual Effects

Kilburn (2000a, 2002b) conducted an environmental study on 236 people exposed to TCE in groundwater in Phoenix, Arizona. Details of the TCE exposure and population are described earlier in Section D.1.1.1 (see Kilburn, 2000a, 2002b). Among other neurological tests, the population and 161 nonexposed controls was tested for color discrimination using the desaturated Lanthony 15-hue test, which can detect subtle changes in color vision deficiencies. Color discrimination errors were significantly increased in the TCE exposed population ($p < 0.05$) with errors scores averaging 12.6 in the TCE exposed in comparison to 11.9 in the control group. This study shows statistically significant differences in visual response between exposed and nonexposed subjects exposed environmentally. Estimates of TCE concentrations in drinking water to individual subjects are lacking.

Reif et al. (2003) conducted a cross sectional environmental study on 143 residents of the Rocky Mountain Arsenal community of Denver whose water was contaminated with TCE and related chemicals from nearby hazardous waste sites between 1981 and 1986. The residents were divided into three groups based on TCE exposure with the lowest exposure group at <5 ppb, the medium exposure group at 5–15 ppb and the high-exposure group defined as >15 ppb TCE. Visual performance was measured by two different contrast sensitivity tests (C and D) and the Benton visual retention test. In the two contrast sensitivity tests, there was a 20–22% decrease in performance between the low and high TCE exposure groups and

approached statistical significance (p = 0.06 or 0.07). In the Benton visual retention test, which measures visual perception and visual memory, scores, dropped by 10% from the lowest exposure to the highest TCE exposure group and was not statistically significant. It should be noted that the residents were potentially exposed to multiple solvents including TCE and a nonexposed TCE group was not included in the study. Additionally, modeled exposure data are only a rough estimate of actual exposures, and possible misclassification bias associated with exposure estimation may limit the sensitivity of the study.

Rasmussen et al. (1993c) conducted a cross-sectional study on 96 metal workers, working in degreasing at various factories in Denmark (industries not specified) with chlorinated solvents. These subjects were identified from a larger cohort of 240 workers. Details of the exposure groups and TCE exposure levels are presented in Section D.1.1.1 [under Rasmussen et al. (1993a)]. Neuropsychological tests including the visual gestalts (test of visual perception and retention) and the stone pictures test (test of visual learning and retention) were administered to the metal workers. In the visual gestalts test, cards with a geometrical figure containing four items were presented and workers had to redraw the figure from memory immediately (learning phase) after presentation and after 1 hour (retention phase). In the learning phase, the figures were redrawn until the worker correctly drew the figure. The number of total errors significantly increased from the low group (3.4 errors) to the high-exposure group (6.5 errors; p = 0.01) during the learning phase (immediate presentation). Similarly, during the retention phase of this task (measuring visual memory), errors significantly increased from an average of 3.2 in the low group to 5.9 in the high group ($p < 0.001$). In the stone pictures test, slides of 10 stones (different shapes and sizes) were shown and the workers had to identify the 10 stones out of a lineup of 25 stones. There were no significant changes in this task, but the errors increased from 4.6 in the low-exposure group to 6.3 in the high-exposure group during the learning phase of this task. Although this study identifies visual performance deficits, a control group (no TCE exposure) was not included in this study and the presented results may actually underestimate visual deficits from TCE exposure.

Troster and Ruff (1990) presented case studies conducted on two occupationally exposed workers to TCE and included a third case study on an individual exposed to 1,1,1-trichloroethane. Case #1 was exposed to TCE (concentration unknown) for 8 months and Case #2 was exposed to TCE over a 3-month period. Each patient was presented with a visual-spatial task (Ruff-Light Trail Learning test as referenced by the authors). Both of the individuals exposed to TCE were unable to complete the visual-spatial task and took the maximum number of trials (10) to attempt to complete the visual task. A control group of 30 individuals and the person exposed to 1,1,1-trichloroethane were able to complete this task accordingly. The lack of quantitative exposure data and a small sample size severely limits the study and does not allow for statistical comparisons.

Vernon and Ferguson (1969) exposed eight male volunteers (ages 21–30 years) to 0, 100, 300, and 1,000 ppm TCE for 2 hours. Each individual was exposed to all TCE concentrations and a span of at least 3 days was given between exposures. The volunteers were presented with six visuo-motor tests during the exposure sessions. When the individuals were exposed to 1,000 ppm TCE (5,500 mg/m^3), significant abnormalities were noted in depth perception as measured by the Howard-Dolman test ($p < 0.01$), but no effects on the flicker fusion frequency test (threshold frequency at which the individual sees a flicker as a single beam of light) or on the form perception illusion test (volunteers presented with an illusion diagram). This is one of the earliest chamber studies of TCE. This study included only healthy young males, is of a small size, limiting statistical power, and reports mixed results on visual testing following TCE exposure.

D.1.5. Cognition

There is a single environmental study in the literature that presents evidence of a negative impact on intelligence resulting from TCE exposure. Kilburn and Warshaw (1993a) (study details in Section D.1.1.1) evaluated the effects on cognition for 544 Arizona residents exposed to TCE in well water. Subjects were recruited and categorized into three groups. Exposed Group 1 consisted of 196 family members with cancer or birth defects. Exposed Group 2 consisted of 178 individuals from families without cancer or birth defects; and exposed Group 3 included 170 parents whose children had birth defects and rheumatic disorders. Sixty-eight referents were used as a comparison group for the clinical memory tests. Several cognitive tests were administered to these residents in order to test memory recall skills and determine if TCE exposure resulted in memory impairment. Working or short-term memory skills were tested by asking each individual to recall two stories immediately after presentation (verbal recall) and also draw three diagrams immediately after seeing the figures (visual recall). Additionally, a digit span test where increasing numbers of digits were presented and then the subject had to recall the digits was conducted to the extent of the short-term memory. Exposed subjects had lower intelligence scores and there were significant impairments in verbal recall ($p = 0.001$), visual recall ($p = 0.03$) and with the digit span test ($p = 0.07$). Significant impairment in short-term memory as measured by three different cognitive test was correlated with TCE exposure. Lower intelligence scores ($p = 0.0001$) as measured by the Culture Fair IQ test may be a possible confounder in these findings. Additionally, the large range of TCE concentrations (6–500 ppb) and exposure durations (1 to 25 years) and overall poor exposure characterization precludes a NOAEL/LOAEL from being estimated from this study on cognitive function.

Rasmussen et al. (1993c, 1993d) and Troster and Ruff (1990) present results of positive findings in occupational studies for cognitive effects of TCE. Rasmussen et al. (1993c) reported an historical cohort study conducted on 96 metal degreasers, identified 2 years previously and were selected from a population of 240 workers from 72 factories in Denmark. They reported

psychoorganic syndrome, a mild syndrome of dementia characterized by cognitive impairment, personality changes, and reduced motivation, vigilance, and initiative, was increased in the three exposure groups. The medium- and high-exposure groups were compared with the low-exposure group. Neuropsychological tests included WAIS (original version, Vocabulary, Digit Symbol, Digit Span), SRT, Acoustic-motor function (Luria), Discriminatory attention (Luria), Sentence Repetition, Paced Auditory Serial Addition Test (PASAT), Text Repetition, Rey's Auditory Verbal Learning, Visual Gestalts, Stone Pictures (developed for this study, nonvalidated), revised Santa Ana, Luria motor function, and Mira. The prevalence of psychoorganic syndrome was 10.5% in low-exposure group; 38.9% in medium-exposure group; 63.4% in high-exposure group. (x^2 trend analysis: low vs. medium exposure $x^2 = 11.0$, $p < 0.001$; low vs. high exposure $x^2 = 19.6$, $p < 0.001$.) Psychoorganic syndrome increased with age ($p < 0.01$). Age was strongly correlated with exposure.

Rasmussen et al. (1993d) used a series of cognitive tests to measure effects of occupational TCE exposure. Short-term memory and retention following an latency period of one hour was evaluated in several tests including a verbal recall (auditory verbal learning test), visual gestalts, visual recall (stone pictures), and the digit span test. Significant cognitive performance decreases were noted in both short-term memory and memory retention. In the verbal recall test, immediate memory and learning were significantly decreased ($p = 0.03$ and 0.04, respectively). No significant effects were noted for retention following a 1-hour latency period was noted. Significant increases in errors were noted in both the learning ($p = 0.01$) and memory ($p < 0.001$) phases for the visual gestalts test. No significant effects were found in the visual recall test in either the learning or memory phases or in the digit span test. As a result, there were some cognitive deficits noted in TCE-exposed individuals as measured through neuropsychological tests.

Troster and Ruff (1990) provides additional supporting evidence in an occupational study for cognitive impairment, although the results reported in a qualitative fashion are limited in their validity. In the two case studies that were exposed to TCE, there were decrements (no statistical analysis performed) in cognitive performance as measured in verbal and visual recall tests that were conducted immediately after presentation (learning phase) and 1 hour after original presentation (retention/memory phase).

Triebig et al. (1977c) presents findings of no impairment of cognitive ability resulting from TCE exposure in an occupational setting. This study was conducted on eight subjects occupationally exposed to TCE. Subjects were seven men and one woman with an age range from 23 to 38 years. Measured TCE in air averaged 50 ppm (260 mg/m^3). Length of occupational exposure was not reported. There was no control group. Results were compared after exposure periods, and compared to results obtained after periods removed from exposure. TCA and TCE metabolites in urine and blood were measured. The testing consisted of the Syndrome Short Test, which consists of nine subtests through which amnesic and simple

perceptive and cognitive functional deficits are detected; the "Attention Load Test" or "d2 Test" from Brickenkamp is a procedure that measures attention, concentration, and stamina; number recall test; letter recall test; the "Letter Reading Test;" and "Word Reading Test." Data were assessed using Wilcoxon and Willcox nonparametric tests. Due to the small sample size, a significance level of 1% was used. The concentrations of TCE, TCOH, and TCA in the blood and total TCE and total TCA elimination in the urine were used to assess exposure in each subject. The mean values observed were 330 mg TCOH and 319 mg TCA/g creatinine, respectively, at the end of a work shift. The psychological tests showed no statistically significant difference in the results before or after the exposure-free time period. The small sample size may limit the sensitivity of the study.

Salvini et al. (1971), Gamberale et al. (1976), and Stewart et al. (1970) reported positive findings for the impairment of cognitive function following TCE exposures in chamber studies. Salvini et al. (1971) reported a controlled exposure study conducted on six male university students. TCE concentration was 110 ppm (550 mg/m^3) for 4-hour intervals, twice per day. Each subject was examined on two different days, once under TCE exposure, and once as self controls, with no exposure. Two sets of tests were performed for each subject corresponding to exposure and control conditions. The test battery included a perception test with tachistoscopic presentation, the Wechsler memory scale test, a CRT test, and a manual dexterity test. Statistically significant results were observed for perception tests learning ($p < 0.001$), mental fatigue ($p < 0.01$), subjects ($p < 0.05$); and CRT learning ($p < 0.01$), mental fatigue ($p < 0.01$), subjects ($p < 0.05$). This is controlled exposure study with measured dose (110 ppm; 600 mg/m^3) and clear, statistically significant impact on neurological functional domains. However, it only assesses acute exposures.

Gamberale et al. (1976) reported a controlled exposure study conducted on 15 healthy men aged 20–31 years old, employed by the Department of Occupational Medicine in Stockholm, Sweden. Controls were within subjects (15 self-controls), described above. Test used included RT addition and short-term memory using an electronic panel. Subjects also assessed their own conditions on a 7-point scale. Researchers used a repeated measures ANOVA for the four performance tests based on a 3 × 3 Latin square design. In the short-term memory test (version of the digit span test), a series of numbers lasting for 1 second was presented to the subject. The volunteer then had to reproduce the numerical sequence after a latency period (not specified). No significant effect on the short-term memory test was observed with TCE exposure in comparison to air exposure. Potential confounders from this study include repetition of the same task for all exposure conditions, volunteers served as their own controls, and TCE exposure preceded air exposure in two of the three exposure experimental designs. This is a well controlled study of short term exposures with measured TCE concentrations and significant response observed for cognitive impairment.

Additional qualitative support for cognitive impairment is provided by Stewart et al. (1970). This was a controlled exposure study conducted on 13 subjects in 10 experiments, which consisted of 10 chamber exposures to TCE vapor of 100 ppm (550 mg/m^3) and 200 ppm (1,100 mg/m^3) for periods of 1 hour to a 5-day work week. Experiments 1–7 were for 7 hours with a mean TCE concentration of 198–200 ppm (1,090–1,100 mg/m^3). Experiments 8 and 9 exposed subjects to 190–202 ppm (1,045–1,110 mg/m^3) TCE for a duration of 3.5 and 1 hour, respectively. Experiment 10 exposed subjects to 100 ppm (550 mg/m^3) TCE for 4 hours. Experiments 2–6 were carried out with the same subjects over 5 consecutive days. Gas chromatography of expired air was measured. There were no self controls. All had normal neurological tests during exposure, but 50% reported greater mental effort was required to perform a normal modified Romberg test on more than one occasion. There were no quantitative data or statistics presented regarding dose and effects of neurological symptoms.

Two chamber studies conducted by Triebig et al. (1977a; 1976) report no impact of TCE exposure on cognitive function. Triebig et al. (1976) was a controlled exposure study conducted on seven healthy male and female students (four females, three males) exposed for 6 hours/day for 5 days to 100 ppm (550 mg/m^3 TCE). The control group was seven healthy students (four females, three males) exposed to hair care products. This was assumed as a zero exposure, but details of chemical composition were not provided. Biochemical and psychological testing was conducted at the beginning and end of each day. Biochemical tests included TCE, TCA, and TCOH in blood. Psychological tests included the d2 test, which was an attention load test; the short test [as characterized in the translated version of Treibig (1976)] is used to record patient performance with respect to memory and attention; daily Fluctuation Questionnaire measured the difference between mental states at the start of exposure and after the end of exposure is recorded; The MWT-A is a repeatable short intelligence test; Culture Fair Intelligence Test (CFT-3) is a nonverbal intelligence test that records the rather “fluid” part of intelligence, that is, finding solution strategies; Erlanger Depression Scale. Results were not randomly distributed. The median was used to describe the mean value. Regression analyses were conducted. In this study the TCE concentrations in blood reported ranged from 4 to 14 μg/mL. A range of 20–60 μg/mL was obtained for TCA in the blood. There was no correlation seen between exposed and unexposed subjects for any measured psychological test results. The biochemical data did demonstrate subjects’ exposures. This is a well-controlled study with excellent exposure data, although the small sample size may have limited sensitivity.

Triebig et al. (1977a) is an additional report on the seven exposed subjects and seven controls evaluated in Triebig et al. (1976). Additional psychological testing was reported. The testing included the Syndrome Short Test, which consists of nine subtests, described above. Statistics were conducted using Whitney Mann. Results indicated the anxiety values of the placebo random sample group dropped significantly more during the course of testing ($p < 0.05$) than those of the active random sample group. No significantly different changes were obtained

with any of the other variables. Both of these studies were well controlled with excellent exposure data, which may provide some good data for establishing a short-term NOAEL. The small sample size may have limited the sensitivity of the study.

Additional reports on the impairment of memory function as a result of TCE exposures have been reported, and provide additional evidence of cognitive impairment. The studies by Chalupa et al. (1960), Rasmussen et al. (1993d, 1993c; 1986), and Troster and Ruff (1990) report impairment of memory resulting from occupational exposures to TCE. Kilburn and Warshaw (1993a) and Kilburn (2002b, a) report impairment of memory following environmental exposures to TCE. Salvini et al. (1971) reports impairment of memory in a chamber study, although Triebig et al. (1976) reports no impact on memory following TCE exposure in a chamber study.

D.1.6. Psychomotor Effects

There is evidence in the literature that TCE can have adverse psychomotor effects in humans. The effects of TCE exposure on psychomotor response have been studied primarily as the impact on RTs, which provide a quantitative measure of the impact TCE exposure has on motor skills. Studies on motor dyscoordination resulting from TCE exposure are more subjective, but provide additional evidence that TCE may cause adverse psychomotor effects. These studies are described below.

D.1.6.1. RT

There are several reports in the literature that report an increase in RTs following exposures to TCE. The best evidence for TCE exposures causing an increase in CRTs comes from environmental studies by Kilburn (2002b, 2002a), Kilburn and Warshaw (1993a), Reif et al. (2003), and Kilburn and Thornton (1996), which were all conducted on populations which were exposed to TCE through groundwater contaminated as the result of environmental spills. Kilburn (2002b, 2002a) (study details described in Section D.1.1) evaluated reaction times in a Phoenix, Arizona population exposed to TCE through groundwater. Volunteers were tested for response rates in the SRT and two CRT tests. Various descriptive statistics were used, as well as analysis of covariance (ANCOVA) and a step-wise adjustment of demographics. The principal comparison, between the 236 exposed persons and the 161 unexposed regional controls, revealed significant differences ($p < 0.05$) indicating that SRTs and CRTs were delayed. Balance was also abnormal with excessive sway speed (eyes closed), but this was not true when both eyes were open. This study shows statistically significant differences in psychomotor responses between exposed and nonexposed subjects exposed environmentally. However, it is limited by poor exposure characterization.

Kilburn and Warshaw (1993a) (study details described in Section D.1.1.1) evaluated reaction times in 170 Arizona residents exposed to TCE in well water. A referent group of

68 people was used for comparison. TCE concentration was from 6 to 500 ppb and exposure ranged from 1 to 25 years. SRT was determined by presenting the subject a letter on a computer screen and measuring the time (in milliseconds [msec]) that it took for the person to type that letter. SRT significantly increased from 281 ± 55 to 348 ± 96 msec in TCE-exposed individuals ($p < 0.0001$). Similar increases were reported for CRT where subjects were presented with two different letters and required to make a decision as to which letter key to press. CRT of the exposed subjects was 93 msec longer in the third trial ($p < 0.0001$) than referents. It was also longer in all trials, and remained significantly different after age adjustment. This study shows statistically significant differences for neurological test results between subjects environmentally exposed and nonexposed to TCE, but is limited by poor exposure data on individual subjects given the ecological design of this study. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Kilburn and Thornton (1996) conducted an environmental study that attempts to use reference values from two control groups in assessing neurological responses for chemically exposed subjects using neurophysiological and neuropsychological testing on three groups. Group A included randomly selected registered voters from Arizona and Louisiana with no exposure to TCE: n = 264 unexposed volunteers aged 18–83 years. Group B included volunteers from California n = 29 (17 males and 12 females) who were used to validate the equations; group C included those exposed to TCE and other chemicals residentially for ≥5 years n = 237. Group A was used to develop the regression equations for SRT and CRT. A similarly selected comparison group B was used to validate the equations. Group C, the exposed population, was submitted to SRT and CRT tests (n = 237) and compared to the control groups. All subjects were screened by a questionnaire. Reaction speeds were measured using a timed computer visual-stimulus generator. No exposure data were presented. The Box-Cox transformation was used for dependent variables and independent variables. They evaluated graphical methods to study residual plots. Cook's distance statistic was used as a measure of influence to exclude outliers with undue influence and none of the data were excluded. Lack-of-fit test was performed on Final model and F statistic was used to compare estimated error to lack-of-fit component of the model's residual sum of squared error. Final models were validated using group B data and paired t-test to compare observed values for SRT and CRT. F statistic was used to test the hypothesis that parameter estimates obtained with group B were equal to those of Group A, the model. The results are as follows: Group A: SRT = 282 ms; CRT = 532 ms. Group B: SRT = 269 ms; CRT = 531 ms. Group C: SRT = 334 ms; CRT = 619 ms. TCE exposure produced a step increase in reaction times (SRT and CRT). The coefficients from Group A were valid for group B. The predicted value for SRT and for CRT, plus 1.5 SDs selected 8% of the model group as abnormal. The model produced consistent measurement ranges with small numerical variation. This study is limited by lack of any exposure data, and does not provide statistics to demonstrate dose-response effects.

Kilburn (2002b, 2002a) conducted an environmental study on 236 residents chronically exposed to TCE-associated solvents in the groundwater resulting from a spill from a microchip plant in Phoenix, Arizona. Details of the TCE exposure and population are described earlier in Section D.1.1.1 (see Kilburn, 2002b, 2002a). The principal comparison, between the 236 exposed persons and the 161 unexposed regional controls, revealed significant differences indicating that SRTs and choice reaction times (CRTs) were increased. SRTs significantly increased from 283 ± 63 msec in controls to 334 ± 118 msec in TCE exposed individuals ($p < 0.0001$). Similarly, CRTs also increased from 510 ± 87 to 619 ± 153 msec with exposure to TCE ($p < 0.0001$). This study shows statistically significant differences in psychomotor responses as measured by reaction times between TCE-exposed and nonexposed subjects. Estimates of TCE concentrations in drinking water to individual subjects were not reported in the paper. Since the TCE exposure ranged from 0.2 to >10,000 ppb in well water, it is not possible to determine a NOAEL for increased reaction times through this study. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Reif et al. (2003) conducted a cross sectional study on 143 residents of the Rocky Mountain Arsenal (RMA) community of Denver exposed environmentally to drinking water contaminated with TCE and related chemicals from nearby hazardous waste sites between 1981 and 1986. The referent group was at the lowest estimated exposure concentration (<5 ppb). The socioeconomic profile of the participants closely resembled those of the community in general.

> A total of 3393 persons was identified through the census, from which an age- and gender-stratified sample of 1267 eligible individuals who had lived at their current residence for at least 2 years was drawn. Random selection was then used to identify 585 persons from within the age–gender strata, of whom 472 persons aged 2–86 provided samples for biomonitoring. Neurobehavioral testing was conducted on 204 adults who lived in the RMA exposure area for a minimum of 2 years. Among the 204 persons who were tested, 184 (90.2%) lived within the boundaries of the LWD and were originally considered eligible for the current analysis. Therefore, participants who reported moving into the LWD after 1985 were excluded from the total of 184, leaving 143 persons available for study.

An elaborate hydraulic simulation model (not validated) was used in conjunction with a GIS to model estimates of residential exposures to TCE. The TCE concentration measured in community wells exceeded the maximum contaminant level of 5 ppb in 80% of cases. Approximately 14% of measured values exceeded 15 ppb. Measured values were used to model actual exposure estimates based on distance of residences from sampled wells. The estimated exposure for the high-exposure group was >15 ppb; the estimate for the low-exposure referent group was <5 ppb. The medium exposure group was estimated at exposures $5 < x < 15$ ppb TCE. The test battery consisted of the Neurobehavioral Core Test Battery (NCTB), which consists of seven neurobehavioral tests including SRT. Results were assessed using the Multivariate Model.

Results were statistically significant ($p < 0.04$) for the SRT tests. The results are confounded by exposures to additional solvents and modeled exposure data, which while highly technical, are still only a rough estimate of actual exposures, and may limit the sensitivity of the study.

Gamberale et al. (1976) conducted a controlled exposure (chamber) study on 15 healthy men aged 20–31 years old, employed by the Department of Occupational Medicine in Stockholm, Sweden. Controls were within subjects (15 self-controls). Subjects were exposed to TCE for 70 minutes via a breathing valve to 540 mg/m^3 (97 ppm), 1,080 mg/m^3 (194 ppm), and to ordinary atmospheric air (0 ppm). Sequence was counterbalanced between the three groups, days, and exposure levels. Concentration was measured with a gas chromatographic technique every third minute for the first 50 minutes, then between tests thereafter. Tests used were RT addition, SRT, CRT and short-term memory using an electronic panel. Subjects also assessed their own conditions on a 7 point scale. The researchers performed Friedman two-way analysis by ranks to evaluate differences between the 3 conditions. The results were nonsignificant when tested individually, but significant when tested on the basis of six variables. Nearly half of the subjects could distinguish exposure/nonexposure. Researchers performed ANOVA for the four performance tests based on a 3 × 3 Latin square design with repeated measures. In the RT-addition test, the level of performance varied significantly between the different exposure conditions ($F[2.24] = 4.35$; $p < 0.05$) and between successive measurement occasions ($F[2.24] = 19.25$; $p < 0.001$). The level of performance declined with increased exposure to TCE, whereas repetition of the testing led to a pronounced improvement in performance as a result of the training effect. No significant interaction effects were observed between exposure to TCE and training. This is a good study of short-term exposures with measured TCE concentrations and significant response observed for RT.

Gun et al. (1978) conducted an occupational study on eight TCE-exposed workers who operated degreasing baths in two different plants. Four female workers were exposed to TCE only in one plant and four female workers were exposed to TCE and nonhalogenated hydrocarbon solvents in the second plant. The control group (n = 8) consisted of four female workers from each plant who did not work near TCE. Each worker worked two separate 4-hour shifts daily, with one shift exposed to TCE and the second 4-hour shift not exposed. Personal air samples were taken continuously over separate 10-minute sessions. Readings were taken every 30 seconds. Eight-choice reaction times were carried out in four sessions; at the beginning and end of each exposure to TCE or TCE + solvents; a total of 40 RT trials were completed. TCE concentrations in the TCE only plant 1 (148–418 ppm [800–2,300 mg/m^3]) were higher than in the TCE + solvent plant 2 (3–87 ppm [16–480 mg/m^3]). Changes in CRTs were compared to level of exposure. The TCE only group showed a mean increase in RT, with a probable cumulative effect. In the TCE + solvent group, mean RT shortened in Session 2, then increased to be greater than at the start. Both control groups showed a shortening in mean CRT in Session 2, which was sustained in Sessions 3 and 4 consistent with a practice effect. This is a

study with well-defined exposures and reports of cause and effect (TCE exposure on RT); however, no statistics were presented to support the conclusions or the significance of the findings, and the small sample size is a limitation of the study.

D.1.6.2. Muscular Dyscoordination

Effects on motor dyscoordination resulting from TCE exposure have been reported in the literature. These impacts are subjective, but may provide additional evidence that TCE can cause adverse psychomotor effects. There are three reports summarized below that suggest that muscular dyscoordination resulted from TCE exposure, although all three have significant limitations due to confounding factors. Rasmussen et al. (1993a) presented findings on muscular dyscoordination as it relates to TCE exposure. This was a historical cohort study conducted on 96 metal degreasers, identified 2 years previously. Subjects were selected from a population of 240 workers from 72 factories in Denmark. Although the papers report a population of 99 participants, tabulated results were presented for a total of only 96. No explanation was provided for this discrepancy. These workers had chronic exposure to fluorocarbon (CFC113) (n = 25) and mostly TCE (n = 70; average duration: 7.1 years). There were no external controls. The range of working full-time degreasing was 1 month to 36 years. Researchers collected data regarding the workers' occupational history, blood and urine tests, as well as biological monitoring for TCE and TCE metabolites. A chronic exposure index (CEI) was calculated based on number of hours/week worked with solvents multiplied by years of exposure multiplied by 45 weeks/year. No TCE air concentrations were reported. Participants were categorized into three groups: (1) "Low exposure:" n = 19, average full-time exposure = 0.5 years; (2) "Medium exposure:" n = 36, average full-time exposure = 2.1 years; or (3) "High exposure:" n = 41, average full-time exposure = 11 years. The mean TCA level in the "high" exposure group was 7.7 mg/L (max = 26.1 mg/L). TWA measurements of CFC113 levels were 260–420 ppm (U.S. and Danish TLV was 500 ppm). A significant trend of dyscoordination from low to high solvent exposure was observed (p = 0.003). This study provides evidence of causality for muscular dyscoordination resulting from exposure to TCE, but no measured exposure data were reported.

Additional evidence of the psychomotor effects caused by exposure to TCE is presented in Gash et al. (2008) and Troster and Ruff (1990). There are, however, significant limitations with each of these studies. In Gash et al. (2008), the researchers evaluated the clinical features of 1 Parkinson's disease patient, identified in a Phase 1 clinical trial study, index case, and an additional 29 coworkers of the patient, all with chronic occupational exposures to TCE. An additional 2 subjects with Parkinson's disease were included, making the total of 3 Parkinson's disease patients, and 27 non-Parkinson's coworkers making up the study population. Coworkers for the study were identified using a mailed questionnaire to 134 former coworkers. No details were provided in the paper on selection criteria for the 134 former coworkers. Of the 134 former workers sent questionnaires, 65 responded. Twenty-one self-reported no symptoms, 23 endorsed

1–2 symptoms, and 21 endorsed ≥3 more signs of parkinsonism. Fourteen of the 21 with three or more signs and 13 of the 21 without any signs agreed to a clinical exam; this group comprises the 27 additional workers examined for parkinsonian symptoms. No details were provided on nonresponders. All subjects were involved in degreasing with long-term chronic exposure to TCE through inhalation and dermal exposure (14 symptomatic: age range = 31–66 years, duration of employment range: 11–35 years) (13 asymptomatic: age range = 46–63 years, duration of employment range: 8–33 years). The data were compared between groups and with data from 110 age-matched controls. Exposure to TCE is self-reported and based on job proximity to degreasing operations. The paper lacks any description of degreasing processes including TCE usage and quantity. Mapping of work areas indicated that workers with Parkinson's disease worked next to the TCE container, and all symptomatic workers worked close to the TCE container. Subjects underwent a general physical exam, neurological exam and Unified Parkinson's Disease Rating Scale (UPDRS), timed motor tests, occupational history survey, and mitochondrial neurotoxicity. ANOVA analysis was conducted, comparing symptomatic vs. nonsymptomatic workers, and comparing symptomatic workers to age-matched, nonexposed controls. No description of the control population (n = 110), nor how data were obtained for this group, was presented. The symptomatic non-Parkinson's group was significantly slower in fine motor hand movements than age-matched nonsymptomatic group ($p < 0.001$). The symptomatic group was significantly slower ($p < 0.0001$) than age-matched unexposed controls as measured in fine motor hand movements on the Movement Analysis Panel. All symptomatic workers had positive responses to 1 or more questions on UPDRS Part II (diminished activities of daily life), and/or deteriorization of motor functions on Part III. The fine motor hand movement times of the asymptomatic TCE-exposed group were significantly slower ($p < 0.0001$) than age-matched nonexposed controls. Also, in TCE-exposed individuals, the asymptomatic group's fine motor hand movements were slightly faster ($p < 0.01$) than those of the symptomatic group. One symptomatic worker had been tested 1 year prior and his UPDRS score had progressed from 9 to 23. Exposures are based on self-reported information, and no information on the control group is presented. One of the Parkinson's disease patients predeceased the study and had a family history of Parkinson's disease.

Troster and Ruff (1990) reported a case study conducted on two occupationally exposed workers to TCE. Patients were exposed to low levels of TCE. There were two groups of n = 30 matched controls (all age and education matched) whose results were compared to the performance of the exposed subjects. Exposure was described as "Unknown amount of TCE for 8 months." Assessment consisted of the San Diego Neuropsychological Test Battery (SDNTB) and "1 or more of" Thematoc Apperception Test (TAT), Minnesota Multiphasic Personal Inventory (MMPI), and Rorschach. Medical examinations were conducted, including neurological, CT scan, and/or chemo-pathological tests, and occupational history was taken, but not described. There were no statistical results reported. Results were reported for each test, but

no tests of significance were included; therefore, the authors presented their conclusions for each "case" in qualitative terms, as such: Case 1: Intelligence "deemed" to drop from premorbid function at 1 year and 10 months after exposure. Impaired functions improved for all but reading comprehension, visuospatial learning and categorization (abstraction). Case 2: Mild deficits in motor speed, but symptoms subsided after removal from exposure.

D.1.7. Summary Tables

The following Tables (D-1 through D-3) provide a detailed summary of all of the neurological studies conducted with TCE in humans. Tables D-1 and D-2 summarize each individual human study where there was TCE exposure. Table D-1 consists of studies where humans were primarily or solely exposed to TCE. Table D-2 contains human studies where there was a mixed solvent exposure and TCE was one of the solvents in the mixture. For each study summary, the study population, exposure assessment, methods, statistics, and results are provided. Table D-3 indicates the neurological domains that were tested from selected references (primarily from Table D-1).

Table D-1. Epidemiological studies: Neurological effects of TCE

<table>
<tr><th>Reference</th><th>Study population</th><th>Exposure assessment and biomarkers</th><th>Tests used</th><th>Statistics</th><th>Results</th></tr>
<tr><td>Barret et al. (1984)</td><td>188 workers exposed to TCE occupationally from small and large factories in France (type of factories not disclosed); average age = 41; 6 yrs average exposure time.

The workers were divided into high- and low-exposure groups for both TCE and urinary TCA. No control group was mentioned.</td><td>Review of medical records and analysis of TCE atmospheric levels (detector tubes) and level of urinary metabolites measurement (TCA). TCE exposure groups included high-exposure group (>150 ppm; n = 54) and low-exposure group <150 ppm; n = 134). Personal factors including age, tobacco use, and alcohol intake were also analyzed; Exposure duration = 7 hrs/d for 7 yrs; no mention was made regarding whether or not the examiners were blind to the subjects' exposure status.</td><td>Complete physical examination including testing visual performance (acuity and color perception), evoked trigeminal potential latencies and audiometry, facial sensitivity, reflexes, and motoricity of the masseter muscles.</td><td>X^2 examined distribution of the different groups for comparing high and low exposed workers, one way ANOVA, Mann Whitney U, and t-test for analyzing personal factors.</td><td>Symptoms for which TCE role is statistically significant include the following: trigeminal nerve impairment was reported in 22.2% (n = 12) of workers in the high-exposure group for TCE, 7.4% (n = 10) in the low-exposure group for TCE, 24.4% (n = 10) in the high-exposure group for TCA, and 8.2% (n = 12) in the low-exposure group for TCA.

<table>
<tr><th>TCE results</th><th>High dose%</th><th>Low dose%</th><th>p</th></tr>
<tr><td>Trigeminal nerve</td><td>22.2</td><td>7.4</td><td><0.01</td></tr>
<tr><td>Impairment asthenia</td><td>18.5</td><td>4.5</td><td><0.01</td></tr>
<tr><td>Optic nerve impairment</td><td>14.8</td><td>0.75</td><td><0.001</td></tr>
<tr><td>Headache</td><td>20.3</td><td>19.4</td><td>NS</td></tr>
<tr><td>Dizziness</td><td>13</td><td>4.5</td><td>0.05 < p <0.06</td></tr>
</table>

Symptoms for which TCE role is possible, but not statistically significant = deafness, nystagmus, GI symptoms, morning cough, change in tumor, eczema, palpitations, and conjunctivitis. Symptoms for which there is a synergistic toxic role for TCE and alcohol ($p < 0.05$) = liver impairment and degreaser flush. TSEPs are suggested as a good screening test.</td></tr>
</table>

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Barret et al. (1987)	104 workers highly exposed to TCE during work as degreaser machine operators in France. Controls: 52 healthy, nonexposed controls of various ages who were free from neurological problems.	Urinary analysis determined TCE and TCA rates. The average of the last five measurements were considered indicative of the average level of past exposure. Mean exposure 8.2 yrs, average daily exposure 7 hrs/d. Mean age 41.6 yrs.	Evoked trigeminal potentials were studied while eyes closed and fully relaxed. Also, physical exams with emphasis on nervous system, a clinical study of facial sensitivity, and of the reflexes depending on the trigeminal nerve were systematically performed. Normal latency and amplitude values for TSEP obtained from data from control population. Normal response characterized from four main peaks, alternating from negative to positive, respective latency of 12.8 ms (SD = 0.6), 19.5 ms (SD = 1.3), 27.6 ms (SD = 1.6), and 36.8 ms (SD = 2.2), mean amplitude of response is 2.5 μv (SD = 0.5 μv). Pathological responses were results 2.5 SDs over the normal value.	Student's t-test and one-way ANOVA used as well as nonparametric tests, Mann-Whitney U test, and Kruskal-Wallis test. Also decision matrix and the analysis of the receiver operating curve to appreciate the accuracy of the TSEP method. The distribution of the different populations was compared by a χ^2 test.	Dizziness (71.4%), headache (55.1%), asthenia (46.9%), insomnia (24.4%), mood perturbation (20.4%), and sexual problems (12.2%) were found. Symptomatic patients had significantly longer exposure periods and were older than asymptomatic patients. 17.3% of patients had trigeminal nerve symptoms. Bilateral hypoesthesia with reflex alterations in nine cases. Hypoesthesia was global and predominant in the mandibular and maxillary nerve areas. Several reflex abolitions were found without facial palsy and without convincing hypoesthesia in nine cases. Corneal reflexes were bilaterally abolished in five cases as were naso-palpebral reflexes in six cases; length of exposure positively correlated with functional manifestations ($p < 0.01$); correlation between symptoms and exposure levels was nonsignificant; 40 (38.4%) subjects had pathological response to TSEP with increased latencies, amplitude, or both; of these, 28 had normal clinical trigeminal exam and 12 had abnormal exam. TSEP was positively correlated with length of exposure ($p < 0.01$) and with age ($p < 0.05$), but not with exposure concentration; trigeminal nerve symptoms (n = 18) were positively correlated with older age ($p < 0.001$).

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Barret et al. (1982)	11 workers with chronic TCE exposure; 9 were suffering effects of solvent intoxication; 2 were work place controls. Control group was 20 unexposed subjects of all ages.	Selected following clinical evaluations of their facial sensitivity and trigeminal nerve reflexes; exposures verified by urinalysis. Presence of TCE and TCA found. (Exposure rates not reported.)	Somatosensory evoked potential (SEP) following stimulation of the trigeminal nerve through the lip alternating right and left by a bipolar surface electrode utilizing voltage, usually 75–80 V, just below what is necessary to stimulate the orbicularis oris muscle. Duration was approximately 0.05 ms stimulated 500 times (2×/sec).	SEP recordings illustrated from trigeminal nerve graphs.	Three pathological abnormalities present in exposed (TCE intoxicated) workers: (1) in eight workers, higher voltage required to obtain normal response; (2) excessive delay in response observed twice; and (3) excessive graph amplitude noted in three cases. One subject exhibited all three abnormalities. Correlation was reported between clinical observation and test results. Most severe SEP alternations observed in subjects with the longest exposure to TCE (although exposure levels or exposure durations are not reported). No statistics presented.
Burg et al. (1995)	From an NHIS TCE subregistry of 4,281 (4,041 living and 240 deceased) residents environmentally exposed to TCE via well water in Indiana, Illinois, and Michigan; compared to NHIS registrants.	Morbidity baseline data were examined from the TCE Subregistry from the NER developed by the ATSDR; were interviewed in the NHIS.	Self report via face-to-face interviews—25 questions about health conditions; were compared to data from the entire NHIS population; neurological endpoints were hearing and speech impairments.	Poisson Regression analysis model used for registrants ≥19 years old. Maximum likelihood estimation and likelihood ratio statistics and Wald CI; TCE subregistry population was compared to larger NHIS registry population.	Speech impairments showed statistically significant variability in age-specific risk ratios with increased reporting for children ≤9 yrs old (RR: 2.45, 99% CI: 1.31, 4.58) and for registrants ≥35 yrs old (data broken down by 10-yr ranges). Analyses suggest a statistically significant increase in reported hearing impairments for children ≤9 yrs old (RR: 2.13, 99% CI: 1.12, 4.06). It was lower for children 10–17 yrs old (RR: 1.12, 99% CI: 0.52, 2.44) and ≤0.32 for all other age groups.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Burg and Gist (1999)	4,041 living members of the National Exposure Registry's TCE Subregistry; 97% white; mean age 34 yrs (SD = 19.9 yrs.); divided in four groups based on type and duration of exposure; analysis reported only for 3,915 white registrants; lowest quartile used as control group.	All registrants exposed to TCE though domestic use of contaminated well water; four exposure Subgroups, each divided into quartiles: (1) Maximum TCE measured in well water, exposure subgroups: 2–12, 12–60, and 60–800 ppb; (2) Cumulative TCE exposure subgroups: <50, 50–500, 500–5,000, and >5,000 ppb; (3) Cumulative chemical exposure subgroups: include TCA, DCE, DCA, in conjunction with TCE, with the same exposure categories as in # 2; and (4) Duration of exposure subgroups: <2, 2–5, 5–10, and >10 yrs; 2,867 had TCE exposure of ≤50 ppb; 870 had TCE exposure of 51–500 ppb; 190 had TCE exposure of 501–5,000 ppb; 35 had TCE exposure >5,000 ppb.	Interviews (occupational, environmental, demographic, and health information); a large number of health outcomes were analyzed, including speech and hearing impairment.	Logistic Regression, ORs; lowest quartile used as reference population.	When the registrants were grouped by duration of exposure to TCE, a statistically significant association (adjusted for age and sex) between duration of exposure and reported hearing impairment was found. The prevalence ORs were 2.32 (95% Cl: 1.18, 4.56) (>2–<5 yrs); 1.17 (95% Cl: 0.55, 2.49) (>5–<10 yrs); and 2.46 (95% Cl:1.30, 5.02) (>10 yrs). Higher rates of speech impairment (not statistically significant) were associated with maximum and cumulative TCE exposure, and duration of exposure.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Buxton and Hayward (1967)	This was a case study on four workers exposed to very high concentrations of TCE, which resulted from an industrial accident. No controls were evaluated.	Case 1 was a 44-yr-old man exposed for 10 min; Case 2 was a 39-yr-old man exposed for 30 min; Case 3 was a 43-yr-old man exposed for 2.5 hrs; Case 4 was a 39-yr-old man exposed for 4 hrs. TCE concentrations were not reported.	Clinical evaluations were conducted by a physician when patients presented with symptoms: numbness of face, ocular pain, enlarged right blind spot, nausea, loss of taste, headache, dizziness, unsteadiness, facial diplesia, loss of gag and swallowing reflex, absence of corneal reflex, and reduction of trigeminal response.	There was no statistical assessment of results presented.	Case 1 exhibited headaches and nausea for 48 hrs, but had a full recovery. Case 2 exhibited nausea and numbness of face, but had a full recovery. Case 3 was seen and treated at a hospital with numbness of face, insensitivity to pin prick over the trigeminal distribution, ocular pain, enlarged right blind spot, nausea, and loss of taste. No loss of mental faculty was observed. Case 4 was seen and treated for headache, nausea, dizziness, unsteadiness, facial diplesia, loss of gag and swallowing reflex, facial analgesia, absence of corneal reflex, and reduction of trigeminal response. The patient died and was examined postmortem. There was demyelination of the 5^{th} cranial nerve evident.
Chalupa et al. (1960)	This was a case study conducted on 22 patients with acute poisoning caused by carbon monoxide and industrial solvents. Six subjects were exposed to TCE (doses not known). Average age 38 years.	No exposure data were reported.	Medical and psychological exams were given to all subjects. These included EEGs, measuring middle voltage theta activity of 5–6 sec duration. Subjects were tested for memory disturbances.	No statistics were performed.	80% of those with pathological EEG displayed memory loss; 30% of those with normal EEGs displayed memory loss. Pathology and memory loss were most pronounced in subjects exposed to carbon monoxide.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
El Ghawabi et al. (1973)	30 money printing shop workers occupationally exposed to TCE; Controls: 20 age and SES matched nonexposed males and 10 control workers not exposed to TCE but exposed to inks used in printing.	Air samples on 30 workers. Mean TCE air concentrations ranged from 41 to 163 ppm throughout the Intalgio process Colorimetric determination of both TCA and TTCs in urine with Fujiware reaction.	Inquiries about occupational, past and present medical histories, and family histories in addition to age and smoking habits. EKGs were performed on 25 of the workers. Lab investigations included complete blood and urine analysis, and routine liver function tests.	Descriptive statistics and central tendency evaluation for metabolites; no statistics reported for neurological symptoms.	Most frequent symptoms: prenarcotic headache (86 vs. 30% for controls), dizziness (67 vs. 6.7% for controls), and sleepiness (53 vs. 6% for controls) main presenting symptoms in addition to suppression of libido. Trigeminal nerve involvement was not detected. The concentration of TTCs increased toward mid-week and was stationary during the last 2 working d. Metabolites of total TCA and TCOH are only proportional to TCE concentrations up to 100 ppm.
Feldman et al. (1988)	21 Massachusetts residents with alleged chronic exposure to TCE in drinking water; 27 laboratory controls.	TCE in residential well water was 30–80 times greater than U.S. EPA maximum contaminant level; maximum reported concentration was 267 ppb; other solvents also present.	Blink reflex used as an objective indicator of neurotoxic effects of TCE; clinical neurological exam, EMGs to evaluate blink reflex, nerve conduction studies, and extensive neuropsychological testing.	Student's t-test used for testing the difference between the group means for the blink reflex component latencies.	Highly significant differences in the conduction latency means of the blink reflex components for the TCE exposed population vs. control population, when comparing means for the right and left side R1 to the controls ($p < 0.001$). The mean R1 blink reflex component latency for the exposed group was 11.35 ms, SD = 0.74 ms, 95% CI: 11.03–11.66. The mean for the controls was 10.21 ms, SD = 0.78 ms, 95% CI: 9.92–10.51; $p < 0.001$. Suggests a subclinical alteration of the trigeminal nerve function due to chronic, environmental exposure to TCE.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Feldman et al. (1992)	18 workers occupationally exposed to TCE; 30 laboratory controls.	Reviewed exposure histories of each worker (job type, length of work) and audited medical records to categorize into three exposure categories: "extensive," "occasional," and "chemical other than TCE."	Blink reflexes using TECA 4 EMG.	Non-Gaussian distribution and high coefficient of variance data were log-transformed and then compared to the log-transformed control mean values. MRV was calculated by subtracting the subjects value (x) from the control group mean (M), and the difference is divided by the control group SD.	The "extensive" group revealed latencies >3 SDs above the nonexposed group mean on R1 component of blink reflex; none of the "occasional" group exhibited such latencies; however, two of them demonstrated evidence of demyelinating neuropathy on conduction velocity studies; the sensitivity, or the ability of a positive blink reflex test to correctly identify those who had TCE exposure, was 50%. However, the specificity was 90%, which means that of those workers with no exposure to TCE, 90% demonstrated a normal K1 latency. Subclinical alteration of the Vth cranial nerve due to chronic occupational exposure to TCE is suggested.
Gash et al. (2008)	30 Parkinson's disease patients and 27 non-Parkinson coworkers exposed to TCE; no unexposed controls.	Mapping of work areas.	General physical exam, neurological exam and UPDRS, timed motor tests, and occupational history survey; mitochondrial neurotoxicity; Questionnaire mailed to 134 former non-Parkinson's workers, (14 symptomatic of parkinsonism: age range = 31–66 yrs, duration of employment range: 11–35 yrs) (13 asymptomatic: age range = 46–63 yrs, duration of employment range: 8–33 yrs).	Workers' raw scores given; ANOVA comparing symptomatic vs. nonsymptomatic workers.	Symptomatic non-Parkinson's group was significantly slower in fine motor hand movements than age-matched nonsymptomatic group ($p < 0.001$). All symptomatic workers had positive responses to one or more questions on UPDRS Part I and Part II, and/or had signs of parkinsonism on Part III One symptomatic worker had been tested 1 yr prior and his UPDRS score had progressed from 9 to 23.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Grandjean et al. (1955)	80 workers employed in 10 different factories of the Swiss mechanical engineering industry exposed to TCE, 7 of whom stopped working with TCE from 3 wks to 6 yrs prior; no unexposed control group.	Vapors were collected in ethylic alcohol 95%. Volume of air was checked using a flowmeter, and quantitatively measured according to the method of Truhaut (1951), which is based on a colored reaction between TCE and the pyridine in an alkaline medium (with modifications). Urine analysis of TCA levels; TCE air concentrations varied from 6 to 1,120 ppm depending on time of day and proximity to tanks, but mainly averaged between 20 and 40 ppm. Urinalysis varied from 30 to 300 mg/L; Could not establish a relationship between TCE eliminated through urine and TCE air levels. Four exposure groups estimated based on air sampling data.	Medical exam, including histories; Blood and biochemical tests, and psychiatric exam. Psychological exam; Meggendorf, Bourdon, Rorschach, Jung, Knoepfel's "thirteen mistakes" test, and Bleuler's test.	Coefficient of determination, Regression coefficient.	Men working all day with TCE showed, on average, larger amounts of TCA than those who worked part time with TCE. Relatively high frequency of subjective complaints, alterations of the vegetative nervous system, and neurological and psychiatric symptoms: 34% had slight or moderate psycho-organic syndrome; 28% had neurological changes. There is a relationship between the frequency of those alterations and the degree of exposure to TCE. There were significant differences ($p = 0.05$) in the incidence of neurological disorder between Groups I and III, while between Groups II and III, there were significant differences ($p = 0.05$) in vegetative and neurological disorders. Based on TCA eliminated in the urine, results show that subjective, vegetative, and neurological disorders were more frequent in Groups II and III than in Group I. Statistical analysis revealed the following significant differences ($p < 0.01$): subjective disorders between I and II; vegetative disorders between I and II and between I and III; neurological disorders between I and (II and III). Vegetative, neurological, and psychological symptoms increased with the length of exposure to TCE. The following definite differences were shown by statistical analysis ($p < 0.03$): vegetative disorders between I and IV; neurological disorders between I and II and between I and IV; and psychological disorders between I and III and between I and IV.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Gun, et al. (1978)	Eight exposed: four female workers from one plant exposed to TCE and four female workers from another plant exposed to TCE + nonhalogenated hydrocarbon solvent used in degreasing; control group (n = 8) consisted of four female workers from each plant who did not work near TCE.	Air sampled continuously over separate 10 min durations drawn into a Davis Halide Meter. Readings taken every 30 sec; ranged from 3 to 419 ppm.	Eight-choice reaction times carried out in four sessions; 40 RT trials completed.	Variations in RT by level of exposure; ambient air exposure TCE concentrations and mean air TCE values.	TCE only group had consistently high mean ambient air TCE levels (which exceeded the 1978 TLV of 100 ppm) and showed a mean increase in RT, with a probable cumulative effect. In TCE + solvent group, ambient TCE was lower (did not exceed 100 ppm) and mean RT shortened in Session 2, then rose subsequently to be greater than at the start. Both control groups showed a shortening in mean CRT in Session 2, which was sustained in Sessions 3 and 4 consistent with a practice effect. No statistics were provided.
Hirsch et al. (1996)	106 residents of Roscoe, a community in Illinois on the Rock River, in direct proximity to an industrial plant that released an unknown amount of TCE into the River. All involved in litigation. Case series report; no unexposed controls.	Random testing of the wells between 1983 and 1984 revealed groundwater in wells to have levels of TCE between 0 and 2,441 ppb; distance of residence from well used to estimate exposure level.	Medical, neurologic, and psychiatric exams and histories. For those who complained of headaches, a detailed headache history was taken, and an extensive exam of nerve-threshold measurements of toes, fingers, face, olfactory threshold tests for phenylethyl methylethyl carbinol, brain map, Fast Fourier Transform (FFT), P300 cognitive auditory evoked response, EEG, visual evoked response, Somato sensory Evoked Potential, BAER, MMPI-II, MCMI-II, and Beck Depression Inventory were also given.	Student t-test, χ^2 analysis, nonparametric t-test and ANOVA, correlating all history, physical exam findings, test data, TCE levels in wells, and distance from plant.	66 subjects (62%) complained of headaches, Diagnosis of TCE-induced cephalagia was considered credible for 57 patients (54%). Retrospective TCE level of well water or well's distance from the industrial site analysis did not correlate with the occurrence of possibly-TCE induced headaches. Studies that were not statistically significant with regard to possible TCE-cephalalgia included P300, FFT, VER, BAER, MMPI, MCMI, Beck Depression Inventory, SSER, and nerve threshold measurements. Headache might be associated with exposure to TCE at lower levels than previously reported. Headaches mainly occurred without sex predominance, gradual onset, bifrontal, throbbing, without associated features; No quantitative data were presented to support statement of headache in relation to TCE exposure levels, except for incidences of headache reporting and measured TCE levels in wells.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn and Thornton (1996)	Group A: Randomly selected registered voters from Arizona and Louisiana with no exposure to TCE: n = 264 unexposed volunteers aged 18–83: Group B volunteers from California n = 29 17 males and 12 females to validate the equations; Group C exposed to TCE and other chemicals residentially for ≥5 yrs n = 237.	No exposure or groundwater analyses reported.	Reaction speed using a timed computer visual-stimulus generator; Compared groups to plotted measured SRT and CRT questionnaire to eliminate those exposed to possibly confounding chemicals.	Box-Cox transformation for dependent and independent variables. Evaluated graphical methods to study residual plots. Cooks distance statistic measured influence of outliers examined. Lack-of-fit test performed on Final model and F statistic to compare estimated error to lack-of-fit component of the model's residual sum of squared error. Final models were validated using Group B data and paired t-test to compare observed values for SRT and CRT. F statistic to test hypothesis that parameter estimates obtained with Group B were equal to those of the model.	Group A: SRT = 282 ms, CRT = 532 ms. Group B: SRT = 269 ms, CRT = 531 ms. Group C: SRT = 334 ms, CRT = 619 ms. Lg(SRT) = 5.620, SD = 0.198. Regression equation for Lg(CRT) = 6.094389 + 0.0037964 × age. TCE exposure produced a step increase in SRT and CRT, but no divergent lines. Coefficients from Group A were valid for Group B. Predicted value for SRT and for CRT, plus 1.5 SDs. selected 8% of the model group as abnormal.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn and Warshaw (1993a)	Well water exposed subjects to 6–500 ppb of TCE for 1–25 yrs; 544 recruited test subjects; Group 1 = 196 exposed family members of subjects with cancer or birth defects; Group 2 = 178 from exposed families without cancer or birth defects; Group 3 = 170 exposed parents whose children had birth defects and rheumatic disorders; Controls: 68 referents and 113 histology technicians (HTs) without environmental exposure to TCE.	Well water was measured from 1957 to 1981 by several governmental agencies, and average annual TCE exposures were calculated and then multiplied by each individual's years of residence for 170 subjects.	Neurobehavioral testing—augmented NBT; Eye Closure and Blink using EMG. Neuropsychological (NPS) test—portions of Wechsler's Memory Scale, and WAIS and embedded figures test, grooved pegboard, Trail Making A and B, POMS, and Culture Fair Test. Neurophysio-logical (NPH) testing—simple visual RT, body balance apparatus, cerebellar function, proprioception, visual, associative links and motor effector function.	Two sided student t-test with a $p < 0.05$. Linear regression coefficients to test how demographic variables or other factors may contribute.	Exposed subjects had lower intelligence scores and more mood disorders. NPH: Significant impairments in sway speed with eyes open and closed, blink reflex latency (R-1), eye closure speed, and two choice visual RT. NPS: Significant impairments in Culture Fair (intelligence) scores, recall of stories, visual recall, digit span, block design, recognition of fingertip numbers, grooved pegboard, and Trail Making A and B. POMS: All subtests, but the fatigue, were elevated. Mean speeds of sway were greater with eyes open at $p < 0.0001$) and with eyes closed $p < 0.05$) in the exposed group compared to the combined referents. The exposed group mean SRT was 67 msec longer than the referent group ($p < 0.0001$). CRT of the exposed subjects was 93 msec longer in the third trial ($p < 0.0001$) than referents. It was also longer in all trials, and remained significantly different after age adjustment. Eye closure latency was slower for both eyes in the exposed and significantly different ($p < 0.0014$) on the right compared to the HT referent group.
Kilburn (2002b, a)	236 residents chronically exposed to TCE and associated solvents, including DCE, perchloroethylene, and vinyl chloride, in the environment from a	Exposure estimate based on groundwater plume based on contour mapping; concentrations between 0.2 and 10,000 ppb of TCE over a 64 km^2 area; additional	SRT, CRT, balance sway speed (with eyes open and eyes closed), color errors, blink reflex latency, Supra orbital	Descriptive statistics; ANCOVA; step-wise adjustment of demographics.	The principal comparison, that was between the 236 exposed persons and the 161 unexposed regional controls, revealed 13 significant differences ($p < 0.05$). SRTs and CRTs were delayed. Balance was abnormal with excessive sway speed (eyes closed), but this was not true when both eyes were open.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn (2002b, a)	nearby microchip plant, some involved in litigation, prior to 1983 and those who lived in the area between 1983 and 1993, during which time dumping of chlorinated solvents had supposedly ceased and clean-up activities had been enacted; Controls: 67 referents from northeast Phoenix, who had never resided near the two plants (mean distance = 2,000 m, range = 1,400–3,600 m from plants) and 161 regional referents from Wickenburg, Arizona up-wind of Phoenix, recruited via random calls made to numbers on voter registration rolls, matched to exposed subjects by age and years of education, records showed no current or past water contamination in the areas.	associated solvents, including DCE, perchloroethylene, and vinyl chloride, No air sampling.	tap (left and right), Culture Fair A, Vocabulary, Pegboard, Trail Making A and B, Immediate verbal recall, POMS; pulmonary function. The same examiners who were blinded to the subjects' exposure status examined the Phoenix group, but the Wickenburg referents' status was known to the examiners. Exact order or timing of testing not stated.		Color discrimination errors were increased. Both right and left blink reflex latencies (R-1) were prolonged. Scores on Culture Fair 2A, vocabulary, grooved pegboard (dominant hand), trail making A and B, and verbal recall (i.e., memory) were decreased in the exposed subjects. Litigation is suggested but not stated and study paid by lawyers. Litigation status may introduce a bias, particularly if no validity tests were used.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn (2002b)	236 residents exposed environmentally from a nearby microchip plant (exact number of litigants not stated); 156 individuals exposed for >10 yrs compared to 80 individuals <10 yrs of exposure; Controls: 58 nonclaimants in 3 areas within exposure zone (Zones A, B, and C).	No discussion of exposure assessment methods and results. Solvents included TCE, DCE, perchloroethylene, and vinyl chloride; concluded exposure is primarily due to groundwater plume rather than air releases.	SRT, CRT, Balance sway speed (with eyes open and eyes closed), color errors, blink reflex latency, Supra orbital tap (left and right), Culture Fair A, Vocabulary, Pegboard, Trail Making A and B, immediate verbal recall, POMS.	Descriptive statistics, regression analysis. Similar study to the one reported above with the exception of looking at the effects of duration of residence, proximity to the microchip plant, and being involved in litigation.	Insignificant effects of longer duration of residence. No effect of proximity and litigation. Effects of longer duration of residence modest and insignificant. No effect of proximity. No litigation effect. Zone A: 100 clients were not different from the nine nonclients. Zone B: nonclients were more abnormal in color different than clients and right-sided blink was less abnormal in nonclients. Zone C: 9 of the 13 measurements were not significantly different. 26 of the original 236 subjects re-tested in 1999: maintained impaired levels of functioning and mood. No tests of effort and malingering used, limiting interpretations. Again, no tests of effort and malingering were used, thus limiting interpretation. Litigation is suggested but not stated and study paid by lawyers. Litigation status may introduce a bias, particularly if no validity tests were used.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Landrigan et al. (1987)	13 Pennsylvania residents exposed through drinking and bathing water contaminated by approximately 1,900 gallon TCE spill; February 1980: Nine workers exposed to TCE while degreasing metal in pipe manufacturing plant and nine unexposed controls (mean ages were 42.7 exposed and 46.4-yr old unexposed; mean durations of employment = 4.4 yrs, exposed, and 9.4 yrs, unexposed. May 1980: 10 exposed workers and same 9 unexposed worker controls from February monitoring.	Community evaluation: Nov 1979—questionnaires on TCE and other chemical exposures, and occurrence of signs and symptoms of exposure to TCE, morning urine samples, urine samples analyzed coloreimetrically for TTCs. Occupational evaluations (In workers): breathing-zone air samples(mean 205 mg/m^3; 37 ppm); medical evaluations, pre- and postshift spot urine samples in February and again in May, mid- and postshift venous blood samples during the May survey.	Community evaluation, occupational evaluations; urine evaluations for TCE metabolites; questionnaires to evaluate neurologic effects and symptoms; ISO concentrations; map of TCE in groundwater.	Descriptive statistics	Community evaluation: No urinary TCA detected in community population except for one resident also working at plant and one resident with no exposure. Occupational evaluation: Range 117–357 mg/m^3–(21–64 ppm). February: Airborne exposures exceeded NIOSH limit by up to 222 mg/m^3 (40 ppm) (NIOSH TWA <135 mg/m^3). (24 ppm). Short-term exposure exceeded NIOSH values of 535 mg/m^3 (96 ppm) by up to 1,465 mg/m^3 (264 ppm). Personal breathing zone of other workers within recommended limits (0.5–125 mg/m^3) (0.1–23 ppm). Seven exposed workers reported acute symptoms, including fatigue, light-headedness, sleepiness, nausea, and headache, consistent with TCE exposure; No control workers reported such symptoms; Prevalence of one or more symptoms 78% in exposed worker group, 0% in control worker group. Symptoms decreased after recommendations were in place for 3 months (May testing) for reduced exposures.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Liu et al. (1988)	103 workers from factories in Northern China, exposed to TCE (79 men, 24 women), during vapor degreasing production or operation. The unexposed control group included 85 men and 26 women.	Exposed to TCE, mostly at <50 ppm; concentration of breathing zone air during entire shift measured by diffusive samplers placed on the chest of each worker; divided into three exposure groups; 1–10, 11–50, and 51–100 ppm. Also, hematology, serum biochemistry, sugar, protein, and occult blood in urine were collected.	Self-reported subjective symptom questionnaire.	Prevalence of affirmative answers = total number of affirmative answers divided by (number of respondents × number of questions); X^2.	Dose-response relationship established in symptoms such as nausea, drunken feeling, light-headedness, floating sensation, heavy feeling of the head, forgetfulness, tremors and/or cramps in extremities, body weight loss, changes in perspiration pattern, joint pain, and dry mouth (all ≥3 times more common in exposed workers); "*bloody strawberry jam-like feces*" was borderline significant in the exposed group and "*frequent flatus*" was statistically significant. Exposure ranged up to 100 ppm; however, most workers were exposed <10 ppm, and some at 11–50 ppm. Contrary to expectations, production plant men had significantly higher levels of exposure (24 had levels of 1–10 ppm, 15 had levels of 11–50 ppm, 4 had levels of 51–100 ppm) than degreasing plant men (31 had levels of 1–10 ppm, 2 had levels of 11–50 ppm, 0 had levels of 51–100 ppm); $p < 0.05$ by χ^2 test. No significant difference ($p > 0.10$) was found in women workers. The differences in exposure intensity between men and women was of borderline significance ($0.05 < p < 0.10$).

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
McCunney (1988)	This is a case study conducted on three young white male workers exposed to TCE in degreasing operations. There were no controls included. Case 1 was a 25-yr-old male, Case 2 was a 28-yr-old white male, Case 3 was a 45-yr-old white male.	Case 1: TCE in air at the work place was measured at 25 ppm, but his TCA in urine was measured at 210 mg/L. This is likely due to dermal exposure while cleaning metal rods in TCE. Case 2: no TCE exposure data presented, TCA at 9 mg/L after 6 months; Case 3: no TCE exposure data presented.	Clinical evaluation of loss of balance, light headedness, resting tremor, blurred vision, and dysdiadochokinesia, change in demeanor and loss of coordination, cognitive changes were noted, as well as depression; CT scan, EEG, nerve conductivity, and visual and somatosensory evoked response. Neurological exams included sensitivity to pinprick over the face. Ophthalmic evaluation.	There were no statistical analyses of results presented.	Case 1 was a 25-yr-old male, who presented with a loss of balance, light headedness, resting tremor, blurred vision, and dysdiadochokinesia. The subject had been in a car accident and suffered head injuries. He later returned with a change in demeanor and loss of coordination. He showed a normal CT scan, EEG, nerve conductivity, and visual and somatosensory evoked response. Neurological exams revealed reduced sensitivity to pinprick over the face, deep tendon reflexes were reduced, and mild to moderate cognitive changes were noted, as well as depression. Ophthalmic evaluation was normal. He was removed from the TCE exposure and appeared to recover. Case 2 was a 28-yr-old white male who presented with numbness and shooting pains in fingers. He exhibited anorexia, and tiredness. He worked in a degreasing operation for a jeweler using open containers filled with TCE in a small, unventilated room. There were no exposure data provided, but his TCA was 9 mg/L at 6 months after exposure. He had been hospitalized with hepatitis previously. No neurological tests were administered. Case 3 was a 45-yr-old white male who presented with numbness in hands and an inability to sleep. He exhibited slurred speech. He was positive for blood in stool, but had a history of duodenal ulcers.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Mhiri et al. (2004)	23 phosphate industry workers exposed to TCE for 6 hrs/d for at least 2 yrs while cleaning walls to be painted; controls: 23 unexposed workers from the department of neurology.	Measurement of urinary metabolites of TCE were performed 3 times/ worker. Blood tests and hepatic enzymes were also collected.	TSEPs recorded using Nihon-Kohden EMG-evoked potential system; baseline clinical evaluations regarding facial burn or numbness, visual disturbances, restlessness, concentration difficulty, fatigue, mood changes, assessment of cranial nerves, quality of life; biological tests described under biomarkers.	Paired or unpaired Student's t-test as appropriate. *p*-value set at <0.05. Spearman rank-correlation procedure was used for correlation analysis.	Abnormal TSEP were observed in six6 workers with clinical evidence of Trigeminal involvement and in nine asymptomatic workers. A significant positive correlation between duration of exposure and the N2 latency ($p < 0.01$) and P2 latency ($p < 0.02$) was observed. Only one subject had urinary TCE metabolite levels over tolerated limits. TCE air contents were over tolerated levels, ranging from 50 to 150 ppm.
Mitchell and Parsons-Smith (1969)	This was a case study of one male patient, age 33 yrs, occupational exposed to TCE during degreasing. There were no controls.	No exposure data were presented.	Trigeminal nerve, loss of taste, X-rays of the skull, EEG, hemoglobin, and Wassermann reaction.	No statistics provided.	The patient had complete analgesia in the right trigeminal nerve and complete loss of taste; patient complained of loss of sensation on right side of face and uncomfortable right eye, as well as vertigo and depression. X-rays of the skull, EEG, hemoglobin, and Wassermann reaction were all normal.
Nagaya et al. (1990)	84 male workers ages 18–61 yrs (mean 36.2 yrs) constantly using TCE in their jobs. Duration of employment (i.e., exposure) 0.1–34.0 yrs, (mean 6.1 yrs; SD = 5.9). Controls: 83 age-matched office workers and students with no exposure.	Workers exposed to about 22-ppm TCE in air. Serum dopamine-β-hydroxylase (DBH) activity levels measured from blood. U-TTCs also measured.	Blood drawn during working time and DBH activities were analyzed; spot urine collected at time of blood sampling and U-TTC determined by alkaline-pyridine method.	Student's t-test and linear correlation coefficient. Results of U-TTC presented by age groups: ≤25; 26–40; and ≥41 yrs.	A slight decrease in serum DBH activity with age was noted in both groups. Significant inverse correlation of DBH activity and age was found in workers ($r = -0.278$, $0.01 < p < 0.02$), but not in controls ($r = -0.182$, $0.05 < p < 0.1$). No significant differences between mean serum DBH activity levels by age groups for workers and corresponding controls in any age group. Workers' U-TTC levels: 3.8–1,066.4 mg/L (M = 133.6 mg/L); U-TTC not detected in controls. Serum DBH activity levels in workers independent of U-TTC levels and duration of employment. Results suggest that chronic occupational exposure to TCE did not influence sympathetic nerve activity.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Reif et al. (2003)	143 residents of the Rocky Mountain Arsenal community of Denver whose water was contaminated with TCE and related chemicals from nearby hazardous waste sites between 1981 and 1986; referent group at lowest concentration (<5 ppb).	Hydraulic simulation model used in conjunction with a GIS estimated residential exposures to TCE; approximately 80% of the sample exposed to TCE exceeding maximum contaminant level of 5 ppb and approximately 14% exceeded 15 ppb. High exposure group >15 ppb, low-exposure referent group <5 ppb, medium exposure group $5 < x < 15$ ppb.	NCTB, tests of visual contrast sensitivity, POMS.	Multivariate model.	Statistical significance was approached as a result of high TCE exposure vs. referent group; poorer performance on the digit symbol ($p = 0.07$), contrast sensitivity C test ($p = 0.06$), and contrast sensitivity D test ($p = 0.07$), and higher mean scores for depression ($p = 0.08$). Alcohol was an effect modifier in high-exposed individuals—statistically significant on the Benton, digit symbol, digit span, and SRT tests, as well as for confusion, depression, and tension.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen and Sabroe (1986)	368 metal workers working in degreasing at various factories in Denmark (industries not specified) with chlorinated solvents; 94 controls randomly selected semiskilled metal workers from same area; mean age: 37.7 yrs (range: 17–65+ yrs). Total 443 men; 19 women.	Questionnaire: categorized in four groups; three exposure groups plus control: (1) currently working with chlorinated solvents (n = 171; average. duration: 7.3 yrs, 16.5 hrs/wk; 57% TCE and 37% 1,1,1-trichloroethane); (2) currently working with other solvents (n = 131; petroleum, gasoline, toluene, xylene); (3) previously (1–5 yrs) worked with chlorinated or other solvents (n = 66); and (4) never worked with organic solvents (n = 94).	Questionnaire: 74 items about neuropsychological symptoms (memory, concentration, irritability, alcohol intolerance, sleep disturbance, fatigue).	χ^2; ORs; t-test; logistic regression.	Neuropsychological symptoms significantly more prevalent in the chlorinated solvents-exposed group; TCE caused the most "inconveniences and symptoms;" dose-response between exposure to chlorinated solvents and chronic neuropsychological symptoms (memory [$p < 0.001$], concentration [$p < 0.02$], irritability [$p < 0.004$], alcohol intolerance [$p < 0.004$], forgetfulness [$p < 0.001$], dizziness [$p < 0.005$], and headache [$p < 0.01$]). Significant associations between previous exposure and consumption of alcohol with chronic neuropsychological symptoms.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen et al. (1993d)	96 Danish workers involved in metal degreasing with chlorinated solvents, mostly TCE (n = 70); (industries not specified), age range: 19–68 yrs; no external controls.	Chronic exposure to TCE (n = 70); CFC (n = 25); HC (n = 1); average duration: 7.1 yrs; range of full-time degreasing: 1 month to 36 yrs; occupational history, blood and urinary metabolites (TCA); biological monitoring for TCE and TCE metabolites; CEI calculated based on number of hrs/wk worked with solvents × yr of exposure × 45 wks per yr; three groups: (1) low exposure: n = 19, average full-time exposure 0.5 yr; (2) medium exposure: n = 36, average full-time exposure 2.1 yrs; and (3) high exposure: n = 41, average full-time exposure 11 yrs. Mean TCA in high exposure group = 7.7 mg/L (maximum = 26.1 mg/L); TWA measurements of CFC113 levels: 260–420 ppm (U.S. and Danish TLV is 500 ppm).	Medical interview, neurological exam, neuropsychological exam. Tests: WAIS: Vocabulary, Digit Symbol; SRT, acoustic-motor function, discriminatory attention, Sentence Repetition, Paced Auditory Serial Addition Test, Text Repetition, Rey's Auditory Verbal Learning, visual gestalt, Stone Pictures (developed for this study, nonvalidated), revised Santa Ana, Luria motor function, Mira; Blind study.	Fisher's exact test, χ^2 trend test, t-test, ANOVA, logistic regression, ORs, χ^2 goodness-of-fit test. Confounders examined: age, primary intellectual level, arteriosclerosis, neurological/ psychiatric disease, alcohol abuse, and present solvent exposure.	After adjusting for confounders, the high-exposure group had significantly increased risk for psychoorganic syndrome following exposure (OR: 11.2); OR for medium exposed group = 5.6; Significant increase in risk with age and with decrease in WAIS Vocabulary scores. Prevalence of psychoorganic syndrome: 10.5% in low-exposure group, 38.9 in medium exposure group, 63.4% in high-exposure group; no significant interaction between age and solvent exposure.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen et al. (1993c)	96 Danish workers involved in metal degreasing with chlorinated solvents (industries not specified), age range: 19–68 yrs; no external controls.	Chronic exposure to TCE (n = 70); CFC (n = 25); HC (n = 1); average duration: 7.1 yrs); range of full-time degreasing: 1 month to 36 yrs; occupational history, blood and urinary metabolites (TCA); biological monitoring for TCE and TCE metabolites; CEI calculated based on number of hrs/wk worked with solvents × yr of exposure × 45 wks per yr; three groups: (1) low exposure: n = 19, average full-time exposure 0.5 yr; (2) medium exposure: n = 36, average full-time exposure 2.1 yrs; and (3) high exposure: n = 41, average full-time exposure 11 yrs. Mean TCA in high-exposure group = 7.7 mg/L (maximum = 26.1 mg/L); TWA measurements of CFC113 levels: 260–420 ppm (U.S. and Danish TLV is 500 ppm).	WAIS (original version): Vocabulary, Digit Symbol, Digit Span; SRT, Acoustic-motor function (Luria), Discriminatory attention (Luria), Sentence Repetition, PASAT, Text Repetition, Rey's Auditory Verbal Learning, Visual Gestalts, Stone Pictures (developed for this study, nonvalidated), revised Santa Ana, Luria motor function, Mira; Blind study.	Linear regression analysis; Confounding variables analyzed: age, primary intellectual function, word blindness, education, arteriosclerosis, neurological/ psychiatric disease, alcohol use, present solvent exposure.	Dose response with 9 of 15 tests; controlling for confounds, significant relationship of exposure was found with Acoustic-motor function ($p < 0.001$), PASAT ($p < 0.001$), Rey AVLT ($p < 0.001$), vocabulary ($p < 0.001$), and visual gestalts ($p < 0.001$); significant age effects.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen et al. (1993a)	96 Danish workers involved in metal degreasing with chlorinated solvents (industries not specified), age range: 19–68 yrs; no external controls.	Chronic exposure to TCE (n = 70); CFC (n = 25); HC (n = 1); average duration: 7.1 yrs); range of full-time degreasing: 1 month to 36 yrs; occupational history, blood and urinary metabolites; biological monitoring for TCE and TCE metabolites; CEI calculated based on number of hrs/wk worked with solvents × yr of exposure × 45 wks per yr; 3 groups: (1) low exposure: n = 19, average full-time exposure 0.5 yr; (2) medium exposure: n = 36, average full-time exposure 2.1 yrs; and (3) high exposure: n = 41, average full-time exposure 11 yrs. Mean TCA in high-exposure group = 7.7 mg/L (maximum = 26.1 mg/L); TWA measurements of CFC113 levels: 260–420 ppm (U.S. and Danish TLV is 500 ppm).	Medical interview, clinical neurological exam, neuropsychological exam.	Multiple regression; Fisher's exact test; Mantel-Haenzel test for linear association.	Significant dose response between exposure and motor dyscoordination remained after controlling for confounders; bivariate analysis showed increased vibration threshold with increased exposure, but with multivariate analysis, age was a significant factor for the increase.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Ruijten et al. (1991)	31 male printing workers exposed to TCE. Mean age 44 yrs; Mean duration 16 yrs; Controls: 28; mean age 45 yrs.	Relied on exposure data from past monitoring activities conducted by plant personnel using gas detection tubes. Estimated 17 ppm for past 3 yrs, 35 ppm for preceding 8 yrs, and 70 ppm before that. Individual cumulative exposure was calculated as time spent in different exposure periods and the estimated exposure in those periods. Mean cumulative exposure = 704 ppm × yrs (SD 583, range: 160–2,150 ppm × yrs.	General questionnaire, cardiotachogram recorded on ink writer to measure autonomic nerve function, including forced respiratory sinus arrhythmia (FRSA), muscle heart reflex (MHR), resting arrhythmia. Trigeminal nerve function measured using masseter reflex and blink reflex; electrophysiological testing of peripheral nerve functioning using motor nerve conduction velocity of the peroneal nerve.	Combined Z score = individual Z scores of the FRSA and MHR; ANCOVA to calculate difference between exposed/ nonexposed workers. Cumulative exposure effect calculated by multiple linear regression analysis. Controlled for age, alcohol consumption, and nationality by including them as covariables. Quetelet-index included for autonomic nerve parameters; Body length and skin temperature used for all peripheral nerve functions; one-sided significance level of 5% used. Non-normal distributions were log or square root transformed.	Slight reduction in Sural nerve conduction velocity was found and a prolongation of the Sural refractory period. Latency of the masseter reflex had increased. No prolongation of the blink reflex was found; no impairment of autonomic or motor nerve function were found. Long-term exposure to TCE at threshold limit values (approximately 35 ppm) may slightly affect the trigeminal and sural nerves.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Smith (1970)	130 (108 males, 22 females); controls: 63 unexposed men working at the same factory matched by age, marital status.	TCA metabolite levels in urine were measured: 60.8% had levels up to 20 mg/L, and 82.1% had levels up to 60 mg/L.	Cornell Medical Index Questionnaire (Psychiatric section), Heron's Personality Questionnaire, Fluency Test, 13-Mistake Test, Serial Sevens, Digit Span, General Knowledge Test, tests of memory.	Descriptive statistics.	Of the 130 subjects exposed, 27% had no complaints of symptoms, 74.5% experienced fatigue, 56.2% dizziness, 17.7% headache, 25.4% GI problems, 7.7% autonomic effects, and 24.9% had other symptoms. The number of complaints reported by subjects were statistically significant between those with ≤20 mg/L TCA (M = 1.8 complaints) and those ≥60 mg/L (M = 2.7 complaints). Each group, however, had a similar proportion of subjects who reported having only 'slight' symptoms. The total time of continuous exposure to TCE (ranging from <1 yr to >10 yrs) appeared to have little influence on frequency of symptoms. No results of the tests were reported. Author postulates that symptom assessment raises the possibility of "errors of subjective judgment."
Triebig et al. (1977c)	This study was conducted on eight subjects occupationally exposed to TCE. Subjects were seven men and one woman with an age range of 23–38 yrs. There was no control group.	Measured TCE in air averaged 50 ppm (260 mg/m^3). Length of occupational exposure was not reported.	Results were compared after exposure periods, and compared to results obtained after periods removed from exposure. TCA and TCE metabolites in urine and blood were measured. Psychological tests included d2, MWT-A, and short test.	Wilcoxon and Willcox nonparametric tests. Due to the small sample size, a significance level of 1% was used.	Mean values observed were 330-mg TCOH and 319-mg TCA/g creatinine, respectively, at the end of a work shift. The psychological tests showed no statistically significant difference in the results before or after the exposure-free time period.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig (1982)	This study was conducted on 24 healthy workers (20 males, 4 females) exposed to TCE occupationally at three different plants. The ages were 17–56 yrs; length of exposure ranged from 1 to 258 months (mean 83 months). A control group of 144 controls used to establish 'normal' responses on the nerve conduction studies. The matched control group consisted of 24 healthy nonexposed individuals (20 males, 4 females), chosen to match the subjects for age and sex.	Length of exposure ranged from 1 to 258 months (mean 83 months). TCE concentrations measured in air at work places ranged from 5 to 70 ppm. TCA, TCE, and TCOH were measured in blood, and TCE and TCA were measured in urine.	Nerve conduction velocities were measured for sensory and motor nerve fibers using the following tests: MCV_{MAX}(U): Maximum NLG of the motor fibers of the N. ulnaris between the wrist joint and the elbow; dSCV (U), pSCV (U), and dSCV (M).	Data were analyzed using parametric and nonparametric tests, rank correlation, and linear regression, with 5% error probability.	Results show no statistically significant difference in nerve conduction velocities between the exposed and unexposed groups. This study has measured exposure data, but exposures/responses were not reported by dose levels.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig (1983)	The exposed group consists of 66 healthy workers selected from a population of 112 workers. Workers were excluded based on polyneuropathy (n = 46) and alcohol consumption (n = 28). The control group consisted of 66 healthy workers with no exposures to solvents.	Subjects were exposed to a mixture of solvents, including TCE, specifically "ethanol, ethyl acetate, aliphatic hydrocarbons (gasoline), MEK, toluene, and trichloroethene." Subjects were divided into three exposure groups based on length of exposure, as follows: 20 employees with "short-term exposure" (7–24 months); 24 employees with "medium-term exposure" (25–60 months); and 22 employees with "long-term exposure" (over 60 months). TCA, TCE, and TCOH were measured in blood, and TCE and TCA were measured in urine.	Nerve conduction velocities were measured for sensory and motor nerve fibers using the following tests: MCV_{MAX}(U): Maximum NLG of the motor fibers of the N. ulnaris between the wrist joint and the elbow; dSCV (U), pSCV (U), and dSCV (M).	Data were analyzed using parametric and nonparametric tests, rank correlation, and linear regression, with 5% error probability.	There was a dose-response relationship observed between length of exposure to mixed solvents and statistically significant reduction in nerve conduction velocities observed for the medium and long-term exposure groups for the NCV.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Troster and Ruff (1990)	Three occupationally exposed workers to TCE or TCA: two patients acutely exposed to low levels of TCE and one patient exposed to TCA; Controls: two groups of n = 30 matched controls (all age and education matched).	"Unknown amount of TCE for 8 months."	SDNTB, "1 or more of:" TAT, MMPI, Rorschach, and interviewing questionnaire, medical examinations (including neurological, CT scan, and/or chemo-pathological tests and occupational history).	Not reported.	Case 1: Intelligence "deemed" to drop from premorbid function at 1 yr and 10 mo after exposure. Impaired functions improved for all but reading comprehension, visuospatial learning, and categorization (abstraction). Case 2: Mild deficits in motor speed, verbal learning, and memory; "marked" deficits in visuospatial learning; good attention; diagnosis of mild depression and adjustment disorder, but symptoms subsided after removal from exposure. Case 3: Manual dexterity and logical thinking borderline impaired; no emotional changes, cognitive function spared, diagnosis of somatoform disorder.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
White et al. (1997)	Group 1: 28 individuals in Massachusetts exposed to contaminated well water; source: tanning factory and chemical plant; age range: 9–55 yrs. Group 2: 12 individuals in Ohio exposed to contaminated well water; source: degreasing; age range: 12–68 yrs. Group 3: 20 individuals in Minnesota exposed to contaminated well water; n = 14 for nerve conduction studies and n = 6 for neuropsychological testing; source: ammunition plant; age range: 8–62 yrs. No controls.	Group 1: two wells tested in 1979: 267 ppb TCE, 21 ppb tetrachloroethylene, 12 ppb chloroform, 29 ppb dichloro-ethylene, 23 ppb trichlorotrifluoro-ethane; 2 yrs average TCE 256 ppb for well G, and 111 ppb for well H. Group 2: 13 wells with 1,1,1-trichloroethane (up to 2,569 ppb) and TCE (up to 760 ppb); blood analysis of individuals 2 yrs after end of exposure and soon after exposure showed normal or mild elevations of TCE, elevations of 1,1,1-trichloroethane, ethylbenzene, and xylenes. Group 3: mean TCE for one well 261 ppb; 1,1-DCE 9.0 ppb; and 1,2-DCE 107 ppb.	Occupational and environmental questionnaire, neurological exam, neuropsychological exam: WAIS-R, WISC-R, WMS, WMS-R, Wisconsin Card Sorting, COWAT, Boston Naming, Boston Visuospatial Quantitative Battery, Milner Facial Recognition Test, Sticks Visuospatial Orientation Task, Word triads, Benton Visual Retention Test, Santa Ana, Albert's Famous Faces, Peabody Picture Vocabulary Test, WRAT, POMS, MMPI, Trail-making, Fingertapping, Delayed Recognition Span Test; Neurophysiological exam: eyeblink, evoked potentials, nerve conduction; and other: EKG, EEG, medical tests.	Data shown in proportion in three communities, clinical diagnostic categories, analysis of central tendencies, and descriptive statistics.	Group 1: Some individuals with subclinical peripheral neuropathy; 92.8% with reflex abnormalities; 75% total diagnosed with peripheral neuropathy; 88.9% with impairment in at least one memory test. Impairments: attention and executive function in 67.9%; motor function in 60.71%, visuospatial in 60.71%, and mild to moderate encephalopathy in 85.7%. Group 2: 25% with abnormal nerve conduction, Impairments: attention and executive function in 83.33%, memory in 58.33%, and language/verbal in 50%. Group 3: 35.7% with peripheral neuropathy; neuropsychological: all six tested had memory impairment, attention and executive function impairment, three had manual motor slowing. Participants younger at time of exposure with wider range of deficits. Language deficits in younger, but not in older, participants.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Winneke (1982)	This is a review article presenting multiple studies that evaluated neurological effects of TCE, and other solvents. Only the TCE results are summarized herein. Experiment 1: 18 subjects (results taken from Schlipköter et al. [(1974)] and summary is based on information from Winneke (1982)]) Experiment 2: 12 subjects (results taken from Winneke et al. (1978; 1976)] and summary is based on information from Winneke (1982)])	Experiment 1: Subjects were exposed to 50 ppm TCE for 3.5 hrs. Experiment 2: Comparative study of effects from (a) 50 ppm TCE for 3.5 hrs and (b) 0.76 mL/kg ethanol.	For both experiments 1 and 2: critical flicker fusion, sustained attention task, auditory evoked potentials	No statistical details were reported.	Significant decrease ($p < 0.05$) in auditory evoked potentials in individuals (experiments 1 and 2) exposed to 50 ppm TCE. No significant effects were noted in the critical flicker fusion or the sustained attention tasks.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
ATSDR (2002)	116 children from registry of 14 hazardous waste sites with TCE in groundwater; under 10 yrs of age at time of registry; control population (n = 177); communities with no evidence of TCE in groundwater (measured below maximum contaminant level); matched by age and race; there were other chlorinated solvents present in the exposed group wells.	Exposures were modeled using tap water TCE concentrations and GIS for spatial interpolation, and LaGrange for temporal interpolation to estimate exposures from gestation to 1990 across the area of subject residences, modeled data were used to estimate lifetime exposures (ppb-yrs) to TCE in residential wells; three exposure level groups; control = 0 ppb; low exposure-group = 0 <23 ppb-yrs; and high-exposure group = >23 ppb-yrs; confounding exposure was a concern.	Fisher Logemann test; OSME-R; CSP; D-COME-T; hearing screening; DPOAE; SCAN.	Screening results as binary variables using logistic regression within SAS; independent variables included exposure measures, age, gender, case history; χ^2 test, Fisher's exact test, t-tests, linear models.	Exposed children had higher abnormalities for D-COME-T ($p < 0.002$), CSP ($p < 0.008$), velopharyngeal function ($p < 0.04$), high palatal arch ($p < 0.04$), and abnormal outer ear cochlear function. No difference observed in exposed and nonexposed populations for speech or hearing function. No difference found in OSH function.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Epidemiological studies: controlled exposure studies; neurological effects of trichloroethylene					
Gamberale et al. (1976)	15 healthy men aged 20–31-yr old employed by the Department of Occupational Medicine in Stockholm, Sweden; Controls: Within Subjects (15 self-controls).	Exposed for TCE 70 min via a breathing valve to 540 mg/m^3 (97 ppm), 1,080 mg/m^3 (194 ppm), and during ordinary atmospheric air. Sequence was counterbalanced between the three groups, days, and exposure levels. Concentration was measured with a gas chromatographic technique every third min for the 1st 50 min, then between tests thereafter.	RT addition, SRT, CRT and short-term memory using an electronic panel. Subjects also assessed their own conditions on a 7-point scale.	Friedman two-way analysis by ranks to evaluate difference between three conditions, nonsignificant when tested individually, but significant when tested on the basis of six variables. Nearly half of the subjects could distinguish exposure/nonexposure. ANOVA for four performance tests based on a 3 × 3 Latin square design with repeated measures.	In the RT-addition test, the level of performance varied significantly between the different exposure conditions ($F[2.24] = 4.35$; $p < 0.051$) and between successive measurement occasions ($tF[2.24] = 19.25$; $p < 0.001$). The level of performance declined with increased exposure to TCE, whereas repetition of the testing led to a pronounced improvement in performance as a result of the training effect. No significant interaction effects between exposure to TCE and training.
Konietzko et al. (1975)	This is a controlled exposure study conducted on 20 healthy male students and scientific assistants with a mean age of 27.2 yrs.	Subjects were exposed to a constant TCE concentration of 95.3 ppm (520 mg/m^3) for up to 12 hrs, and blood concentrations of TCE were also analyzed at hourly intervals.	Evaluated for changes in alpha waves (<14 Hz) in the EEG recordings; EEG recordings were performed hourly for a period of 1 min with the eyes closed. This was used as a potential measure of psychomotor disturbance.		The alpha segment increased over time of exposure (from 0800 to 0900 and 1,000 hrs [military time]) ($p = 0.05$). There were no significant differences for the other time spans or for other parameters. Subjects with highest and lowest TCE blood levels, <2 and >5 µg/mL, were compared to determine if they showed different responses, but in no case were the differences statistically different.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kylin et al. (1967)	12 subjects exposed to 1,000 ppm TCE for 2 hrs in a 1.5 × 2 × 2 meters chamber; 2 subjects were given alcohol (0.7 gm of body weight); controls: 7 of the 12 were tested some days prior to exposure and 5 of the 12 were tested some days after exposure.	1,000 ppm of TCE was blown into a chamber via an infusion unit and vaporizing system. Ostwald's distribution factor for TCE—the quotient of the amount of solvent in the blood by the amount of alveolar air.	Optokinetic nystagmus; venus blood and alveolar air specimens were taken at various times after exposure and analyzed in a gas chromatograph with a flame ionization detector.	Ostwald's distribution factor for TCE (the quotient of the amount of solvent in the blood in mg/L by the amount of the alveolar air in mg/L) = 9.7; significant relationship between TCE in air and blood (0.88).	"A number" of subjects showed reduction in Fusion limit although more pronounced in the two subjects who consumed alcohol. "Others," however, showed little if any effect. No statistics.
Salvini et al. (1971)	This is a controlled exposure study conducted on six male university students. Each subject was examined on 2 different d, once under TCE exposure, and once as self controls, with no exposure.	TCE concentration was 110 ppm for 4-hr intervals, twice per day. 0-ppm control exposure for all as self controls.	Two sets of tests were performed for each subject corresponding to exposure and control conditions. Perception test with tachistoscopic presentation, Wechsler memory scale, CRT test, and manual dexterity test.	ANOVA	A decrease in function for all measured effects was observed. Statistically significant results were observed for perception tests learning ($p < 0.001$), mental fatigue ($p < 0.01$), subjects ($p < 0.05$); and CRT learning ($p < 0.01$), mental fatigue ($p < 0.01$), subjects ($p < 0.05$).

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Stewart et al. (1970)	13 subjects in 10 experiments	Ten chamber exposures to TCE vapor (100 ppm and 200 ppm) for periods of 1 h to a 5-d work week. Experiments 1–7 were for a duration of 7 hrs with a mean TCE concentration of 198–200 ppm. Experiments 8 and 9 exposed subjects to 202 ppm TCE for a duration of 3.5 and 1 hr, respectively. Experiment 10 exposed subjects to 100 ppm TCE for 4 hrs. Experiments 2–6 were carried out with the same subjects over 5 consecutive d; gas chromatography of expired air; no self controls.	Physical examination 1 hr prior to exposure. Blood analysis for complete blood cell count, sedimentation rate, total serum lipid, total serum protein, serum electrophoresis, serum glutamic oxaloacetic transaminase, and serum glutamic pyruvic transaminase. 24-hr urine collection for urobilinogen, TCA and TCE. Also a preexposure expirogram, tidal volume measurement, and an alveolar breath sample for TCE; short neurological exam including modified Romberg test, heel-to-toe test, finger-to-nose test.	Descriptive statistics.	Ability to perceive TCE odor diminished as duration of expo increased; 40% had dry throat after 30-min exposure; 20% reported eye irritation. Urine specimens showed progressive increase in amounts of TCE metabolites over the five consecutive exposures. Concentrations of TCA and TCE decreased exponentially after last exposure, but were still present in abnormal amounts in urine specimens 12 d after exposure. Loss of smelling TCE: >1 hr = 33%; >2 hrs = 80%; >6.5 hrs = 100%. Symptoms of lightheadedness, headache, and eye, nose, and throat irritation. Prominent fatigue and sleepiness by all after 200 ppm. These symptoms may be of clinical significance. All had normal neurological tests during exposure, but 50% reported greater mental effort was required to perform a normal modified Romberg test on more than one occasion.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig (1976)	This was a controlled exposure study conducted on seven healthy male and female students (four females, three males). The control group was seven healthy students (four females, three males).	Subjects exposed for 6 hrs/d for 5 d to 100 ppm (550 mg/m^3 TCE). Controls were exposed in chamber to zero TCE. Biochemical tests included TCE, TCA, and TCOH in blood. In this study, the TCE concentrations in blood reported ranged from 4 to 14 µg/mL. A range of 20–60 µg/mL was obtained for TCA in the blood.	Psychological tests were: the d2 test was an attention load test; the short test is used to record patient performance with respect to memory and attention; daily Fluctuation Questionnaire measured the difference between mental states at the start of exposure and after the end of exposure is recorded; the MWT-A is a repeatable short intelligence test; the Freiburg Personality Inventory is a test for 12 independent personality traits; CFT-3 is a nonverbal intelligence test; Erlanger Depression Scale.	Regression analyses were conducted.	There was no correlation seen between exposed and unexposed subjects for any measured psychological test results. The biochemical data did demonstrate that exposed subjects' exposures.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig et al. (1977a)	This was a controlled exposure study conducted on seven healthy male and female students (four females, three males) The control group was seven healthy students (four females, three males).	Subjects exposed for 6 hrs/d for 5 d to 100 ppm (550 mg/m^3 TCE). Controls were exposed in chamber to zero TCE. Biochemical tests included TCE, TCA, and TCOH in blood. In this study, the TCE concentrations in blood reported ranged from 4 to 14 µg/mL. A range of 20–60 µg/mL was obtained for TCA in the blood.	The testing consisted of: the Syndrome Short Test; the "Attention Load Test" or "d2 Test;" Number recall test, letter recall test, The "Letter Reading Test," "Word Reading Test," Erlanger Depression Scale. Scale for Autonomic Dysfunction, Anxiety Scale, Pain Short Scale, and Information on Daily Fluctuations.	Statistics were conducted using Whitney Mann.	Results indicated the anxiety values of the placebo random sample group dropped significantly more during the course of testing ($p < 0.05$) than those of the active random sample group. No significantly different changes were obtained with any of the other variables.
Vernon and Ferguson (1969)	Eight male volunteers age range 21–30; self controls: 0 dose.	TCE administered as Trilene air-vapor mixtures through spirometers administered at random concentrations of 0, 100, 300, or 1,000 ppm of TCE for 2 hrs at a time, during which testing took place. Concentrations were measured with a halide meter. Medical history, exam including CBC, urinalysis, BUN, and SGOT.	Flicker Fusion with Krasno-Ivy Flicker Photometer, Howard-Dolman depth perception apparatus, Muller-Lyer two-dimensional illusion, groove-type steadiness test, Purdue Pegboard, Written "code substitution," blood studies.	ANOVAs, Dunnett's test.	TCE did not produce any appreciable effects at lower concentrations. Compared to controls, participants exposed to 1,000 ppm of TCE had adverse effects on the Howard-Dolman, steadiness, and part of the pegboard, but no effects on Flicker Fusion, from perception or code substitution. No appreciable changes in CBC, urinalysis, SGOT, or BUN.

Table D-1. Epidemiological studies: neurological effects of TCE (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Windemuller and Ettema (1978)	Pilot study: 24 healthy male volunteers; age range = 19–26 yr, four groups with six volunteers in each: (1) control; (2) exposed to TCE; (3) exposed to alcohol; and (4) exposed to TCE and alcohol; final study: 15 other volunteers, each exposed to all four conditions.	Chamber study; Group 1 no exposure; Group 2 TCE exposure: 2.5 hrs with 200 ppm; Group 3 alcohol exposure: 0.35 g/kg body weight; Group 4 TCE and alcohol: same as above levels. Blood alcohol levels taken with breathalyzer; exhaled air sampled for levels of TCE and TCOH; TCE exposure: average measured TCE in exhaled air = 29 µg/L (SD = 3); TCE and alcohol exposure average measured TCE in exhaled air = 63 µg/L (SD = 12).	Binary Choice Task (Visual); Pursuit Rotor; Recording of heart rate, sinus arrhythmia, breathing rate; Questionnaire (15 items on subjective feelings).	K-sample trend test; two-tailed Wilcoxon test.	Pilot study: no systematic effect of exposure on test perform. Alcohol group had higher heart rate than TCE group, and TCE and alcohol group; minimal effect of mental load on heart rate; sinus arrhythmia suppressed as mental load increased with higher suppression in exposed groups (all 3) compared to controls (differences possibly due to existing group differences); Final Study: pursuit-rotor task "somewhat impaired by exposure condition;" authors acknowledge possibility of sequence effects; no significant difference between conditions on questionnaire responses; performing mental tasks resulted in higher heart rate in the TCE + alcohol condition than in Alcohol alone condition; Mental load suppressed sinus arrhythmia, especially in TCE + alcohol condition; Conclusion: TCE and alcohol together impair mental capacity more than each one alone.

NIOSH = National Institute of Occupational Safety and Health

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Albers et al. (1999)	30 railroad workers with toxic encephalopathy; involved in litigation; long-term exposure to solvents (n = 20 yrs.; range = 10–29 yrs.); Historical controls matched by gender, age, and body mass.	Most common solvents included TCE, trichloroethane, perchloroethylene; respirator not typically used.	Neurologic exams (cranial nerves, motor function, alternate motion range, subjective sensory function, Romberg test, reflexes), occupational history, medical history, sensory and motor nerve conduction studies (NCS).	Log transformations of amplitude data; Mann-Whitney U Test for NCS; t-test; simple linear regression and stepwise regression for dose response.	Three workers met clinical polyneuropathy criteria; NCS values not influenced by exposure duration or job title; no significant difference in NCS between presence or absence of polyneuropathy symptoms, disability status, severity or type of encephalopathy, or prior polyneuropathy diagnosis.
Antti-Poika (1982)	87 patients (painters, paint and furniture factory workers, carpet and laundry workers) diagnosed 3–9 yrs prior with chronic solvent exposure (mean age 38.6 yrs). Control: 29 patients with occupational asthma.	Mean duration of exposure 10.4 yrs; solvents: TCE, perchloroethylene, solvent mixture; based on patients' and/or employers' reports; Nine worksites visited for environmental measures; biological measures at One worksite; exposure classified as low, moderate, or high.	Interview, neurologic exam, EEG, electroneuromyographs, psychological examination (intellectual, short-term memory, sensory and motor functions).	Correlation coefficients for prognosis and factors influencing diagnosis.	Reported symptoms: fatigue, headaches, memory disturbances, pain, numbness, paresthesias; 1st exam: 87 patients with objective and subjective neurological signs, 61 with psychological disturbance, 58 abnormal EEG, 25 clinical abnormalities, 57 PNS symptoms; 69 patients had neurophysiological or psychological disturbances identified by neurologist in only 4 patients; 2nd exam: 42 with clinical neurological signs, 21 patients deteriorated, 23 improved, 43 same; poor correlation between prognosis of examinations; no significant correlation between prognosis and age, sex, exposure duration and level, alcohol use, or other diseases.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Aratani et al. (1993)	437 exposed workers from various industries (not specified); 394 males, 43 females and 1,030 male clerical workers as controls; age range: 16–72 yrs.	Exposed to Thinner, G/5100, TCE, xylene, toluene, methylchloride, and gasoline.	Vibrometer (VPT); urinary metabolites.	Spearman correlation.	Positive correlations between age and VPT 7; between job experience and VPT; urinary metabolites not significantly correlated with VPT; no dose-effect for subjective symptoms and neurological signs.
Binaschi and Cantu (1983)	35 patients with occupational exposure to organic solvents; Industry not specified; no controls.	Occupational history provided by patients; descriptions of jobs and conditions provided by employer; workplace observations. Some available measurements of solvents in air; 9 patients exposed to TCE; 11 exposed to toluene and xylene; 15 exposed to mixtures of solvents; all exposures described to be under TLV-TWA, but short exposure might have exceeded ACGIH limit for short time.	Examination of provoked and spontaneous vestibular symptoms; pure tone threshold measurement; EEG; psychiatric interviews and psychiatric history; prevalence of 37 psychiatric symptoms.	Not stated.	All patients had subjective symptoms (fatigue, psychic disturbances, dizziness, vegetative symptoms, vertigo); vestibular system affected in most cases, with lesions in nucleo-reticular substance and brain stem; EEG change with diffuse and focal slowing; 71% of patients had mild neurasthenic symptoms (fatigue, emotional instability, memory and concentration difficulties).
Bowler et al. (1991)	67 former microelectronics workers exposed to multiple organic solvents; controls (n = 157) were recruited from the same region; 67 pairs were matched on the basis of age, sex, ethnicity, educational level, sex, and number of children.	Self-report and work history from microelectronics workers. Exposures and risks were estimated. Solvents include TCE, TCA, benzene, toluene, methylene chloride, and n-hexane.	California Neuropsychological Screening Battery.	t-test for matched pairs; Wilcoxon Signed Rank test.	Exposed workers performed significantly worse on tests of attention, verbal ability, memory, visuospatial, visuomotor speed, cognitive flexibility, psychomotor speed, and RT; no significant differences in mental status, visual recall, learning, and tactile function.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Colvin et al. (1993)	Final sample: 67 workers (43 exposed; 24 unexposed) in a paint manufacturing plant employed there for at least 5 yrs; all black males; exclusion criteria: encephalopathy, head injury with ≥24 hrs unconsciousness, psychotropic medication, alcohol/drug dependence history, epilepsy, mental illness.	Chronic exposure was assessed through self-reported detailed work history for each worker; past and current industrial hygiene measurements of solvent levels in air; "total cumulative expo" in the factory and "average lifetime exposures" were calculated; visitations to establish areas with "homogeneous exposure;" All exposures below the ACGIH limit. Solvents include MEK, benzene, TCE, methyl isobutyl ketone, toluene, butyl acetate, xylene, cellosolve acetate, isophorone, and white spirits.	Work and personal history interview; brief neurological evaluation, WHO Neurobehavioral Core Test Battery (all tests except POMS); Computer-administered tests: RT, Fingertapping, Continuous Performance Test, Switching attention, Pattern Recognition Test, Pattern Memory; UNISA Neuropsychological Assessment Procedure: Four word memory test, Paragraph memory, Geometric Shape drawing; symptom and health questionnaires.	Division into exposed and unexposed; Student's t-test; Multiple linear regression.	Exposed group performed worse than unexposed on 27/33 test results; only significant difference was on latency times of two switching attention tests; no difference in subjects' symptom reporting between groups when questions analyzed separately or analyzed as a group; Average lifetime exposure was a significant predictor for continuous performance latency time, Switching attention latency time, mean RT, pattern memory; fine visuomotor tracking speed significantly associated with cumulative exposure; effects of exposure concluded to be "relatively mild" and subclinical.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Daniell et al. (1999)	89 retired male workers (62–74 yrs old) with prior long-term exposure to solvents including 67 retired painters and 22 aerospace manufacturing workers. Controls: 126 retired carpenters with minimal solvent exposure.	Chronic occupational exposure; structured clinical interview about past and present exposure to solvents; Cumulative Exposure Index was constructed. Solvents not specified.	Psychiatric interview; questionnaires; physical exam; blood cell counts, chemistry panel, blood lead levels, Neuropsychological: BDI, verbal fluency test. WAIS-R: Vocabulary, Similarities, Block Design, Digit Span, Digit Symbol; Wisconsin Card Sorting; verbal aphasia screening test, Trails A and B, Fingertapping; WMS-R: logical memory and visual subtests; Rey Auditory Verbal Learning; Benton Visual Retention test; d2 test; Stroop; Grooved pegboard; SRT.	OR, logarithmic transformation of non-Gaussian data, standardization of test scores, ANCOVA, Multiple Linear regression; Kruskal Wallis test for differences in blood lead concentration.	CEI was similar for painters and aerospace workers. Painters reported greater alcohol use than carpenters; painters also had lower scores on WAIS-R Vocabulary subtest. Controlling for age, education, alcohol use, and vocabulary score, painters performed worse on motor, memory, and reasoning ability tests; painters reported more symptoms of depression and neurological symptoms; painters more likely to have more abnormal test scores (OR: 3.1) as did aerospace workers (OR: 5.6); no dose effect with increasing exposure and neuropsychological tests.
Donoghue et al. (1995)	16 patients diagnosed with organic-solvent-induced toxic encephalopathy with various occupations compared to age-stratified normal groups (n = 38); average age: 43 yrs (range = 31–58); exclusion criteria: diabetes mellitus, ocular disease impairing vision, visual acuity with existing refractive correction of less than 4/6, abnormal direct ophthalmoscopic exam.	Average exposure duration was 19 yrs (range = 5–36 yrs); Solvents include TCE, MEK, toluene, thinners, unidentified hydrocarbons.	Visual acuity measured with a 4-m optotype chart; Contrast sensitivity measured with Vistech VCTS 6,500 chart; monocular thresholds, pupil diameter.	χ^2 test.	Six participants (37.5%) with abnormal contrast sensitivity; two of the six (33%) had monocular abnormalities; abnormalities occurred at all tested spatial frequencies; significant difference between groups at 3, 6, and 12 cpd frequencies.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Elofsson et al. (1980)	Epidemiologic study of car or industrial spray painters (male) exposed long-term to low levels of organic solvents (n = 80); two groups of matched controls; 80 nonexposed male industrial workers in each control group.	Long term, low-level expo to multiple solvents. Assessed by interviews, on-the-job measurements, and a 1955 workshop model. Blood analysis: mean values were within normal limits for both groups. Exposed group had significantly higher values for alkaline phosphates, hemoglobin, hematocrit, and erythrocytes; early exposure TLVs in Sweden were significantly lower; solvents include TCE, TCA, methylene chloride, and others.	Self-administered psychiatric questionnaires, Eysenck's Personality Inventory, psychosocial structured interview, Comprehensive Psychopathological Rating Scale; visual evoked responses; EEG; Electroneurography; Vibration Sense Threshold estimations; Neurological exam.	Calculation of z values; Pearson correlation; Multiple Regression Analysis.	Significant differences between controls and exposed in symptoms of neurasthenic syndrome, in RT, manual dexterity, perceptual speed, and short-term memory; no significant differences on verbal, spatial, and reasoning ability; some differences on EEG, VER, ophthalmologic, and CT.
Gregersen (1988)	Workers exposed to organic solvents (paint, lacquer, photogravure, and polyester boat industries). Controls: warehousemen electricians; 1st follow-up 5.5 yrs after initial evaluation (59 exposed, 30 unexposed); 2nd follow-up: 10.6 yrs after initial evaluation (53 exposed, 30 unexposed controls).	1st follow-up: data about working conditions, materials and exposure in prior 5 yrs used for exposure index; 2nd follow-up: nine questions asking about exposure to solvents in the prior 5 yrs; TCE, toluene, styrene, white spirits.	1st follow-up: structured interviews on occupational, social, medical history; clinical exam, neurological exam; 2nd follow-up: mailed questionnaire (49 follow-up issues to 1st follow-up).	Wilcoxon-Mann-Whitney tests; Kruskal-Wallis test; χ^2; Spearman Rank Partial Correlation Coefficient.	More acute neurotoxic symptoms in exposed group at both follow-ups, but fewer symptoms at 2nd follow-up than at 1st follow-up; at both follow-ups exposed participants had more encephalopathy symptoms, especially memory and concentration; no encephalopathy symptoms in control group; symptoms and signs of peripheral, sensory, and motor neuropathy significantly worse in participants still exposed. Exposure index showed dose-effect with memory and concentration. Both follow-ups: improvement in acute symptoms; aggravation in CNS; more symptoms of peripheral nervous system and social consequences.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Juntunen et al. (1980)	37 patients with suspected organic solvent poisoning (mean age = 40.1 yrs); selection based on pneumoencephalography; no controls.	Patients were exposed to carbon disulphide (n = 6), TCE (5), styrene (1), thinner (2), toluene (1), methanol (1), and carbon tetrachloride (2), and mixtures (19). Exposure was assessed by patients' and employers' reports and measurements of air concentrations when available.	Neurologic examination, pneumoencephalographic exam, EEG, tests assessing intelligence, memory and learning, motor function, and personality.	Descriptive Statistics.	Clinical neurological findings of slight psychoorganic alterations, cerebellar dysfunction, and peripheral neuropathy; 63% had indication of brain atrophy; 23 of the 28 patients examined with electroneuromyography showed signs of peripheral neuropathy; 94% had personality changes, 80% had psychomotor deficits, 69% had impaired memory, and 57% had intelligence findings; no dose-effect found.
Juntunen et al. (1982)	80 (41 women, 39 men) Finnish patients diagnosed 3–9 yrs prior with chronic solvent exposure (mean age = 38.6 yrs); 31 had slight neurological signs; no controls.	Assessed by patients' occupational history, employers' workplace description, observations and data collected at workplace, environmental measurements, biological tests; TCE, perchloroethylene, or mixed solvent exposures.	Neurologic examination; EEG and ENMG; tests of intellectual function, memory, learning, personality, and psychomotor performance.	χ^2, Maxwell-Stuart, correlation and multiple linear regression analyses.	Significant correlations between prognosis of disturbances in gait ($p < 0.05$) and station and length of follow-up, duration and level of exposure and multiplying the two; no gender effects. Common subjective symptoms; headaches, fatigue, and memory problems. Impairment in fine motor skills, gait, and cerebellar functions. Subjective symptoms decreased during follow-up, but clinical signs increased.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Laslo-Baker et al. (2004)	32 mothers with occupational exposure to organic solvents during pregnancy and their children (3–9 yrs of age); included if exposure started in 1st trimester and lasted for at least 8 wks of pregnancy (32 mother-child pairs). Controls: 32 unexposed control mothers matched on age, child age, child sex, SES, and reported cigarette use and their children (32 mother-child pairs).	Exposure information collected at 3 times: (1) during pregnancy; (2) when contacted for study participation later in pregnancy; and (3) at time of assessment. Information collected included types of solvent, types of setting, duration of exposure during pregnancy, use of protection, symptoms, and ventilation. Solvents include toluene (n = 12 women), xylene (10), ethanol (7), acetone (6), methanol (5), TCE (3), etc. (a total of 78 solvents were reported).	Children: Wechsler Preschool and Primary Scale of Intelligence, WISC, Preschool Language Scale, Clinical Evaluations of Language Fundamentals, Beery-Buktenica Developmental test of Visuo-Motor Integration, Grooved Pegboard Test, Child Behavior Checklist (Parent Version), Connor's Rating Scale-Revised (Parent Version), Behavioral Style Questionnaire; Mothers: WASI.	Power analysis, Multiple linear regression.	Verbal IQ was lower (104) in children exposed in utero vs. unexposed children controls (110); Children did not differ between groups in birth weight, gestational age, or developmental milestones; Children in the exposed group had significantly lower VIQ (108) and Full IQ (108) than controls (VIQ = 116 and Full IQ = 114; No significant difference in PIQ; Performance on expressive language, total language, and receptive language was significantly worse in children from exposed group.
Lee et al. (1998)	40 Korean female shoe factory workers employed there for at least 5 yrs; cases with head injury, neurological or psychological disorder, or hearing or visual impairment were excluded. Controls: 28 (housekeepers); no in-plant controls available.	Four workers wore passive personal air samplers for a full 8-hr shift. Detected solvents: toluene, methyl ethyl ketone, *n*-hexane, *c*-hexane, cyclohexane, DCE, TCE, benzene, and xylene. In frame-making, air concentration of solvents was 0.46–0.71 ppm. In adhesive process, solvent air concentrations were 1.83–2.39 ppm; three exposure indices were calculated: current exposures, exposure duration (yrs), and Cumulative Exposure Estimate (CEE) (yrs × average exposures).	Questionnaire; Neurobehavioral Core Test Battery (includes POMS, SRT, Santa Ana Dexterity test, Digit Span, Benton Visual Retention Test, Pursuit aiming motor steadiness test); POMS was excluded because of cultural inapplicability.	Multivariate ANOVA for tests with 2 outcomes; ANOVA for tests with 1 outcome; education was adjusted in analyses.	Significant differences between groups based on exposure index. Differences in performance between controls and participants on Santa Ana were found only in the CEE (participants performed worse). CEE is a more sensitive measure of exposure to organic solvents.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Lindstrom (1973)	168 male workers with suspected occupational exposure to solvents. Group I with solvent poisoning (n = 42). Group II with solvent exposure, undergoing mandatory periodic health check (n = 126). Control: 50 healthy nonexposed male volunteers working in a viscose factory. Group IV: 50 male workers with carbon disulfide poisoning.	44 exposed to TCE, 8 to tetrachloroethylene, 26 to toluene, 25 to toluene and xylene, 44 to thinners, 21 to "miscellaneous;" solvent-exposed group had an average of 6 yrs of exposure; CS_2 group had average of 9 yrs of exposure.	WAIS: Similarities, Picture Completion, Digit Symbol; Bourdon-Wiersma vigilance test, Santa Ana, Rorschach Inkblot test, Mira test.	Student's t-test.	The solvent-exposed group and CS_2 group had significantly worse "psychological performances" than controls; greatest differences in sensorimotor speed and psychomotor function; solvent-exposed and CS_2 groups had deteriorated visual accuracy.
Lindstrom (1980)	56 male workers diagnosed with occupational disease caused by solvents. Controls: 98 styrene-exposed workers; 43 nonexposed construction workers.	Chronic "excessive" exposure: mean duration of exposure = 9.1 yrs (SD = 8.3); exposed to halogenated and aromatic hydrocarbons, paint solvents, alcohols, and aliphatic hydrocarbons (TCE n = 14). Individual exposure levels estimated as TWAs, based on information provided by subjects, employer, or workplace measurements, were categorized as low (3 patients), intermediate (26 patients), and high (27 patients).	WAIS subtests: Similarities, Digit Span, Digit Symbol, Picture Completion, Block Design; WMS subtests: Visual Reproduction; Benton Visual Retention test; Symmetry Drawing; Santa Ana Dexterity test; Mira test.	Factor analysis; Student's t-test; Multivariate Discriminant analysis.	Significant decline in visuomotor performance and freedom from distractibility (attention) in the solvent-exposed participants; significant relationship between duration of solvent exposure and visuomotor performance; solvent exposure level was not significant; psychological test performance of styrene-exposed control was only slightly different from nonexposed controls.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Lindstrom et al. (1982)	86 patients with prior diagnosis of solvent intoxication (mean age 38.6 yrs); 40 male, 46 female; 52 exposed to mixed solvents; 21 exposed to TCE or perchloroethylene; 13 exposed to both; results at follow-up compared to those at initial diagnosis.	Mean duration of exposure 10.4 yrs; solvents: TCE, perchloroethylene, solvent mixture; based on patients' and/or employers' reports.	Intellectual Function: from WAIS – Similarities, Block Design, Picture Completion; Short Term Memory: from WMS – Digit Span, Logical Memory, Visual Reproduction; Benton Visual Retention test; Sensory and Motor Functions: Bourdon Wiersma Vigilance Test, Symmetry Drawing, Santa Ana Dexterity test, Mira test.	Frequency distributions, Student's t-test for paired data, stepwise linear regression.	All patients grouped together regardless of types of past solvent exposure; on follow-up, significant learning effects for similarities when compared to results at initial diagnosis; group mean for intellectual functioning increased; no significant change in memory test results; group means for sensory and motor tasks were lower; prognosis was better for longer follow-up and younger age and poorer for users of medicines with neurological effects.
Marshall et al. (1997)	All singleton births in 1983–1986 in 188 New York State counties (total number not specified); 473 CNS-defect births and 3,305 musculoskeletal-defect births; controls: 12,436 normal births. Exclusion criteria: Trisomy 13, 18, or 21, birth weight of <1,000 g, sole diagnosis of hydrocephaly or microencephalopathy, hip subluxation.	Information on inactive waste sites was examined, including air vapor, air particulates, groundwater exposure via wells, and groundwater exposure. via basements; exposure was categorized as "high," "medium," "low," or unknown based on probability of exposure; proximity to waste sites was also considered; Most common solvents: TCE, toluene, xylenes, tetrachloroethene, 1,1,1-trichloroethane; Most common metals found lead, mercury, cadmium, chromium, arsenic, and nickel.		OR, Fisher's exact test, χ^2, unconditional logistic regression.	13 CNS cases and 351 controls with potential exposures; crude OR. When controlling for mother's education, prenatal care, and exposure to a TCE facility, OR was 0.84; CNS and solvents OR: 0.8; CNS and metals OR: 1.0, musculoskeletal defects and solvents OR: 0.9, musculoskeletal defects and pesticides OR: 0.8; higher risk for CNS defects when living close to solvent-emitting facilities.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
McCarthy and Jones (1983)	384 industrial workers with solvent poisoning; 103 operated degreasing baths, 62 maintained degreasing baths, 37 used TCE in portable form, 37 miscellaneous; no controls.	Individuals poisoned with TCE, perchloroethylene, and methylchloroform were examined retrospectively; medical record review; 288 exposed to TCE, 44 to perchloroethylene, 52 to 1,1,1-trichloroethane.	Symptoms reported in occupational/medical records from industrial poisoning incidents; data from 1961 to 1980 on demographics, occupation, work process, type of industry, if incident caused fatality.		17 fatality cases, with 10 in confined spaces; most common symptoms include effects on CNS; gastrointestinal and respiratory symptoms; no strong evidence for cardiac and hepatic toxicity; no change in affected number of workers in 1961 to 1980; greatest effect due to narcotic properties.
Mergler et al. (1991)	54 matched pairs; Matching on the basis of age, sex, ethnicity, educational level, sex, and number of children taken from180 former microelectronics workers exposed to multiple organic solvents and control population of 157 recruited from the same region.	Average duration of employment: 6.1 yrs (range: 1–15 yrs); information about products used and chemical make-up from employer; chemicals: chlorofluorocarbons, chlorinated hydrocarbons, glycol ethers, isopropanol, acetone, toluene, xylene, and ethyl alcohol.	Sociodemographic questionnaire; monocular examination of visual function: Far visual acuity using a Snellen chart, near visual acuity using a National Optical Visual Chart, color vision using Lanthony D-15, near contrast sensitivity using Vistech grating charts.	Signed-rank Wilcoxon test; Mann-Whitney; χ^2 test for matched pairs; Multiple Regression; Stepwise regression.	Significant difference in near contrast sensitivity: 75% of exposed workers with poorer contrast sensitivity at most frequencies than the matched controls (no difference in results based on smoking, alcohol use, and near visual acuity loss). Significant differences on near visual acuity, color vision, and rates of acquired dyschromatopsia for one eye only. No difference between groups in near or far visual acuity.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Morrow et al. (1989)	22 male patients with exposure to multiple organic solvents; 4 involved in litigation. Exclusion: neurologic or psychiatric disorder prior to assessment, alcohol consumption more than two drinks/d. Average yrs education 12 (range: 10–16 yrs); average age 38 yrs (range: 27–61); compared to responses of WWII prisoner of war (POW) population with posttraumatic stress disorder (PTSD).	Exposure assessed with questionnaire (duration, type of solvents, weeks since last exposure, cases of excessive exposure); Average exposure duration = 7.3 yrs (range: 2 months–19 yrs); average wks since last exposure was 19.8 (range: 1–84 wks); 28% had at least one instance of excessive exposure.	Exposure questionnaire, Group form of the MMPI.	Stepwise multiple regression.	All profiles valid; 90% with at least two elevated scales above T score of 70 (clinically significant); highest elevations on scales 1, 2, 3, and 8; only one case within normal limits; when compared to a group of nonpsychiatric patients, exposed patients had more elevations, although both groups have physical complaints. When compared with WWII POW (1/2 diagnosed with PTSD) with similar SES and education, both groups have similar profiles; no age effects found; significant positive correlation between scale 8 and duration of exposure; no significant difference based on time since last exposure or on experiencing excessive exposure.
Morrow et al. (1992)	Nine men and three women occupationally exposed to multiple organic solvents with CNS complaints; all met criteria for mild toxic encephalopathy; exposed group average age was 47 yrs; Controls: 19 (healthy male volunteers); 26 psychiatric controls (male patients with chronic schizophrenia) average age unexposed controls: 34 yrs; average age schizophrenic patients: 36 yrs.	Exposure assessed with occupational and environmental exposure questionnaire; mean duration of exposure = 3 yrs (range = <1 d–30 yrs); average time between last exposure and assessment was 2 yrs (range; 2 months–10 yrs); solvents toluene, TCE.	Auditory event-related potentials under the oddball paradigm: counting and CRT tasks.	Repeated measures ANOVA.	Exposed patients had significant delays in N250 and P300 compared to normal controls and in P300 compared to psychiatric controls. Exposed patients had higher amplitudes for N100, P200, and N250; no difference in P300 amplitude between groups; for the exposed group, P300 positively correlated with exposure duration; findings indicate that solvent exposure affects neural networks.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Seppäläinen and Antti-Poika (1983)	87 patients with solvent poisoning (40 male and 47 female) with occupational exposure to solvents; follow-up 3–9 yrs after initial diagnosis; mean age at diagnosis 38.6 (range: 20–59 yrs); no control population.	Chronic exposure with average duration of 10.7 yrs (range: 1–33); patients were exposed to TCE (n = 21), perchloroethylene (n = 12), mixtures of solvents (n = 53), mixtures and TCE or perchloroethylene (n = 13). Exposure of 54 patients stopped after diagnosis, 33 continued to be exposed; at follow-up, only 5 working with potential of some exposure.	EEG using 10/20 system with 25–30 min of recording, 3 min hyperventilation and intermittent photic stimulation; ENMG.	χ^2, hypergeometric distribution, McNemar test.	Significantly more ENMG abnormalities at follow-up than at initial diagnosis. Most common finding: slight polyneuropathy; 43% showed improved ENMG, 33% had deteriorated, and 18 points. with similar ENMG findings (six normal at both exams); at follow-up, slow-wave abnormalities decreased and paroxysmal abnormalities increased; 41 with improved EEG, 28 with similar EEG (19 had normal EEG at diagnosis), and 18 with deteriorated EEG; EEG pattern of change compared to external head injuries.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Shlomo et al.(2002)	Male industrial workers; mercury exposure group (n = 40); average age 49.7 (±6.4) yrs; chlorinated hydrocarbons exposure group (n = 37) average age 46.0 (±4.73); controls, unexposed (*n* = 36) average age 49.8 (±5.8), matched by age; (industries not specified).	Interview and record review; urine samples collected at end of work shift prior to testing and tested for mercury and TCA; chlorinated hydrocarbons: TCE (n = 7), perchloroethylene (n = 8), trichloroethane (n = 22). Mean duration of CH exposure 15.8 (±7.2) yrs. Mean duration of mercury exposure 15.5 (±6.4) yrs. Air sampling: mercury: 0.008 mg/m^3 (TLV = 0.025); TCE: 98 ppm (TLV = 350); perchloroethylene: 12.7 ppm (TLV = 25); and trichloroethane: 14.4 ppm (TLV = 200). Blood levels: mercury (B-hg) 0.5 g% (±0.3); TCA urine levels: 1–80% of Biologic Exposure Index (BEI); CH urine levels: 0.11–0.2 of BEI.	Medical history, Neurological tests assessing cranial nerves and cerebellar function; Otoscopy, review of archival data from pure-tone audiometric tests; Auditory brain stem responses (ABR).	Student's t-test, proportions test.	Significant differences between exposed and controls: 33.8% of CH-exposed workers with abnormal IPL I-III; 18% of controls; authors suggest ABRs are sensitive for detecting subclinical CNS effects of CH and mercury.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Till et al. (2001b)	The children of mothers who had contacted a Canadian pregnancy risk counseling program during pregnancy and reported occupational exposure to solvents (n = 33); children age range: 3–7 yrs; Mothers' occupations: lab technicians, factory workers, graphic designers, artists, and dry cleaning. Controls: 28 matched on age, gender, parental SES, and ethnicity; children of mothers exposed to nonteratogenic agents.	Structured questionnaire about exposure; method: weight assigned to each exposure parameter (length of exposure, frequency of exposure, symptoms); sum of scores for each parameter used as exposure index; median split used to categorize in low (n = 19) and high (n = 14) exposures; solvents include benzene, toluene, methane, ethane, TCE, methyl chloride, etc.	NEPSY: Visual Attention, Statue, Tower, Body Part Naming, Verbal Fluency, Speeded Naming, Visuomotor Precision, Imitating Hand Positions, Block Construction, Design Copying, Arrows; Peabody Picture Vocabulary Test; WRAVMA Pegboard test; Child Behavior Checklist (Parent form); Continuous Performance Test.	Mantel Haenszel test, t-test, ANCOVA, Hierarchical multiple linear regression.	Lower composite neurobehavioral scores as exposure increased after adjusting for demographics in receptive language, expressive language, graphomotor ability. Significantly more exposed children rated with mild-severe problems. No significant difference between groups in attention, visuo-spatial ability, and fine-motor skills. Mean difference on broad- and narrow-band scales of Child Behavior Checklist scores not significant.
Till et al. (2001b)	Children of mothers who had contacted a Canadian pregnancy risk counseling program during pregnancy and reported occupational exposure to solvents (n = 32); children age range: 3–7 yrs. Mothers' occupations: lab technicians, factory workers, graphic designers, artists, and dry cleaning. Controls: 27 matched on age, gender, parental SES, and ethnicity; children of mothers exposed to nonteratogenic agents.	Structured questionnaire about exposure; method: weight assigned to each exposure parameter (length of exposure, frequency of exposure, symptoms); sum of scores for each parameter used as exposure index; median split used to categorize in low (n = 19) and high (n = 14) exposures; solvents include benzene, toluene, methane, ethane, TCE, methyl chloride, etc.	Minimalist test to assess color vision; Cardiff Cards to assess visual acuity.	Independent samples t-tests, Mantel Haenszel Chi test; Wilcoxon-Mann-Whitney test; Kruskal-Wallis χ^2.	Significantly higher number of errors on red-green and blue-yellow discrimination in exposed children compared to controls; exposed children had poorer visual acuity than controls. No significant dose-response relationship between exposure index and color discrimination and visual acuity.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Till et al. (2005)	21 infants (9 male, 12 female) of mothers who contacted a Canadian pregnancy risk counseling program and reported occupational exposure to solvents (occupations: factory, laboratory, dry cleaning. Controls: 27 age-matched infants (17 male, 10 female) of mothers contacted the program due to exposure during pregnancy to nonteratogenic substances).	Structured questionnaire about exposure; method: weight assigned to each exposure parameter (length of exposure, frequency of exposure, symptoms); sum of scores for each parameter used as exposure index; median split used to categorize in low and high exposures; exposure groups: (1) aliphatic and/or aromatic hydrocarbons (n = 9); (2) alcohols (n = 3); (3) multiple solvents (n = 6); and (4) perchloroethylene, (n = 3); mean duration of exposure during pregnancy 27.2 wks. (SD 7.93, range = 12–40); solvents include benzene, toluene, methane, ethane, TCE, methyl chloride, etc.	1st visit: Sweep VEP to assess contrast sensitivity and grating acuity; 2nd visit (2 wks after 1st): Transient VEPs to assess chromatic and achromatic mechanisms; ophthalmological exam, physical and neurological exam; testers masked to exposure status of infant.	Median split; Multiple Linear Regression; χ^2, t-test, Mann-Whitney U test, Multivariate ANCOVA, Pearson correlation, Logistic Regression.	Significant decline of contrast sensitivity in low and intermediate spatial frequencies in exposed infants when compared with controls. Significant effect of exposure level on grating acuity, 26.3% of exposed (but 0% of controls) with abnormal VEP to red-green onset stimulus. No differences between groups in latency and amplitude of chromatic and achromatic response.
Valic et al. (1997)	138 occupationally exposed and 100 unexposed controls. Exclusion criteria: congenital color vision loss, severe ocular disease, significant vision impairment, tainted glasses or contact lenses, diabetes mellitus, neurological disease, prior severe head or eye injuries, alcohol abuse, medication impairing color vision.	Solvents: TCE, perchloroethylene, toluene, xylene; historical data on duration of exposure protective equipment use, subjective evaluation of exposure, nonoccupational solvent exposure, solvent-related symptoms at work, alcohol and smoking, drug intake. Mean urinary levels of TCA: 1.55 (± 1.75) mg/L.	Lanthony D15.	Polytomous logistic regression.	Significant effect of age in exposed group; with alcohol of <250 g/wk no significant correlation between color confusion and solvent exposure. Significant interaction between solvent exposure and alcohol intake. Color Confusion Index significantly higher in exposed group with alcohol use of >250 g/wk.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Windham et al. (2006)	Children born in 1994 in San Francisco Bay Area with ASDs (n = 284) and controls (n = 657), matched on basis of gender and month of birth.	Birth addresses were geocoded and linked to hazardous air pollutant database; exposure levels assigned for 19 chemicals; chemicals were grouped based on mechanistic and structural properties; Summary index scores were calculated; risk of ASD calculated in upper quartiles of groups or individual chemical concentrations; adjustment for demographic factors.	Archival data.	Pearson correlation, Logistic Regression.	Elevated adjusted ORs for ASD (by 50%) in top quartile of chlorinated solvents, but not for aromatic solvents; AOR for TCE in 4th quartile = 1.47; lessened when adjusted for metals; correlation between hydrocarbon and metals exposures; when adjusted, increased risk for metals (in 3rd quartile = 1.95; in 4th quartile = 1.7). Contributing compounds: mercury, cadmium, nickel, TCE, vinyl chloride. Results interpreted to suggest relationship between autism and estimated metal and solvent concentrations in air around place of birth residence.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Epidemiological studies: controlled exposure studies; neurological effects of trichloroethylene/mixed solvents					
Levy et al. (1981)	Nine participants (eight males and one female) recruited through newspaper ad; 8 hrs fasting before testing; no control.	Experiment 1: alcohol consumption (three doses)—blood alcohol levels were measured with breath analyzer pre- (multiple baselines) and post-test (multiple). Experiment 2: CH administered orally over 2 min in either 500 or 1,500 mg dose; multiple baseline smooth pursuit eye movement (SPEM) tests and multiple posttests after exposure; no control dose administered.	SPEM tests of following a sinusoidally oscillated target at 0.4 Hz; eye movements were recorded through electrodes at each eye.	t-tests; ANOVA.	Experiment 1: prealcohol all subjects had intact SPEM; no significant effect for 1.5 mL/kg of alcohol; significant decline in SPEM at 2.0 and 3.0 mL/kg alcohol; significant dose-effect. Experiment 2: at 500 mg CH, no significant change in pursuit was noted; at 1,500 mg CH, qualitative disruptions in pursuit in all participants (4); at 500 mg, participants observed to be drowsy. When number reading was added SPEM impairment was 'attenuated' in both alcohol and CH conditions.

Table D-2. Epidemiological studies: neurological effects of TCE/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Stopps and McLaughlin (1967)	Chamber study using two healthy male volunteers exposed to Freon-113; one volunteer exposed to TCE; No control.	Exposure booth was constructed; TCE in air: TCE concentrations: 100, 200, 300, or 400 ppm (1965 TLV: 100 ppm for 8-hr exposure) in ascending and descending order; total time in chamber: 2.75 hrs; Freon-113 concentrations: 1,500, 2,500, 3,500, or 4,500 ppm (1965 TLV: 1,000 ppm for 8-hr exposure), duration 1.5 hrs; TCE and Freon-113: (1) reduction of weight of compound during exposure was calculated; (2) continuous air sampling in the chamber; and (3) gas chromatography on air captured in bottles sealed in the chamber; no control dose given.	Crawford Small Parts Dexterity Test, Necker Cube Test, Card Sorting, Card Sorting with an Auxiliary Task, Dial Display (TCE participant only); Short Employment Test-Clerical (Freon-113 participants only).	Descriptive statistics for air measurement plots by % of TCE change in groups.	No TCE effect at 100 ppm, but test performance deteriorated with increase of TCE concentration. No effect of Freon-113 on psychomotor function at 1,500 ppm, deterioration at 2,500 ppm, as concentration increased, performance deteriorated.

Table D-3. Literature review of studies of TCE and domains assessed with neurobehavioral/neurological methods

Authors	Year	Study type	Participants no. (N = exposed C = nonexposed)	Dur	PM/RT	VM	Cogn	M&L	M&P	Symp†	Sen††	Resp	Dose effect √√ urinary metabolites √	TCE levels
ATSDR	(2003a)	E	N = 116, C = 177	**C**	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	**A**	*ne*	*ne*	0 → 23 ppb in dg water
Barret et al.	(1984)	O	N = 188	C	*ne*	*ne*	*ne*	*ne*	*ne*	H, D	T, N, V	*ne*	√	150 ppm
Barret et al.	(1987)	O	N = 104, C = 52	C	*ne*	*ne*	*ne*	*ne*	√	H, D, S, I	T, N	*ne*	√	*ne*
Barret et al.	(1982)	O	N = 11, C = 2	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	T	*ne*	√	*ne*
Burg et al.	(1995)	E	N = 4,281	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	A, N	√	√	*ne*
Burg and Gist	(1999)	E	N = 3,915	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	A, N	√	√√	4 gps: 2–75,000 ppb
El Ghawabi et al.	(1973)	O	N = 30, C = 30	C	*ne*	*ne*	*ne*	*ne*	*ne*	H, S	(-)	*ne*	√	165 ppm
Feldman et al.	(1988)	E	N = 21, C = 27	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	T	*ne*	*ne*	*ne*
Feldman et al.	(1992)	O	N = 18, C = 30	A,C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	T, N	*ne*	*ne*	*ne*
Gamberale et al.	(1976)	C	N = 15	A	√	*ne*	√	(-)	*ne*	*ne*	*ne*	*ne*	*ne*	540–1,080 mg^3
Gash et al.	(2008)	O	N = 30	C	√	*ne*	*ne*	*ne*	*ne*	M, N		*ne*	*ne*	*ne*
Grandjean et al.	(1955)	O	N = 80	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	N	*ne*	√, √√	6–1,120 ppm
Gun et al.	(1978)	O	N = 8, C = 8	C	√	*ne*	√	*ne*	*ne*	*ne*	N	*ne*	*ne*	3–418 ppm
Hirsch et al.	(1996)	E	N = 106	C	*ne*	*ne*	*ne*	*ne*	*ne*	H	*ne*	*ne*	*ne*	0–2,441 ppb
Kilburn and Thornton	(1996)	E	N = 237, C = 264	C	√	*ne*	√	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*
Kilburn and Warshaw	(1993a)	E	N = 544, C = 181	C	√	√	√	√	√	M	T, N	*ne*	*ne*	6–500 ppb
Kilburn	(2002a)	E	N = 236, C = 228	C	*ne*	*ne*	√	*ne*	*ne*	M	B	*ne*	*ne*	6–500 ppb
Kilburn	(2002b)	E	N = 236, C = 58	C	(-)	*ne*	*ne*	*ne*	(-)	*ne*	*ne*	*ne*	*ne*	0.2–1,000 ppb
Konietzko et al.	(1975)	C	N = 20	A	*ne*	*ne*	*ne*	*ne*	*ne*	M	N	*ne*	√	953 ppm
Kylin, et al.	(1967)	C	N = 12	A	√	*ne*	*ne*	*ne*	*ne*	*ne*	N	*ne*	*ne*	1,000 ppm
Landrigan, et al.	(1987)	O	Residents and 12 W	A,C	*ne*	*ne*	√	*ne*	*ne*	H, D	*ne*	*ne*	√√	≥183,000 ppb
Liu, et al.	(1988)	O	N = 103, C = 111	C	*ne*	*ne*	*ne*	√	*ne*	D, N	N	*ne*	√√	1–100 ppm
Mhiri et al.	(2004)	O	N = 23, C = 23	A	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	T	*ne*	√, √√	*ne*
Nagaya et al.	(1990)	O	N = 84, C = 83	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	N	*ne*	√	22 ppm

Table D-3. Literature review of studies of TCE and domains assessed with neurobehavioral/neurological methods (continued)

Authors	Year	Study type	Participants no. (N = exposed C = nonexposed)	Dur	PM/RT	VM	Cogn	M&L	M&P	Symp†	Sen††	Resp	Dose effect √√ urinary metabolites√	TCE levels
Rasmussen and Sabroe	(1986)	O	N = 240, C = 350	C	*ne*	*ne*	*ne*		√	H,D, I, M	*ne*	*ne*	*ne*	*ne*
Rasmussen et al.	(1993d)	O	N = 96	C	*ne*	*ne*	√	*ne*	*ne*	*ne*	*ne*	*ne*	√√	*ne*
Rasmussen et al.	(1993c)	O	N = 96	C	*ne*	√	√	*ne*	*ne*	*ne*	*ne*	*ne*	√√	*ne*
Rasmussen et al.	(1993a)	O	N = 99	C	√	*ne*	*ne*	*ne*	*ne*	*ne*	N	*ne*	√√	*ne*
Reif et al.	(2003)	E	N = 143	C	√	√	*ne*	*ne*	√	M	M	*ne*	√√	5–15 ppb
Ruijten et al.	(1991)	O	N = 31, C = 28	C	√	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	17–70 ppm
Smith	(1970)	O	N = 130, C = 63	C	*ne*	*ne*	*ne*	*ne*	*ne*	H, D	**N**	*ne*	√, √√	*ne*
Stewart et al	(1970)	C	N = 13	A	*ne*	*ne*	√	*ne*	*ne*	H	*ne*	*ne*	√	100–202 ppm
Triebig et al.	(1976)	C	N = 7, C = 7	A	*ne*	*ne*	√	√	√	(-)	*ne*	*ne*	√, √√	0–100 ppm
Triebig et al.	(1977a)	C	N = 7, C = 7	A	*ne*	*ne*	√	√	√	M	(-)	*ne*	√, √√	0–100 ppm
Triebig et al.	(1977c)	O	N = 8	A,C	*ne*	√	√	√	*ne*	*ne*	*ne*	*ne*	√	50 ppm
Triebig et al.	(1982)	O	N = 24, C = 24	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	N	*ne*	√, √√	5–70 ppm
Triebig et al.	(1983)	O	N = 66, C = 66	C	*ne*	*ne*	*ne*	*ne*	*ne*	N, H	N	*ne*	√	10–600 mg/m^3
Troster and Ruff	(1990)	O	N = 3, C = 60	A	√	√	√	√	√	*ne*	N	*ne*	*ne*	*ne*
Vernon and Ferguson	(1969)	C	N = 8	A	√	√	*ne*	*ne*	*ne*	*ne*	N	*ne*	√√	0–1,000 ppm
Windemuller and Ettema	(1978)	C	N = 39	A	√	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	200 ppm
Winneke	(1982)	O	Not reported	*ne*	(-)	(-)	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	50 ppm

†H = Headaches; **D** = Dizziness; **I** = Insomnia; **S** = Sex Probls; **M** = Mood; **N** = Neurological.
††**A** = Audition; **B** = Balance; **V** = Vision; **T** = Trigeminal nerve; **N** = Other Neurological.

Study: **C** = Chamber; **E** = Environmental; **O** = Occupational.
Duration: **A** = Acute, **C** = Chronic.

√ = positive findings; (-) = findings not significant; *ne* = not examined or reported; Dur = duration; PM/RT = psychomotor/reaction time; VM = visuo-motor; Cogn = cognitive; M&L = memory and learning; M&P = mood and personality; Symp = symptoms; Sen = sensory; Resp = respiratory

D.2. CNS TOXICITY IN ANIMAL STUDIES FOLLOWING TCE EXPOSURE

In vivo studies in animals and in vitro models have convincingly demonstrated that TCE produces functional and physiological neurological changes. Overall, these effects collectively indicate that TCE has CNS depressant-like effects at lower exposures and causes anesthetic-like effects at high exposures. Studies of TCE toxicity in animals have generally not evaluated whether or not adverse effects seen acutely persist following exposure or whether there are permanent effects of exposure. Exceptions to the focus on acute impairment while under TCE intoxication include studies of hearing impairment and histopathological investigations focused primarily on specific neurochemical pathways, hippocampal development, and demyelination. These persistent TCE effects are discussed initially followed by the results of studies that examined the acute effects of this agent. Summary tables for all of the animal studies are at the end of this section.

D.2.1. Alterations in Nerve Conduction

There is little evidence that TCE disrupts trigeminal nerve function in animal studies. Two studies demonstrated that TCE produces morphological changes in the trigeminal nerve at a dose of 2,500 mg/kg-day for 10 weeks (1992; 1991). However, dichloroacetylene, a degradation product formed during the volatilization of TCE was found to produce more severe morphological changes in the trigeminal nerve and at a lower dose of 17 mg/kg-day (Barret et al., 1992; Barret et al., 1991). Only one study (Albee et al., 2006) evaluated the effects of TCE on trigeminal nerve function, and a subchronic inhalation exposure did not result in any significant functional changes. A summary of these studies is provided in Table D-4.

Barret et al. (1992; 1991) conducted two studies evaluating the effects of both TCE and dichloroacetylene on trigeminal nerve fiber diameter and internodal length as well as several markers for fiber myelination. Female Sprague-Dawley rats (n = 7/group) were dosed with 2,500 mg/kg TCE or 17 mg/kg-day dichloroacetylene by gavage for 5 days/week for 10 weeks. These doses were selected based upon the ratio of the LD_{50} values (dose at which there is 50% lethality) for these two agents. Two days after administration of the last dose, a morphometric approach was used to study the diameter of teased fibers from the trigeminal nerve. The fibers were classified as Class A or Class B and evaluated for internode length and fiber diameter. TCE-dosed animals only exhibited changes in the smaller Class A fibers where internode length increased marginally (<2%) and fiber diameter increased by 6%. Conversely, dichloroacetylene-treated rats exhibited significant and more robust decreases in internode length and fiber diameter in both fiber classes A and B. Internode length decreased 8% in Class A fibers and 4% in Class B fibers. Fiber diameter decreased 10% in Class A fibers and 6% in Class B fibers. Biochemical data are presented for fatty acid composition from total lipid extractions from the trigeminal nerve. These two studies identify a clear effect of dichloroacetylene on trigeminal nerve fibers, but the effect by TCE is quite limited.

Albee et al. (2006) evaluated the effects of a subchronic inhalation TCE exposure in F344 rats (10/sex/group). Rats were exposed to 0, 250, 800, and 2,500 ppm TCE for 6 hours/day, 5 days/week for 13 weeks. At the 11th week of exposure, rats were surgically implanted with epidural electrodes over the somatosensory and cerebellar regions, and TSEPs were collected 2–3 days following the last exposure. TSEPs were generated using subcutaneous needle electrodes to stimulate the vibrissal pad (area above the nose). The resulting TSEP was measured with electrode previously implanted over the somatosensory region. The TCE exposures were adequate to produce permanent auditory impairment, even though TSEPs were unaffected. While TCE appears to be negative in disrupting the trigeminal nerve, the TCE breakdown product, dichloroacetylene, does impair trigeminal nerve function.

Albee et al. (1997) reported that dichloroacetylene disrupted trigeminal nerve somatosensory evoked potentials in F344 male rats. The subjects were exposed to a mixture of 300 ppm dichloroacetylene, 900 ppm acetylene, and 170 ppm TCE for a single 2.25-hour period. This dichloroacetylene was generated by decomposing TCE in the presence of potassium hydroxide and stabilizing with acetylene. A second treatment group was exposed to a 175 ppm TCE/1,030 ppm acetylene mix with no potassium hydroxide present. Therefore, no dichloroacetylene was present in the second treatment group, providing an opportunity to determine the effects on the trigeminal nerve somatosensory evoked potential in the absence of dichloroacetylene. Evoked potentials from the dichloroacetylene/TCE/acetylene-exposed rats were about 17% smaller measured between peaks I and II and 0.13 msec slower in comparison to the preexposure measurements. Neither latency nor amplitude of this potential changed significantly between the pre- and postexposure test in the air-exposed animals (control). The dichloroacetylene-mediated evoked potential changes persisted at least until day 4 postexposure. No changes in evoked potentials were observed in the 175 ppm TCE/1,030 ppm acetylene mix group. It is noteworthy that dichloroacetylene treatment produced broader evidence of toxicity as witnessed by a persistent drop in body weight among subjects over the 7-day postexposure measuring period. In light of the differences observed between the effects of TCE and dichloroacetylene on the trigeminal nerve, it would be instructive to calculate the dose of TCE that would be necessary to produce comparable tissue levels of dichloroacetylene produced in the Albee et al. (1997) study.

Kulig (1987) also measured peripheral (caudal nerve) nerve conduction time in male Wistar rats and failed to show an effect of TCE with exposures as high as 1,500 ppm for 16 hours/day, 5 days/week for 18 weeks.

D.2.2. Auditory Effects

D.2.2.1. Inhalation

The ability of TCE to disrupt auditory function and produce inner ear histopathology abnormalities has been demonstrated in several studies using a variety of test methods. Two

different laboratories have identified NOAELs for auditory function of 1,600 ppm following inhalation exposure for 12 hours/day for 13 weeks in Long-Evans rats (n = 6–10) (Rebert et al., 1991) and 1,500 ppm in Wistar-derived rats (n = 12) exposed by inhalation for 18 hours/day, 5 days/week for 3 weeks (Jaspers et al., 1993). The LOAELs identified in these and similar studies are 2,500–4,000-ppm TCE for periods of exposure ranging from 4 hours/day for 5 days to 12 hours/day for 13 weeks (e.g., Albee et al., 2006; Boyes et al., 2000; Muijser et al., 2000; Fechter et al., 1998; Crofton and Zhao, 1997; Rebert et al., 1995; Crofton et al., 1994; Rebert et al., 1993). Rebert et al. (1993) estimated acute blood TCE levels associated with permanent hearing impairment at 125 μg/mL by methods that probably underestimated blood TCE values (rats were anaesthetized using 60% carbon dioxide). A summary of these studies is presented in Table D-5.

Rebert et al. (1991) evaluated auditory function in male Long-Evans rats (n = 10) and F344 rats (n = 4–5) by measuring brainstem auditory-evoked responses (BAERs) following stimulation with 4-, 8-, and 16-kHz sounds. The Long-Evans rats were exposed to 0, 1,600, or 3,200 ppm TCE, 12 hour/day for 12 weeks and the F344 rats were exposed to 0, 2,000, or 3,200 ppm TCE, 12 hours/day for 3 weeks. BAERs were measured every 3 weeks during the exposure and then for an additional 6 weeks following the end of exposure. For the F344 rats, both TCE exposures (2,000 and 3,200 ppm) significantly decreased BAER amplitudes at all frequencies tested. In comparison, Long-Evans rats exposed to 3,200 ppm TCE also had significantly decreased BAER amplitude, but exposure to 1,600 ppm did not significantly affect BAERs at any stimulus frequency. These data suggest a LOAEL of 2,000 ppm for the F344 rats and a NOAEL of 1,600 ppm for the Long-Evans rats. In subsequent studies, Rebert et al. (1995; 1993) again demonstrated TCE significantly decreases BAER amplitudes and significantly increases the latency of the initial peak (identified as P1).

Jaspers et al. (1993) exposed Wistar-derived WAG-Rii/MBL rats (n = 12) to 0, 1,500 and 3,000 ppm TCE exposure for 18 hours/day, 5 days/week for 3 weeks. Auditory function for each frequency was assessed by reflex modification (recording the decibel threshold required to generate a startle response from the rat). Three tones (5, 20, and 35 kHz) were used to test auditory function. The startle measurements were made prior to exposure and at 1, 3, 5, and 6 weeks after exposure. A selective impairment of auditory threshold for animals exposed to 3,000-ppm TCE was observed at all postexposure times at 20 kHz only. No significant effects were noted in rats exposed to 1,500 ppm TCE. This auditory impairment was persistent up through 6 weeks after exposure, which was the last time point presented. There was no impairment of hearing at either 5 or 25 kHz for animals exposed to 1,500 or 3,000 ppm TCE. This study indicates TCE selectively produces a persistent mid-frequency hearing loss and identifies a NOAEL of 1,500 ppm. Similarly, Crofton et al. (1994) exposed male Long-Evans rats (n = 7–8) to 3,500 ppm TCE, 8 hours/day for 5 days. Auditory thresholds were determined

by reflex modification audiometry 5–8 weeks after exposure. TCE produced a selective impairment of auditory threshold for mid frequency tones, 8 and 16 kHz.

Muijser et al. (2000) evaluated the ability of TCE to potentiate the damaging effect of noise on hearing. Wistar rats (n = 8 per group) were exposed by inhalation to 0 or 3,000 ppm TCE alone for 18 hours/day, 5 days/week for 3 weeks (no noise) or in conjunction with 95-dB broad band noise. The duration of noise exposure is not specified, but presumably was also 18 hours/day, 5 days/week for 3 weeks. Pure tone auditory thresholds were determined using reflex modification audiometry 1 and 2 weeks following the exposures. Significant losses in auditory sensitivity were observed for rats exposed to noise alone at 8, 16, and 20 kHz, for rats exposed to TCE alone at 4, 8, 16, and 20 kHz and for combined exposure subjects at 4, 8, 16, 20, and 24 kHz. The loss of hearing sensitivity at 4 kHz is particularly striking for the combined exposure rats, suggesting a potentiation effect at this frequency. Impairment on this auditory test suggests toxicity at the level of the cochlea or brainstem.

Fechter et al. (1998) exposed Long-Evans rats inhalationally to 0 or 4,000 ppm TCE 6 hours/day for 5 days. Three weeks later, auditory thresholds were assessed by reflex modification audiometry (n = 12), and then 5–7 weeks later, cochlear function was assessed by measuring compound action potentials (CAPs) and the cochlear microphonic response (n = 3–10). Cochlear histopathology was assessed at 5–7 weeks (n = 4) using light microscopy. Reflex modification thresholds were significantly elevated at 8 and 18 kHz, as were CAP thresholds. The growth of the N1 evoked potential was reduced in the TCE group, and they failed to show normal N1 amplitudes even at supra-threshold tone levels. There was no effect on the sound level required to elicit a cochlear microphonic response of 1 μV. Histological data suggest that TCE produces a loss of spiral ganglion cells.

Albee et al. (2006) exposed male and female F344 rats to TCE at 250, 800, or 2,500 ppm for 6 hours/day, 5 days/week, for 13 weeks. At 2,500 ppm TCE, mild frequency-specific hearing deficits were observed, including elevated tone-pip auditory brainstem response thresholds. Focal loss of hair cells in the upper basal turn of the cochlea was observed at 2,500 ppm; this was apparently based upon midmodiolar sections, which lack power in quantification of hair cell death. Except for the cochleas of rats at 2,500 ppm, no treatment-related lesions were noted during the neuro-histopathologic examination. The NOAEL for this study was 800 ppm based on ototoxicity at 2,500 ppm.

The relationship between dose and duration of exposure with respect to producing permanent auditory impairment was presented in Crofton and Zhao (1997) and again in Boyes et al. (2000). The LOAELs identified in Long-Evans rats (n = 10–12) were 6,000 ppm for a 1-day exposure, 3,200 ppm per day for both the 1- and 4-week exposures, and 2,400 ppm per day for the 13-week exposure. It was estimated from these data that the LOAEL for a 2-year long exposure would be 2,100 ppm. Auditory thresholds were determined for a 16-kHz tone 3–5 weeks after exposure using reflex modification audiometry. Results replicated previous

findings of a hearing loss at 16 kHz for all exposure durations. One other conclusion reached by this study is that TCE concentration and not concentration × duration of exposure is a better predictor of auditory toxicity. That is, the notion that total exposure represented by the function, concentration (C) × time (t), or Haber's law, is not supported. Therefore, higher exposure concentrations for short durations are more likely to produce auditory impairment than are lower concentrations for more protracted durations when total dosage is equated. Thus, consideration needs to be given not only to total C × t, but also to peak TCE concentration.

Crofton and Zhao (1997) also presented a BMD for which the calculated dose of TCE would yield a 15-dB loss in auditory threshold. This BMR was selected because a 15-dB threshold shift represents a significant loss in threshold sensitivity for humans. The benchmark concentrations for a 15-dB threshold shift are 5,223 ppm for 1 day, 2,108 ppm for 5 days, 1,418 ppm for 20 days, and 1,707 ppm for 65 days of exposure. While more sensitive test methods might be used and other definitions of a benchmark effect chosen with a strong rationale, these data provide useful guidance for exposure concentrations that do yield hearing loss in rats.

These data demonstrate that the ototoxicity of TCE was less than that predicted by a strict concentration × time relationship. These data also demonstrate that simple models of extrapolation (i.e., $C \times t = k$, Haber's Law) overestimate the potency of TCE when extrapolating from short-duration to longer-duration exposures. Furthermore, these data suggest that, relative to ambient or occupational exposures, the ototoxicity of TCE in the rat is a high-concentration effect; however, the selection of a 15-dB threshold for detecting auditory impairment along with tests at a single auditory frequency may not capture the most sensitive reliable measure of hearing impairment.

With the exception of a single study performed in the Hartley guinea pig (n = 9–10) (Yamamura et al., 1983), there are no data in other laboratory animals related to TCE-induced ototoxicity. Yamamura et al. (1983) exposed Hartley guinea pigs to TCE at doses of 6,000, 12,000, and 17,000 ppm for 4 hours/day for 5 days and failed to show an acute impairment of auditory function. However, despite the negative finding in this study, it should be considered that auditory testing was performed in the middle of a laboratory and not in an audiometric sound attenuating chamber. The influence of extraneous and uncontrolled noise on cochlear electrophysiology is marked and assesses auditory detection thresholds in such an environment unrealistic. Although the study has deficiencies, it is important to note that the guinea pig has been reported to be far less sensitive than the rat to the effects of ototoxic aromatic hydrocarbons such as toluene.

It may be helpful to recognize that the effects of TCE on auditory function in rats are quite comparable to the effects of styrene (e.g., Campo et al., 2006; Crofton et al., 1994; Pryor et al., 1987), toluene (e.g., Campo et al., 1999; Pryor et al., 1983), ethylbenzene (e.g, Fechter et al., 2007; Cappaert et al., 2000; Cappaert et al., 1999), and *p*-xylene (e.g., Gagnaire et al., 2001; Pryor et al., 1987). All of these aromatic hydrocarbons produce reliable impairment at the

peripheral auditory apparatus (inner ear), and this impairment is associated with death of sensory receptor cells, the outer hair cells. In comparing potency of these various agents to produce hearing loss, it appears that TCE is approximately equipotent to toluene and less potent than, in order, ethylbenzene, *p*-xylene, and styrene. Occupational epidemiological studies do appear to identify auditory impairments in workers who are exposed to styrene (Morata et al., 2002; Morioka et al., 2000; Sliwińska-Kowalska et al., 1999) and those exposed to toluene (Morata et al., 1997; Abbate et al., 1993), particularly when noise is also present.

D.2.2.2. Oral and Injection Studies

No experiments were identified in which auditory function was assessed following TCE administration by either oral or injection routes.

D.2.3. Vestibular System Studies

The effect of TCE on vestibular function was evaluated by either: (1) promoting nystagmus (vestibular system dysfunction) and comparing the level of effort required to achieve nystagmus in the presence and absence of TCE or (2) using an elevated beam apparatus and measuring the balance. Overall, it was found that TCE disrupts vestibular function as presented below. A summary of these studies is found in Table D-6.

Tham et al. (1984; 1979) demonstrated disruption in the stimulated vestibular system in rabbits and Sprague-Dawley rats during i.v. infusion with TCE. It is difficult to determine the dosage of TCE necessary to yield acute impairment of vestibular function since testing was performed under continuing infusion of a lipid emulsion containing TCE, and therefore, blood TCE levels were increasing during the course of the study. Tham et al. (1979), for example, infused TCE at doses of 1–5 mg/kg-minute reaching arterial blood concentrations as high as 100 ppm. They noted increasing numbers of rabbits experiencing positional nystagmus as blood TCE levels increased. The most sensitive rabbit showed nystagmus at a blood TCE concentration of about 25 ppm. Similarly, the Sprague-Dawley rats also experienced increased nystagmus with a threshold effect level of 120 ppm as measured in arterial blood (Tham et al., 1984). Animals demonstrated a complete recovery in vestibular function when evaluated for nystagmus within 5–10 minutes after the i.v. infusion was stopped.

Niklasson et al. (1993) showed acute impairment of vestibular function in male and female pigmented rats during acute inhalation exposure to TCE (2,700–7,200 ppm) and to trichloroethane (500–2,000 ppm). Both of these agents were able to promote nystagmus during optokinetic stimulation in a dose related manner. While there were no tests performed to assess persistence of these effects, Tham et al. (1984; 1979) did find complete recovery of vestibular function in rabbits (n = 19) and female Sprague-Dawley rats (n = 11) within minutes of terminating a direct arterial infusion with TCE solution.

The finding that TCE can yield transient abnormalities in vestibular function is not unique. Similar impairments have been shown for toluene, styrene, along with trichloroethane (Niklasson et al., 1993) and by Tham et al. (1984) for a broad range of aromatic hydrocarbons. The concentration of TCE in blood at which effects were observed for TCE (0.9 mM/L) was quite close to that observed for most of these other vestibulo-active solvents.

D.2.4. Visual Effects

Changes in visual function have also been demonstrated in animal studies following acute (Boyes et al., 2005a; Boyes et al., 2003) and subchronic exposure (Blain et al., 1994). Summary of all TCE studies evaluating visual effects in animals can be found in Table D-6. In these studies, the effect of TCE on visual-evoked responses to patterns (Boyes et al., 2005a; Boyes et al., 2003; Rebert et al., 1991) or a flash stimulus (Blain et al., 1994; Rebert et al., 1991) were evaluated. Overall, the studies demonstrated that exposure to TCE results in significant changes in the visual evoked response, which is reversible once TCE exposure is stopped. Only one study (Rebert et al., 1991) did not demonstrate changes in visual system function with a subchronic TCE exposure, but visual testing was conducted 10 hours after each exposure.

Boyes et al. (2005a; 2003) found significant reduction in the VEP acutely while Long-Evans male rats were being exposed to TCE concentrations of 500, 1,000, 2,000, 3,000, 4,000, and 5,000 ppm for intervals ranging from 4 to 0.5 hours, respectively. In both instances, the degree of effect correlated more with brain TCE concentrations than with duration of exposure.

Boyes et al. (2003) exposed adult, male Long-Evans rats to TCE in a head-only exposure chamber while pattern onset/offset VEPs were recorded. Exposure conditions were designed to provide C × t products of 0 ppm/hour (0 ppm for 4 hours) or 4,000 ppm/hour created through four exposure scenarios: 1,000 ppm for 4 hours; 2,000 ppm for 2 hours; 3,000 ppm for 1.3 hours; or 4,000 ppm for 1 hour (n = 9–10/concentration). Blood TCE concentrations were assessed by GC with ECD, and brain TCE concentrations were estimated using a PBPK model. The amplitude of the VEP frequency double component (F2) was decreased significantly ($p < 0.05$) by exposure. The mean amplitude (± SEM in μV) of the F2 component in the control and treatment groups measured 4.4 ± 0.5 (0 ppm/4 hours), 3.1 ± 0.5 (1,000 ppm/4 hours), 3.1 ± 0.4 (2,000 ppm/2 hours), 2.3 ± 0.3 (3,000 ppm/1.3 hours), and 1.9 ± 0.4 (4,000 ppm/1 hour). A PBPK model was used to estimate the concentrations of TCE in the brain achieved during each exposure condition. The F2 amplitude of the VEP decreased monotonically as a function of the estimated peak brain concentration but was not related to the area under the curve of the brain TCE concentration. These results indicate that an estimate of the brain TCE concentration at the time of VEP testing predicted the effects of TCE across exposure concentrations and duration.

In a follow-up study, Boyes et al. (2005a) exposed Long-Evans male rats (n = 8–10/concentration) to TCE exposures of 500 ppm for 4 hours, 1,000 ppm for 4 hours, 2,000 ppm for 2 hours, 3,000 ppm for 1.3 hours, 4,000 ppm for 1 hour, and 5,000 ppm for 0.8 hour. VEP

recordings were made at multiple time points, and their amplitudes were adjusted in proportion to baseline VEP data for each subject. VEP amplitudes were depressed by TCE exposure during the course of TCE exposure. The degree of VEP depression showed a high correlation with the estimated brain TCE concentration for all levels of atmospheric TCE exposure.

This transient effect of TCE on the peripheral visual system has also been reported by Blain (1994) in which New Zealand albino rabbits were exposed by inhalation to 350 and 700 ppm TCE 4 hours/day, 4 days/week for 12 weeks. ERGs and OPs were recorded weekly under mesopic conditions. Recordings from the 350 and 700 ppm exposed groups showed a significant increase in the amplitude of the a- and b-waves (ERG). The increase in the a-wave was dose related increasing 30% at the low dose and 84% in the high dose. For the b-wave, the lower exposure dose yielded a larger change from baseline (52%) than did the high dose (33%). The amplitude of the OPs was significantly decreased at 350 ppm (57%) and increased at 700 ppm (117%). The decrease in the OPs shown in the low-dose group appears to be approximately 25% from 9 to 12 weeks of exposure. These electroretinal changes were reversed to the baseline value within 6 weeks after the inhalation stopped.

Rebert et al. (1991) evaluated VEPs (flash evoked potentials and pattern reversal evoked potentials) in male Long-Evans rats that received 1,600 or 3,200 ppm TCE for 3 weeks 12 hours/day. No significant changes in flash evoked potential measurements were reported following this exposure paradigm. Limited shifts in pattern reversal VEPs were reported during subchronic exposure, namely a reduction in the N1-P1 response amplitude that reached statistical significance following 8, 11, and 14 weeks of exposure. The drop in response amplitude ranged from approximately 20% after 8 weeks to nearly 50% at week 14. However, this potential recovered completely during the recovery period.

D.2.5. Cognitive Function

There have been a number of reports (e.g., Kishi et al., 1993; Kulig, 1987; Kjellstrand et al., 1980) showing alteration in performance in learning tasks such as a change in speed to complete the task, but little evidence that learning and memory function are themselves impaired by exposure. Table D-7 presents the study summaries for animal studies evaluating cognitive effects following TCE exposure. Such data are important in efforts to evaluate the functional significance of decreases in myelinated fibers in the hippocampus reported by Isaacson et al. (1990) and disruption of long-term potentiation discovered through in vitro testing (Ohta et al., 2001) since the hippocampus has been closely tied to memory formation.

Kjellstrand et al. (1980) exposed Mongolian gerbils (n = 12/sex**)** to 900 ppm TCE by inhalation for 9 months. Inhalation was continuous except for 1–2 hours/week for cage cleaning. Spatial memory was tested using the radial arm maze task. In this task, the gerbils had to visit each arm of the maze and remember which arm was visited and unvisited in selecting an arm to visit. The gerbils received training and testing in a radial arm maze starting after 2 months of

TCE exposure. There was no effect of TCE on learning or performance on the radial arm maze task.

Kishi et al. (1993) acutely exposed Wistar rats to TCE at concentrations of 250, 500, 1,000, 2,000, and 4,000 ppm for 4 hours. Rats were tested on an active (light) signaled shock avoidance operant response. Rats exposed to 250 ppm TCE showed a significant decrease both in the total number of lever presses and in avoidance responses at 140 minutes of exposure compared with controls. The rats did not recover their pre-exposure performance until 140 minutes after the exhaustion of TCE vapor. Exposures in the range 250–2,000 ppm TCE for 4 hours produced concentration related decreases in the avoidance response rate. No apparent acceleration of the RT was seen during exposure to 1,000 or 2,000 ppm TCE. The latency to a light signal was somewhat prolonged during the exposure to 2,000–4,000 ppm TCE. It is estimated that there was depression of the CNS with slight performance decrements and the corresponding blood concentration was 40 μg/mL during exposure. Depression of the CNS with anesthetic performance decrements was produced by a blood TCE concentration of about 100 μg/mL. In general, the authors observed dose related reductions in total number of lever presses, but these changes may be more indicative of impaired motor performance than of cognitive impairment. In any event, recovery occurred rapidly once TCE exposure ceased.

Isaacson et al. (1990) studied the effects of oral TCE exposure in weanling rats at exposure doses of 5.5 mg/day for 4 weeks, followed by an additional 2 weeks of exposure at 8.5 mg/day. No significant changes were observed in locomotor activity in comparison to the control animals. This group actually reported improved performance on a Morris swim test of spatial learning as reflected in a decrease in latency to find the platform from 14 seconds in control subjects to 12 seconds in the lower dose TCE group to a latency of 9 seconds in the higher TCE group. The high-dose group differed significantly from the control and low-dose groups while these latter two groups did not differ significantly from each other. This improvement relative to the control subjects occurred despite a loss in hippocampal myelination, which approached 8% and was shown to be significant using Duncan's multiple range test.

Likewise, Umezu et al. (1997) exposed ICR strain male mice acutely to doses of TCE ranging from 62.5 to 1,000 mg/kg depending upon the task. They reported a depressed rate of operant responding in a conditioned avoidance task that reached significance with i.p. injections of 1,000 mg/kg. Increased responding during the signaled avoidance period at lower doses (250 and 500 mg/kg) suggests an impairment in ability to inhibit responding or failure to attend to the signal. However, all testing was performed under TCE intoxication.

D.2.6. Psychomotor Effects

Changes in psychomotor activity such as loss of righting reflex, FOB changes, and locomotor activity have been demonstrated in animals following exposure to TCE. Summaries for some of these studies can be found below and are presented in detail in Table D-8.

D.2.6.1. Loss of Righting Reflex

Kishi et al. (1993) evaluated the activity and performance of male Wistar rats in a series of tasks following an acute 4-hour exposure to 250, 500, 1,000, 2,000, and 4,000 ppm. They reported disruption in performance at the highest test levels with CNS depression and anesthetic performance decrements. Blood TCE concentrations were about 100 µg/mL in Wistar rats (such blood TCE concentrations were obtained at inhalation exposure levels of 2,000 ppm).

Umezu et al. (1997) studied disruption of the righting reflex following acute injection of 250, 500, 1,000, 2,000, 4000, and 5,000 mg/kg TCE in male ICR mice. At 2,000 mg/kg, loss of righting reflex (LORR) was observed in only 2/10 animals injected. At 4,000 mg/kg, 9/10 animals experienced LORR, and 100% of the animals experienced LORR at 5,000 mg/kg. Shih et al. (2001) reported impaired righting reflexes at exposure doses of 5,000 mg/kg in male Mf1 mic although lower exposure doses were not included. They showed, in addition, that pretreatment prior to TCE with DMSO or disulfiram (which is a CYP2E1 inhibitor) in DMSO could delay loss of the righting reflex in a dose related manner. By contrast, the alcohol dehydrogenase inhibitor, 4-metylpyradine did not delay loss of the righting reflex that resulted from 5,000 mg/kg TCE. These data suggest that the anesthetic properties of TCE involve its oxidation via CYP2E1 to an active metabolite, a finding that is consistent with the anesthetic properties of CH.

D.2.6.2. FOB and Locomotor Activity Studies

D.2.6.2.1. FOB and locomotor activity studies with TCE.

A number of papers have measured locomotor activity and used FOBs in order to obtain a more fine grained analysis of the motor behaviors that are impaired by TCE exposure. While exposure to TCE has been shown repeatedly to yield impairments in neuromuscular function acutely, there is very little evidence that the effects persist beyond termination of exposure.

One of the most extensive evaluations of TCE on innate neurobehavior was conducted by Moser et al. (2003; 1999) using FOB testing procedures. Moser et al. (1995) evaluated the effects of acute and subacute (14-day) gavage administration of TCE in adult female F344 rats. Testing was performed both 4 hours post TCE administration and 24 hours after TCE exposure, and a comparison of these two time points along with comparison between the first day and the last day of exposure provides insight into the persistence of effects observed. Various outcome measures were grouped into five domains: autonomic, activity, excitability, neuromuscular, and sensorimotor. Examples of tests included in each of these groupings are as follows: autonomic—lacrimation, salivation, palpebral closure, pupil response, urination, and defecation; activity—rearing, motor activity counts home cage position; excitability—ease of removal, handling reactivity, arousal, clonic, and tonic movements; and neuromuscular—gait score, righting reflex, fore- and hindlimb grip strength, and landing foot splay; sensorimotor-tail-pinch

response, click response, touch response, and approach response. Scoring was performed on a 4-point scale ranging from "1" (normal) to "4" (rare occurrence for control subjects). In the acute exposure, the exposure doses utilized were 150, 500, 1,500, and 5,000 mg/kg TCE in corn oil. These doses represent 3, 10, 30, and 56% of the limit dose. For the 14-day subacute exposure, the doses used were 50, 150, 500, and 1,500 mg/kg. Such doses represent 1, 3, 10, and 30% of the limit dose for TCE.

The main finding for acute TCE administration is that a significant reduction in activity level occurred after the highest dose of TCE (5,000 mg/kg) only. This effect showed substantial recovery 24 hours after exposure though residual decrements in activity were noted. Neuromuscular function as reflected in the gait score was also severely affected only at 5,000-mg/kg dose and only at the 4-hour test period. Sensorimotor function reflected in response to a sudden click, was abnormal at both 1,500 and 5,000 mg/kg with a slight difference observed at 1,500 mg/kg and a robust difference apparent at 5,000 mg/kg. Additional effects noted, but not shown quantitatively were abnormal home-cage posture, increased landing foot splay, impaired righting and decreased fore and hind limb grip strength. It is uncertain at which doses such effects were observed.

With the exception of sensorimotor function, these same categories were also disrupted in the subacute TCE administration portion of the study. The lack of effect of TCE on sensorimotor function with repeated TCE dosing might reflect either habituation, tolerance, or an unreliable measurement at one of the time points. Given the absence of effect at a range of exposure doses, a true dose-response relationship cannot be developed from these data.

In the subacute study, there are no clearly reliable dose-related differences observed between treated and control subjects. Rearing, a contributor to the activity domain, was elevated in the 500-mg/kg dose group, but was normal in the 1,500-mg/kg group. The neuromuscular domain was noted as significantly affected at 15 days, but it is not clear which subtest was abnormal. It appears that the limited group differences may be random among subjects unrelated to exposure condition.

In a follow-up study, Moser et al. (2003) treated female F344 rats with TCE by gavage for periods of 10 days at doses of 0, 40, 200, 800, and 1,200 mg/kg-day, and testing was undertaken either 4 hours following the first or 10^{th} dose as well as 24 hours after these two time points. The authors identified several significant effects produced by TCE administration including a decrease in motor activity, tail pinch responsiveness, reactivity to handling, hind limb grip strength, and body weight. Rats administered TCE also showed significantly more piloerection, higher gait scores, lethality, body weight loss, and lacrimation compared to controls. Only effects observed 4 hours after the 10^{th} exposure dose were presented by the authors, and no quantitative information of these measurements is provided.

Albee et al. (2006) exposed male and female F344 rats to 250, 800, and 2,500 ppm TCE for 6 hours/day, 5 days/week for 13 weeks. FOB was performed 4 days prior to exposure and

then monthly. Auditory impairments found by others (e.g., Boyes et al., 2000; Muijser et al., 2000; Fechter et al., 1998; Crofton and Zhao, 1997; Rebert et al., 1995; Crofton et al., 1994) were replicated at the highest exposure dose, but treatment related differences in grip strength or landing foot splay were not demonstrated. The authors report slight increases in handling reactivity among female rats and slightly more activity than in controls at an intermediate time point, but apparently did not conduct systematic statistical analyses of these observations. In any event, there were no statistically significant effects on activity or reactivity by the end of exposure.

Kulig (1987) also failed to show significant effects of TCE inhalation exposure on markers of motor behavior. Wistar rats exposed to 500, 1,000, and 1,500 ppm for 16 hours/day, 5 days/week for 18 weeks failed to show changes in spontaneous activity, grip strength, or coordinated hind limb movement. Measurements were made every three weeks during the exposure period and occurred between 45 and 180 minutes following the previous TCE inhalation exposure. This study establishes a NOAEL of 1,500 ppm TCE with an exposure duration of 16 hours/day.

D.2.6.2.2. **Acute and subacute oral exposure to DCA on functional observational batteries (FOB).**

Moser et al. (1999) conducted a series of experiments on DCA ranging from acute to chronic exposures. The exposure doses used in the acute experiment were 100, 300, 1,000, and 2,000 mg/kg. In the repeated exposure studies (8 weeks–24 months), doses varied between 16 and 1,000 mg/kg-day. The authors showed pronounced neuromuscular changes in Long-Evans and F344 rats dosed orally with the TCE metabolite, DCA, over a period ranging from 9 weeks to 24 months at different exposure doses. Using a multitude of exposure protocols, which most commonly entailed daily exposures to DCA either by gavage or drinking water, the authors identify effects that were "mostly limited" to the neuromuscular domain. These included disorders of gait, grip strength, foot splay, and righting reflex that are dose and duration dependent. Data on gait abnormality and grip strength are presented in greatest detail. In adults exposed to DCA by gavage, gait scores were "somewhat abnormal" at the 7-week test in both the adult Long-Evans rats receiving 300 mg/kg-day and those receiving 1,000 mg/kg-day. There was no adverse effect in the rats receiving 100 mg/kg-day. In the chronic study, which entailed intake of DCA via drinking water yielding an estimated daily dose of 137 and 235 mg/kg-day, "moderately to severely abnormal" gait was observed within 2 months of exposure and dosing was either reduced or discontinued because of the severity of toxicity. For the higher DCA dose, gait scores remained "severely abnormal" at the 24-month test time even though the DCA had been discontinued at the 6-month test time. Hindlimb grip strength was reduced to about half the control value in both exposure doses and remained reduced throughout the 24 months of testing

even though DCA administration ceased at 6 months for the 235 mg/kg-day group. Forelimb grip strength showed a smaller and apparently reversible effect among DCA-treated rats.

D.2.6.3. Locomotor Activity

Wolff and Siegmund (1978) administered 182 mg/kg TCE (i.p.) in AB mice and observed a decrease in spontaneous locomotor activity. In this study, AB mice were injected with TCE 30 minutes prior to testing for spontaneous activity at one of four time points during a 24 hours/day (0600, 1200, 1800, and 2400 hours). Marked decreases (estimated 60–80% lower than control mice) in locomotor activity were reported in 15-minute test periods. The reduction in locomotion was particularly profound at all time intervals save for the onset of light (0600). Nevertheless, even at this early morning time point, activity was markedly reduced from control levels (60% lower than controls as approximated from a graph).

Moser et al. (2003; 1995) included locomotor activity as one of their measures of neurobehavioral effects of TCE given by gavage over a 10–14-day period. In the 1995 paper, female F344 rats were dosed either acutely with 150, 500, 1,500 or 5,000 mg/kg TCE or for 14 days with 50, 150, 500 or 1,500 mg/kg. In terms of the locomotor effects, they report that acute exposure produced impaired locomotor scores only at 5,000 mg/kg while in the subacute study, locomotion was impaired at the 500 mg/kg dose, but not at the 1,500 mg/kg dose. In the Moser (2003) study, it appears that 200 mg/kg TCE may actually have increased locomotor activity, while the higher test doses (800 and 1,200 mg/kg) decreased activity in a dose related manner. What is common to both studies, however, is a depression in motor activity that occurs acutely following TCE administration and which may speak to the anesthetic, if not CNS depressive, effects of this solvent.

There are also a number of reports (Waseem et al., 2001; Fredriksson et al., 1993; Kulig, 1987) that failed to demonstrate impairment of motor activity or ability following TCE exposure. Waseem et al. (2001) failed to show effects of TCE given in the drinking water of Wistar rats over the course of a 90-day trial. While nominal solvent levels were 350, 700, and 1,400 ppm in the water, no estimate is provided of daily TCE intake or of the stability of the TCE solution over time. However, assuming a daily water intake of 25 mL/day and body weight of 330 g, these exposures would be estimated to be approximately 26, 52, and 105 mg/kg. These doses are far lower than those studied by Moser and colleagues.

Fredriksson et al. (1993) studied the effects of TCE given by gavage to male NMRI mice at doses of 50 and 290 mg/kg-day from PNDs 10 to 16 on locomotion assessed either on the day following exposure or at age 60 days. They found no significant effect of TCE on locomotor activity and no consistent effects on other motor behaviors (e.g., rearing).

Waseem et al. (2001) studied locomotor activity in Wistar rats exposed for up to 180 days to 376-ppm TCE by inhalation for 4 hours/day, 5 days/week and acutely intoxicated with TCE. Here, the authors report seemingly inconsistent effects of TCE on locomotion. After 30 days of

exposure, the treated rats show an increase in locomotor activity relative to control subjects. However, after 60 days of exposure, they note a significant *increase* in distance traveled found among experimental subjects, but a decrease in horizontal activity in this experimental group. Moreover, the control subjects vary substantially in horizontal counts among the different time periods. No differences between the treatment groups are found after 180 days of exposure. It is difficult to understand the apparent discrepancy in results reported at 60 days of exposure.

D.2.7. Sleep and Mood Disorders

D.2.7.1. Effects on Mood: Laboratory Animal Findings

It is difficult to obtain comparable data of emotionality in laboratory studies. However, Moser et al. (2003) and Albee et al. (2006) both report increases in handling reactivity among rats exposed to TCE. In the Moser study, female F344 rats received TCE by gavage for periods of 10 days at doses of 0, 40, 200, 800, and 1,200 mg/kg-day, while Albee et al. (2006) exposed F344 rats to TCE by inhalation at exposure doses of 250, 800, and 2,500 ppm for 6 hours/day, 5 days/week for 13 weeks.

D.2.7.2. Sleep Disturbances

Arito et al. (1994) exposed male Wistar rats to 50, 100, and 300 ppm TCE for 8 hours/day, 5 days/week for 6 weeks and measured EEG responses. EEG responses were used as a measure to determine the number of awake (wakefulness hours) and sleep hours. Exposure to all of the TCE levels significantly decreased amount of time spent in wakefulness during the exposure period. Some carry over was observed in the 22-hour postexposure period with significant decreases in wakefulness seen at 100-ppm TCE. Significant changes in wakefulness-sleep elicited by the long-term exposure appeared at lower exposure levels. These data seem to identify a low dose of TCE that has anesthetic properties and established a LOAEL of 50 ppm for sleep changes.

D.2.8. Mechanistic Studies

D.2.8.1. Dopaminergic Neurons

In two separate animal studies, subchronic administration of TCE has resulted in a decrease of dopaminergic cells in both rats and mice. Although the mechanism for dopaminergic neurons resulting from TCE exposure is not elucidated, disruption of dopaminergic-containing neurons has been extensively studied with respect to Parkinson's disease and parkinsonism. In addition to Parkinson's disease, significant study of MPTP and of high-dose manganese toxicity provides strong evidence for extrapyramidal motor dysfunction accompanying loss of dopamine neurons in the substantia nigra. These databases may provide useful comparisons to the highly limited database with regard to TCE and dopamine neuron effects. The studies are presented in Table D-9.

Gash et al. (2008) assessed the effects of subchronic TCE administration on dopaminergic neurons in the CNS. F344 male rats were orally administered by gavage

1,000 mg/kg TCE in olive oil, 5 days/week for 6 weeks. Degenerative changes in dopaminergic-containing neurons in the substantia nigra were reported as indexed by a 45% decrease in the number of tyrosine hydroxylase positive cells. Additionally, there was a decrease in the ratio of 3,4-dihydroxyphenylacetic acid, a metabolite of dopaminergic, to dopaminergic levels in the striatum. This shift in ratio, on the order of 35%, was significant by Student's t-test, suggesting a decrease in release and utilization of this neurotransmitter. While it is possible that long-term adaptation might occur with regard to release rates for dopaminergic, the loss of dopaminergic cells in the substantia nigra is viewed as a permanent toxic effect. The exposure level used in this study was limited to one high dose and more confidence in the outcome will depend upon replication and development of a dose-response relationship. If the results are replicated, they might be important in understanding mechanisms by which TCE produces neurotoxicity in the CNS. The functional significance of such cellular loss has not yet been determined through behavioral testing.

Guehl (1999) also reported persistent effects of TCE exposure on dopaminergic neurons. In this study, OF1 male mice (n = 10) were injected i.p. daily for 5 days/week for 4 weeks with TCE (400 mg/kg-day). Following a 7-day period when the subjects did not receive TCE, the mice were euthanized and tyrosine hydroxylase immunoreactivity was used to measure neuronal death in the substantia nigra pars compacta. Treated mice presented significant dopaminergic neuronal death (50%) in comparison with control mice based upon total cell counts conducted by an examiner blinded as to treatment group in six samples per subject. The statistical comparison appears to be by Student's t-test (only means, SDs, and a probability of $p < 0.001$ are reported). While this study appears to be consistent with that of Gash et al. (2008), there are some limitations of this study. Specifically, no photomicrographs are provided to assess adequacy of the histopathological material. Additionally, no dose-response data are available to characterize dose-response relationships or identify either a BMD or NOAEL. Behavioral assessment aimed at determining functional significance was not determined.

The importance of these two studies suggesting death of dopaminergic neurons following TCE exposure may be addressable by human health studies because they suggest the potential for TCE to produce a parkinsonian syndrome.

D.2.8.2. GABA and Glutamatergic Neurons

Disruption of GABAergic and glutamatergic neurons by toxicants can represent serious impairment as GABA serves as a key inhibitory neurotransmitter while glutamate is equally important as an excitatory neurotoxicant. Moreover, elevations in glutamatergic release have been identified as an important process by which more general neurotoxicity can occur through a process identified as excitotoxicity. The data, with regard to TCE exposure and alteration in GABA and glutamate function, are limited. The studies are presented in Table D-10.

Briving et al. (1986) conducted a chronic inhalation exposure in Mongolian gerbils to 50- and 150-ppm TCE continuously for 12 months and reported the changes in amino acids levels in the hippocampus and cerebellar vermis and on high affinity uptake of GABA and glutamate in those same structures. A dose-related elevation of glutamine in the hippocampus of approximately 20% at 150 ppm was reported, but no other reliable changes in amino acids in either of these two structures. With regard to high affinity uptake of glutamate and GABA, there were no differences in the hippocampal uptake between control and treated gerbils although in the cerebellar vermis there was a dose related elevation in the high affinity uptake for both of these neurotransmitter. Glutamate uptake was increased about 50% at 50 ppm and 100% at 150 ppm. The corresponding increases for GABA were 69 and 74%. Since control tissue uptake is identified as being 100% rather than as an absolute rate, the ability to assess quality of the control data are limited. It is unclear if this finding in cerebellar vermis is also present in other brain tissues and should be studied further. If these findings are reliable, then the changes in high affinity uptake in cerebellum for GABA and glutamate might represent alterations that could have functional outcomes. For example, alteration in GABA release and reuptake from the cerebellum might be consistent with acute alteration in vestibular function described below. However, there are presently no compelling data to support such a relationship.

The change in hippocampal glutamine levels is not readily interpretable. What is not clear from this paper is whether the alterations observed were acute effects observable only while subjects were intoxicated with TCE or whether they would persist once TCE had been removed from the neural tissue. This study used inhalation doses that were at least 1 order of magnitude lower than those required to produce auditory impairment.

A study by Shih et al. (2001) provides indirect evidence in male Mf1 mice that TCE exposure by injection might alter GABAergic function. The mice were injected i.p. with 250, 500, 1,000 and 2,000 mg/kg TCE in corn oil and the effect of these treatments on susceptibility to seizure induced by a variety of drugs was observed. Shih et al. (2001) reported that doses of TCE as low as 250 mg/kg could reduce signs of seizure induced by picrotoxin, bicuculline, and pentylenetetrazol. These drugs are all GABAergic antagonists. TCE treatment had a more limited effect on seizure threshold induced by non-GABAergic convulsant drugs such as strychnine (glycine receptor antagonist), 4-aminopyridine (alcohol dehydrogenase inhibitor), and N-methyl-d-aspartate (glutamatergic agonist) than was observed with the GABAergic antagonists. While these data suggest the possibility that TCE could act at least acutely on GABAergic neurons, there are no direct measurements of such an effect. Moreover, there is no obvious relationship between these findings and those of Briving et al. (1986) with regard to increased high affinity uptake of glutamate and GABA in cerebellum. Beyond that fact, this study does not provide information regarding persistent effects of TCE on either seizure susceptibility or GABAergic function as all measurements were made acutely shortly following a single injection of TCE.

D.2.8.3. Demyelination Following TCE Exposure

Because of its anesthetic properties and lipophilicity, it is hypothesized that TCE may disrupt the lipid-rich sheaths that cover many central and peripheral nerves. This issue has also been studied both in specific cranial nerves known to be targets of TCE neurotoxicity (namely the trigeminal nerve) and in the CNS including the cerebral cortex, hippocampus, and cerebellum in particular. For peripheral and cranial nerves, there are limited nerve conduction velocity studies that are relevant as a functional measure. For central pathways, the most common outcomes studied include histological endpoints and lipid profiles.

A significant difficulty in assessing these studies concerns the permanence or persistence of effect. There is a very large literature unrelated to TCE, which demonstrates the potential for repair of the myelin sheath and at least partial if not full recovery of function. In the studies where nerve myelin markers are assessed, it is not possible to determine if the effects are transient or persistent.

There are two published manuscripts (Isaacson et al., 1990; Isaacson and Taylor, 1989) that document selective hippocampal histopathology when Sprague-Dawley rats are exposed to TCE within a developmental model. Both of these studies employed oral TCE administration via the drinking water. In Isaacson and Taylor (1989), a combined prenatal and neonatal exposure was used while Isaacson's et al. (1990) report focused on a neonatal exposure. In addition, Ohta et al. (2001) presented evidence of altered hippocampal function in an in vitro preparation following acute in vivo TCE intoxication. The latter most manuscript details a shift in long term potentiation elicited by tetanic shocks to hippocampal slices in vitro. In the two developmental studies, the exposure doses are expressed in terms of the concentration of TCE placed in the drinking water and the total daily dose is then estimated based upon average water intake by the subjects. However, since the subjects' body weight is not provided, it is not possible to estimate dosage on a mg/kg body weight basis.

Isaacson and Taylor (1989) examined the development of the hippocampus in neonatal rats that were exposed in utero and in the preweaning period to TCE via their dam. TCE was added to the drinking water of the dam and daily maternal doses are estimated based upon water intake of the dam as being 4 and 8.1 mg/day. Based upon body weight norms for 70-day-old female Sprague-Dawley rats, which would predict body weights of about 250 g at that age, such a dose might approach 16–32 mg/kg-day initially during pregnancy. Even if these assumptions hold true, it is not possible to determine how much TCE was received by the pups, although the authors do provide an estimate of fetal exposure expressed as μg/mL of TCE, TCOH, and TCA. The authors reported a 40% decline in myelinated fibers in the CA1 region of the hippocampus of the weanling rats. There was no effect of TCE treatment on myelination in several other brain regions including the internal capsule, optic tract, or fornix and this effect appears to be restricted to the CA1 region of the hippocampus at the tested exposures.

In a second manuscript by that group (Isaacson et al., 1990), weanling rats were exposed to TCE via their drinking water at doses of 5.5 mg/day for 4 weeks or 5.5 mg/day for 4 weeks, a 2-week period with no TCE and then a final 2 weeks of exposure to 8.5 mg/day TCE. Spatial learning was studied using the Morris water maze and hippocampal myelination was examined histologically starting 1 day postexposure. The authors report that the subjects receiving a total of 6 weeks exposure to TCE showed *better* performance in the Morris swim test ($p < 0.05$) than did controls, while the 4-week-exposed subjects performed at the same level as did controls. Despite this apparent improvement in performance, histological examination of the hippocampus demonstrated a dose-dependent relationship with hippocampal myelin being significantly reduced in the TCE exposed groups, while normal myelin patterns were found in the internal capsule, optic tract, and fornix. The authors did not evaluate the signs of gross toxicity in treated animals such as growth rate, which might have influenced hippocampal development.

Ohta et al. (2001) administered 300 or 1,000 mg/kg TCE, i.p., to male ddY mice. Twenty-four hours after TCE administration, the mice were sacrificed and hippocampal sections were prepared from the excised brains and long-term potentiation was measured in the slices. A dose-related reduction in the population spike was observed following a tetanic stimulation relative to the size of the population spike elicited in the TCE mice prior to tetany. The spike amplitude was reduced 14% in the 300 mg/kg TCE group and 26% in the 1,000 mg/kg group. Precisely how such a shift in excitability of hippocampal CA1 neurons relates to altered hippocampal function is not certain, but it does demonstrate that injection with 300 mg/kg TCE can have lingering consequences on the hippocampus at least 24 hours following i.p. administration.

A critical area for future study is the potential that TCE might have to produce demyelination in the CNS. While it is realistic to imagine that an anesthetic and lipophilic agent such as TCE might interact with lipid membranes and produce alterations, for example, in membrane fluidity at least at anesthetic levels, the data collected by Kyrklund and colleagues suggest that low doses of TCE (50 and 150 ppm chronically for 12 months, 320 ppm for 90 days, 510 ppm 8 hours/day for 5 months) might alter fatty acid metabolism in Sprague-Dawley rats and Mongolian gerbils. Because they have not included high doses in their studies and because the low doses produce only sporadic significant effects and these tend to be of very small magnitude (5–10%), it is not certain that they are truly observing events with biological significance or whether they are observing random effects. A key problem in determining whether the effects under study are spurious or are due to ongoing exposure is that the magnitude and direction of the effect does not grow larger as exposure continues. It could be hypothesized that the alterations in fatty acid metabolism could be an underlying mechanism for demyelination. However, there is not enough evidence to determine if the changes in the lipid profiles lead to demyelination or if the observed effects are purely due to chance. Similarly, the size of statistically significant effects (5–12%) is generally modest. A broad dose-response

analysis or the addition of a positive control group that is treated with an agent well-known to produce central demyelination would be important in order to characterize the potency of TCE as an agent that disrupts CNS lipid profiles.

Kyrklund and colleagues (e.g., 1986) have generally evaluated the hippocampus, cerebral cortex, cerebellum, and in some instances, brainstem in adult gerbil. It is not apparent that one brain region is more vulnerable to the effects of TCE than is another region. While this group does not report significant changes in levels of cholesterol, neutral and acidic phospholipids, or total lipid phospholipids, they do suggest a shift in lipid profiles between treated and untreated subjects. Similarly, inhalation exposure to trichloroethane at 1,200 ppm for 30 days (Kyrklund and Haglid, 1991) leads to sporadic changes in fatty acid profiles in Sprague-Dawley rats. However, these changes are small and are not always in the same direction as the changes observed following TCE exposure. In the case of trichloroethane, a NOAEL of 320 ppm for 30 days 24 hours/day was observed and no other doses were evaluated (Kyrklund et al., 1988).

D.2.9. Summary Tables

Tables D-4 through D-8 summarize the animal studies by neurological domains (Table D-4—trigeminal nerve; Table D-5—ototoxicity; Table D-6—vestibular and visual systems; Table D-7—cognition; and Table D-8—psychomotor function and locomotor activity). For each table, the reference, exposure route, species, dose level, effects, and NOAEL/LOAEL values are provided. Tables D-9 through D-11 summarize mechanistic (Tables D-9 and D-11) and neurochemical studies (Table D-10). Brief summaries of developmental neurotoxicity studies are provided in Table D-12.

Table D-4. Summary of mammalian in vivo trigeminal nerve studies

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL: LOAEL	Effects
Barret et al. (1991)	Direct gastric administration	Rat, Sprague-Dawley, female, 21	0, 2.5 g/kg, acute administration	LOAEL: 2.5 g/kg	Morphometric analysis was used for analyzing the trigeminal nerve. Increase in external and internal fiber diameter as well as myelin thickness was observed in the trigeminal nerve after TCE treatment.
Barret et al. (1992)	Direct gastric administration	Rat, Sprague-Dawley, female, 18	0, 2.5 g/kg; 1 dose/d, 5 d/wk, 10 wks	LOAEL: 2.5 g/kg	Trigeminal nerve analyzed using morphometric analysis. Increased internode length and fiber diameter in class A fibers of the trigeminal nerve observed with TCE treatment. Changes in fatty acid composition also noted.
Albee et al. (2006)	Inhalation	Rat, F344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm	NOAEL: 2,500 ppm	No effect on trigeminal nerve function was noted at any exposure level.

Table D-5. Summary of mammalian in vivo ototoxicity studies

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Rebert et al. (1991)	Inhalation	Rat, Long-Evans, male, 10/group	Long-Evans: 0, 1,600, 3,200 ppm; 12 hrs/d, 12 wks	Long-Evans: NOAEL: 1,600 ppm; LOAEL: 3,200 ppm	BAERs were measured. Significant decreases in BAER amplitude and an increase in latency of appearance of the initial peak (P1).
		Rat, F344, male, 4–5/group	F344: 0, 2000, 3200 ppm; 12 hrs/d, 3 wks	F344: LOAEL: 2,000 ppm	
Rebert et al. (1993)	Inhalation	Rat, Long-Evans, male, 9/group	0, 2,500, 3,000, 3,500 ppm; 8 hrs/d, 5 d	NOAEL: 2,500 ppm LOAEL: 3,000 ppm	BAERs were measured 1–2 wks postexposure to assess auditory function. Significant decreases in BAERs were noted with TCE exposure.
Rebert et al. (1995)	Inhalation	Rat, Long-Evans, male, 9/group	0, 2,800 ppm; 8 hrs/d, 5 d	LOAEL: 2,800 ppm	BAER measured 2–14 d postexposure at a 16-kHz tone. Hearing loss ranged from 55 to 85 dB.
Crofton et al. (1994)	Inhalation	Rat, Long-Evans, male, 7–8/group	0, 3,500 ppm TCE; 8 hrs/d, 5 d	LOAEL: 3,500 ppm	BAER measured and auditory thresholds determined 5–8 wks postexposure. Selective impairment of auditory function for mid-frequency tones (8 and 16 kHz).
Crofton and Zhao (1997); Boyes et al. (2000)	Inhalation	Rat, Long-Evans, male, 9–12/group	0, 4,000, 6,000, 8,000 ppm; 6 hrs	NOAEL: 6,000 ppm LOAEL: 8,000 ppm	Auditory thresholds as measured by BAERs for the 16-kHz tone increased with TCE exposure.
		Rat, Long-Evans, male, 8–10/group	0, 1,600, 2,400, 3,200 ppm; 6 hrs/d, 5 d	NOAEL: 2,400 ppm LOAEL: 3,200 ppm	
		Rat, Long-Evans, male, 8–10/group	0, 800, 1,600, 2,400, 3,200 ppm; 6 hrs/d, 5 d/wk, 4 wks	NOAEL: 2,400 ppm LOAEL: 3,200 ppm	
		Rat, Long-Evans, male, 8–10/group	0, 800, 1,600, 2,400, 3,200 ppm; 6 hrs/d, 5 d/wk, 13 wks	NOAEL: 1,600 ppm LOAEL: 2,400 ppm	

Table D-5. Summary of mammalian in vivo ototoxicity studies (continued)

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Fechter et al. (1998)	Inhalation	Rat, Long-Evans, male, 12/group	0, 4,000 ppm; 6 hrs/d, 5 d	LOAEL: 4,000 ppm	Cochlear function measured 5–7 wks after exposure. Loss of spiral ganglion cells noted. Auditory function was significantly decreased as measured by compound action potentials.
Jaspers et al. (1993)	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 12/group	0, 1,500, 3,000 ppm; 18 hrs/d, 5 d/wk, 3 wks	LOAEL: 1,500 ppm	Auditory function assessed repeatedly 1–5 wks postexposure for 5-, 20-, and 35-kHz tones. No effect at 5 or 35 kHz. Decreased auditory sensitivity at 20 kHz.
Muijser et al. (2000)	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 8	0, 3,000 ppm	LOAEL: 3,000 ppm	Auditory sensitivity decreased with TCE exposure at 4-, 8-, 16-, and 20-kHz tones.
Albee et al. (2006)	Inhalation	Rat, F344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm	NOAEL:800 ppm LOAEL: 2,500 ppm	Mild frequency specific hearing deficits. Focal loss of hair cells and cochlear lesions.
Yamamura et al. (1983)	Inhalation	Guinea Pig, albino Hartley, male, 7–10/group	0, 6,000, 12,000, 17,000 ppm; 4 hrs/d, 5 d	NOAEL: 17,000 ppm	No change in auditory sensitivity at any exposure level as measured by cochlear action potentials and microphonics.

Table D-6. Summary of mammalian sensory studies—vestibular and visual systems

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Vestibular system studies					
Tham et al. (1979)	i.v.	Rabbit, strain unknown, sex unspecified, 19	1–5 mg/kg-min	–	Positional nystagmus developed once blood levels reached 30 ppm.
Tham et al. (1984)	i.v.	Rat, Sprague-Dawley, female, 11	80 µg/kg-min	–	Excitatory effects on the vestibule-oculomotor reflex. Threshold effect at blood (TCE) of 120 ppm or 0.9 mM/L.
Niklasson et al. (1993)	Inhalation	Rat, strain unknown, male and female, 28	0, 2,700, 4,200, 6,000, 7,200 ppm; 1 hr	LOAEL: 2,700 ppm	Increased ability to produce nystagmus.
Umezu et al. (1997)	i.p.	Mouse, ICR, male, 116	0, 250, 500, 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 250 mg/kg LOAEL: 500 mg/kg	Decreased equilibrium and coordination as measured by the Bridge test (staying time on an elevated balance beam).
Visual system studies					
Rebert et al. (1991)	Inhalation	Rat, Long-Evans, male, 10/group	0, 1,600, 3,200 ppm; 12 hrs/d, 12 wks	NOAEL: 3,200 ppm	No effect on visual function as measured by VEP changes.
		Rat, F344, male, 4–5/group	0, 2,000, 3,200 ppm; 12 hrs/d, 3 wks	NOAEL: 3,200 ppm	
Boyes et al. (2003)	Inhalation	Rat, Long-Evans, male, 9–10/group	0 ppm, 4 hrs; 1,000 ppm, 4 hrs; 2,000 ppm, 2 hrs; 3,000 ppm, 1.3 hrs; 4,000 ppm, 1 hr	LOAEL: 1,000 ppm, 4 hrs	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed VEPs.
Boyes et al. (2005a)	Inhalation	Rat, Long-Evans, male, 8–10/group	0 ppm, 4 hrs; 500 ppm, 4 hrs; 1,000 ppm, 4 hrs; 2,000 ppm, 2 hrs; 3,000 ppm, 1.3 hrs; 4,000 ppm, 1 hr; 5,000 ppm, 0.8 hr	LOAEL: 500 ppm, 4 hrs	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed VEPs.
Blain et al. (1994)	Inhalation	Rabbit, New Zealand albino, male, 6–8/group	0, 350, 700 ppm; 4 hrs/d, 4 d/wk, 12 wks	LOAEL: 350 ppm	Significant effects noted in visual function as measured by ERG and OPs immediately after exposure. No differences in ERG or OP measurements were noted at 6 wks post-TCE exposure.

Table D-7. Summary of mammalian cognition studies

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Kjellstrand et al. (1980)	Inhalation	Gerbil, Mongolian, males and females, 12/sex/dose	0, 320 ppm; 9 months, continuous (24 hrs/d) except 1–2 hrs/wk for cage cleaning	NOAEL: 320 ppm	No significant effect on spatial memory (radial arm maze).
Kulig et al. (1987)	Inhalation	Rat, Wistar, male, 8/dose	0, 500, 1,000, 1,500 ppm; 16 hrs/d, 5 d/wk, 18 wks	NOAEL: 500 ppm LOAEL: 1,000 ppm	Increased latency time in the two-choice visual discrimination task (cognitive disruption and/or motor activity related effect).
Isaacson et al. (1990)	Oral, drinking water	Rat, Sprague-Dawley, male, 12/dose	(1) 0 mg/kg-d, 8 wks; (2) 5.5 mg/d (47 mg/kg-d[a]), 4 wks + 0 mg/kg/d, 4 wks; (3) 5.5 mg/d, 4 wks (47 mg/kg-d[a]) + 0 mg/kg-d, 2 wks + 8.5 mg/d (24 mg/kg-d),[a] 2 wks	NOAEL: 5.5 mg/d, 4 wks spatial learning LOAEL: 5.5 mg/d hippocampal demyelination	Decreased latency to find platform in the Morris water maze (Group #3). Hippocampal demyelination observed in all TCE-treated groups.
Kishi et al. (1993)	Inhalation	Rats, Wistar, male, number not specified	0, 250,500, 1,000, 2,000, 4,000 ppm, 4 hrs	LOAEL: 250 ppm	Decreased lever presses and avoidance responses in a shock avoidance task.
Umezu et al. (1997)	i.p.	Mouse, ICR, male, 6 exposed to all treatments	0, 125, 250, 500, 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg	Decreased response rate in an operant response-cognitive task.
Ohta et al. (2001)	i.p.	Mouse, ddY, male, 5/group	0, 300, 1,000 mg/kg, sacrificed 24 hrs after injection	LOAEL: 300 mg/kg	Decreased response (LTP response) to tetanic stimulation in the hippocampus.
Oshiro et al. (2004)	Inhalation	Rat, Long-Evans, male, 24	0, 1,600, 2,400 ppm; 6 hrs/d, 5 d/wk, 4 wks	NOAEL: 2,400 ppm	No change in RT in signal detection task and when challenged with amphetamine, no change in response from control.

[a]mg/kg-day conversion estimated from average male Sprague-Dawley rat body weight from ages 21–49 days (118 g) for the 5.5 mg dosing period and ages 63–78 days (354 g) for the 8.5 mg dosing period.

Table D-8. Summary of mammalian psychomotor function, locomotor activity, and RT studies

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Savolainen et al. (1977)	Inhalation	Rat, Sprague-Dawley, male, 10	0, 200 ppm; 6 hrs/d, 4 d	LOAEL: 200 ppm	Increased frequency of preening, rearing, and ambulation. Increased preening time.
Wolff and Siegmund (1978)	i.p.	Mouse, AB, male, 144	0, 182 mg/kg, tested 30 min after injection	LOAEL: 182 mg/kg	Decreased spontaneous motor activity.
Kulig et al. (1987)	Inhalation	Rat, Wistar, male, 8/dose	0, 500, 1,000, 1,500 ppm; 16 hrs/d, 5 d/wk, 18 wks	NOAEL: 1,500 ppm	No change in spontaneous activity, grip strength or hindlimb movement.
Motohashi and Miyazaki (1990)	i.p.	Rat, Wistar, male, 44	0, 1.2 g/kg, tested 30 min after injection	LOAEL: 1.2 g/kg	Increased incidence of rats slipping in the inclined plane test.
			0, 1.2 g/kg-d, 3 d	LOAEL: 1.2 g/kg	Decreased spontaneous motor activity.
Fredriksson et al. (1993)	Oral	Mouse, NMRI, male, 12 (3–4 litters)	0, 50, 290 mg/kg-d, at d 10–16	–	Decreased rearing. No evidence of dose response.
Moser et al. (1995)	Oral	Rat, F344, female, 8/dose	0, 150, 500, 1,500, 5,000 mg/kg, 1 dose	NOAEL: 500 mg/kg LOAEL: 1,500 mg/kg	Decreased motor activity. Neuro-muscular and sensorimotor impairment.
			0, 50, 150, 500, 1,500 mg/kg-d, 14 d	NOAEL: 150 mg/kg-d LOAEL: 500 mg/kg-d	Increased rearing activity.
Bushnell (1997)	Inhalation	Rat, Long-Evans, male, 12	0, 400, 800, 1,200, 1,600, 2,000, 2,400 ppm, 1-hr/test d, 4 consecutive test d, 2 wks	NOAEL: 800 ppm LOAEL: 1,200 ppm	Decreased sensitivity and increased response time in the signal detection task.

Table D-8. Summary of mammalian psychomotor function, locomotor activity, and RT studies (continued)

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Umezu et al. (1997)	i.p.	Mouse, ICR, male, 6 exposed to all treatments	0, 2,000, 4,000, 5,000 mg/kg—loss of righting reflex measure	LOAEL: 2,000 mg/kg—loss of righting reflex	Loss of righting reflex, decreased operant responses, increased punished responding.
			0, 62.5, 125, 250, 500, 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg—operant behavior NOAEL: 125 mg/kg LOAEL: 250 mg/kg—punished responding	
Bushnell and Oshiro (2000)	Inhalation	Rat, Long-Evans, male, 32	0, 2,000, 2,400 ppm; 70 min/d, 9 d	LOAEL: 2,000 ppm	Decreased performance on the signal detection task. Increased response time and decreased response rate.
Nunes et al. (2001)	Oral	Rat, Sprague-Dawley, male, 10/group	0, 2,000 mg/kg-d, 7 d	LOAEL: 2,000 mg/kg-d	Increased foot splay. No change in any other FOB parameter (e.g., piloerection, activity, reactivity to handling).
Waseem et al. (2001)	Oral	Rat, Wistar, male, 8/group	0, 350, 700, 1,400 ppm in drinking water for 90 d	NOAEL: 1,400 ppm	No significant effect on spontaneous locomotor activity.
	Inhalation	Rat, Wistar, male, 6/group	0, 376 ppm for up to 180 d	LOAEL: 376 ppm	Changes in locomotor activity but not consistent when measured over the 180-d period.
Moser et al. (2003)	Oral	Rat, F344, female, 10/group	0, 40, 200, 800, 1,200 mg/kg-d, 10 d	–	Decreased motor activity; Decreased sensitivity; Increased abnormality in gait; Adverse changes in several FOB parameters.
Albee et al. (2006)	Inhalation	Rat, F344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm	NOAEL: 2,500 ppm	No change in any FOB measured parameter.

Table D-9. Summary of mammalian in vivo dopamine neuronal studies

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Guehl et al. (1999)	i.p. administration	Mouse, OF1, male, 10	0, 400 mg/kg	LOAEL: 400 mg/kg	Significant dopaminergic neuronal death in substantia nigra.
Gash et al. (2008)	Oral	Rat, F344, male, 17/group	0, 1,000 mg/kg	LOAEL: 1,000 mg/kg	Degeneration of dopamine-containing neurons in substantia nigra.

Table D-10. Summary of neurochemical effects with TCE exposure

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
In vivo studies					
Shih et al. (2001)	i.p.	Mouse, Mf1, male, 6/group	0, 250 500, 1,000, 2,000 mg/kg, 15 min; followed by tail infusion of PTZ (5 mg/mL), picrotoxin (0.8 mg/mL), bicuculline (0.06 mg/mL), strychnine (0.05 mg/mL), 4-AP (2 mg/mL), or NMDA (8 mg/mL)	–	Increased threshold for seizure appearance with TCE pretreatment for all convulsants. Effects strongest on the $GABA_A$ antagonists, PTZ, picrotoxin, and bicuculline suggesting $GABA_A$ receptor involvement. NMDA and glycine Rc involvement also suggested.
Briving et al. (1986)	Inhalation	Gerbils, Mongolian, male and female, 6/group	0, 50, 150 ppm, continuous, 24 hrs/d, 12 months	NOAEL: 50 ppm; LOAEL: 150 ppm for glutamate levels in hippocampus NOAEL: 150 ppm for glutamate and GABA uptake in hippocampus LOAEL: 50 ppm for glutamate and GABA uptake in cerebellar vermis	Increased glutamate levels in the hippocampus. Increased glutamate and GABA uptake in the cerebellar vermis.
Subramoniam et al. (1989)	Oral	Rat, Wistar, female,	0, 1,000 mg/kg, 2 or 20 hrs 0, 1,000 mg/kg-d, 5 d/wk, 1 yr	–	PI and PIP2 decreased by 24 and 17% at 2 hrs. PI and PIP2 increased by 22 and 38% at 20 hrs. PI, PIP, and PIP2 reduced by 52, 23, and 45% in 1-yr study.
Kjellstrand et al. (1987)	Inhalation	Mouse, NMRI, male	0, 150, 300 ppm, 24 hrs/d, 4 or 24 d	LOAEL: 150 ppm, 4 and 24 d	Sciatic nerve regeneration was inhibited in both mice and rats.
		Rat, Sprague-Dawley, female	0, 300 ppm, 24 hrs/d, 4 or 24 d	NOAEL: 300 ppm, 4 d LOAEL: 300 ppm, 24 d	

Table D-10. Summary of neurochemical effects with TCE exposure (continued)

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Haglid et al. (1981)	Inhalation	Gerbil, Mogolian, male and female, 6–7/group	0, 60, 320 ppm, 24 hrs/d, 7 d/wk, 3 months	LOAEL: 60 ppm, brain protein changes NOAEL: 60 ppm; LOAEL: 320 ppm, brain DNA changes	(1) Decreases in total brain soluble protein whereas increase in S100 protein. (2) Elevated DNA in cerebellar vermis and sensory motor cortex.

Table D-11. Summary of in vitro ion channel effects with TCE exposure

Reference	Cellular system	Neuronal channel/ receptor	Concentrations	Effects
In vitro studies				
Shafer et al. (2005)	PC12 cells	Voltage sensitive calcium channels (VSCC)	0, 500, 1,000, 1,500, 2,000 μM	Shift of VSCC activation to a more hyperpolarizing potential. Inhibition of VSCCs at a holding potential of -70 mV.
Beckstead et al. (2000)	*Xenopus* oocytes	Human recombinant Glycine receptor α1, $GABA_A$ receptors, α1β1, α1β2γ2L	0, 390 μM	50% potentiation of the $GABA_A$ receptors; 100% potentiation of the glycine receptor.
Lopreato et al. (2003)	*X.* oocytes	Human recombinant serotonin 3A receptor	Not provided	Potentiation of serotonin receptor function.
Krasowski and Harrison (2000)	Human embryonic kidney 293 cells	Human recombinant Glycine receptor α1, $GABA_A$ receptors α2β1	Not provided	Potentiation of glycine receptor function with an EC_{50} of 0.65 ± 0.05 mM. Potentiation of $GABA_A$ receptor function with an EC_{50} of 0.85 ± 0.2.

EC_{50} = median effective concentration

Table D-12. Summary of mammalian in vivo developmental neurotoxicity studies—oral exposures

Reference	Species/strain/ sex/number	Dose level/ exposure duration	Route/vehicle	NOAEL; LOAEL[a]	Effects
Fredriksson et al. (1993)	Mouse, NMRI, male pups, 12 pups from 3 to 4 different litters/group	0, 50, 290 mg/kg-d PNDs 10–16	Gavage in a 20% fat emulsion prepared from egg lecithin and peanut oil	Developmental LOAEL: 50 mg/kg-d	Rearing activity statistically significant ↓ at both dose levels on PND 60.
George et al. (1986)	Rat, F334, male and female, 20 pairs/ treatment group, 40 controls/sex	0, 0.15, 0.30, 0.60% microencapsulated TCE. Breeders exposed 1 wk premating, then for 13 wks; pregnant ♀s throughout pregnancy (i.e., 18-wk total).	Dietary	LOAEL: 0.15%	Open field testing in pups: a statistically significant dose-related trend toward ↑ time required for male and female pups to cross the first grid in the test device.
Isaacson and Taylor (1989)	Rat, Sprague-Dawley, females, 6 dams/group	0, 312, 625 mg/L. (0, 4.0, 8.1 mg/d)[b] Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Developmental LOAEL: 312 mg/L	Statistically significant ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the hippocampus.
Noland-Gerbec et al. (1986)	Rat, Sprague-Dawley, females, 9–11 dams/group	0, 312 mg/L (Avg. total intake of dams: 825 mg TCE over 61 d.)[b] Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Developmental LOEL: 312 mg/L	Statistically significant ↓ uptake of [^{3}H]-2-DG in whole brains and cerebella (no effect in hippocampus) of exposed pups at 7, 11, and 16 d, but returned to control levels by 21 d.
Taylor et al. (1985)	Rat, Sprague-Dawley, females, number dams/group not reported	0, 312, 625, 1,250 mg/L Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Developmental LOAEL: 312 mg/L	Exploratory behavior statistically significant ↑ in 60- and 90-d old male rats at all treatment levels. Locomotor activity was higher in rats from dams exposed to 1,250 ppm TCE.

[a]NOAEL, LOAEL, and LOEL are based upon reported study findings.
[b]Dose conversions provided by study author(s).

E. ANALYSIS OF LIVER AND CO-EXPOSURE ISSUES FOR THE TCE TOXICOLOGICAL REVIEW

The purpose of this Appendix is to provide scientific support and rationale for the hazard and dose-response sections of the *Toxicological Review of Trichloroethylene (TCE)* regarding liver effects and those of co-exposures. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of TCE. Please refer to the *Toxicological Review of Trichloroethylene (TCE)* for characterization of EPA's overall confidence in the quantitative and qualitative aspects of hazard and dose-response for TCE-induced liver effects. Matters considered in this appendix include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the scientific issues regarding the data and mode-of-action considerations for experimental animal data for liver effects in the TCE assessment.

E.1. BASIC PHYSIOLOGY AND FUNCTION OF THE LIVER—A STORY OF HETEROGENEITY

The liver is a complex organ whose normal function and heterogeneity are key to understanding and putting into context perturbations by TCE, cancer biology, and variations in response observed, and anticipated for susceptible lifestages and background conditions.

E.1.1. Heterogeneity of Hepatocytes and Zonal Differences in Function and Ploidy

Malarkey et al. (2005) state that: (1) the liver transcriptome (i.e., genes expressed as measured by mRNA) is believed only second to the brain in its complexity and includes about 25–40% of the approximately 50,000 mammalian genes; (2) during disease states, the transcriptome can double or triple and its increased complexity is due not only to differential gene expression (up- and downregulation of genes) but also to the mRNA contributions from the heterogeneous cell populations in the liver; and (3) when one considers that over a dozen cell types comprise the liver in varying proportions, particularly in disease states, knowledge about the cell types and cell-specific gene expression profiles help unravel the complex genomic and proteomic data sets. Gradients of gene and protein activity varying from the periportal region to the centrilobular region also exist for sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, and the matrix in the space of Disse. Malarkey et al. (2005) also estimate that hepatocytes constitute 60%, sinusoidal endothelial cells 20%, Kupffer cells 15%, and stellate cells 5% of liver cells. Therefore, in experimental paradigms where liver homogenates are used for the determination of "changes in liver," gene expression, or other parameters, the individual changes from cells residing in differing zones and by differing cell type is lost. Malarkey et al. (2005) define the need to better characterize the histological cellular components of the tissues from

which mRNA and protein is extracted and referred to "phenotypic anchoring" and cite acetaminophen as a "model hepatotoxicant under study to assess the strengths and weaknesses of genomics and proteomics technologies" as well as "a good example for understanding and utilizing phenotypic anchoring to better understand genomics data." After acetaminophen exposure "there is an unexplained and striking inter and intralobular variability in acute hepatic necrosis with some regions having massive necrosis and adjacent areas within the same lobe or other lobes showing no injury at all." Malarkey et al. (2005) go on to cite similar lobular variability in response for "copper distribution, iron and phosphorous, chemical and spontaneous carcinogenesis, cirrhosis and regeneration" and suggest that although uncertain "factors such as portal streamlining of blood to the liver, redistribution of blood to core of the liver secondary to nerve stimulation, and exposures during fetal development and possibly lobular gradients are important." Hepatic interlobe differences exist for initiating agents in terms of DNA alkylation and cell replication. In the rat, diethylnitrosamine (DEN) alkylation has been reported to occur preferentially in the left and right median lobes, while cell replication was higher in the right median and right anterior lobes (Richardson et al., 1986). Richardson et al. (1986) reported that exposure to DEN induced a 100% incidence of HCC in the left, caudate, left median, and right median lobes of the liver by 20 weeks vs. only 30% in the right anterior and right posterior hepatic lobes. There was a reported interlobe difference in adduct formation, cell proliferation, liver lobe weight gain, number and size of γ-glutamyltranspeptidase (GGT)+ foci, and carbon 14 labeling from a single dose of DEN. Richardson et al. (1986) suggest that many growth-selection studies utilizing the liver to evaluate the carcinogenic potential of a chemical often focus on only one or two of the hepatic lobes, which is especially true for partial hepatectomy, and that for DEN and possibly other chemicals, this procedure removes the lobes most likely to get tumors. Thus, the "distribution of toxic insult may not be correctly assessed with random sampling of the liver tissue for microarray gene expression analysis" (Malarkey et al., 2005) and certainly any such distributional differences are lost in studies of whole-liver homogenates.

The liver is normally quiescent with few hepatocytes undergoing mitosis and, as described below, normally occurring in the periportal areas of the liver. Mitosis is observed only in approximately 1 in every 20,000 hepatocytes in adult liver (Columbano and Ledda-Columbano, 2003). The studies of Schwartz-Arad et al. (1989), Zajicek et al. (1991), Zajicek and Schwartz-Arad (1990), and Zajicek et al. (1989) have specifically examined the birth, death, and relationship to zone of hepatocytes as the "hepatic streaming theory." They report that hepatocytes and littoral cells continuously stream from the portal tract toward the terminal hepatic vein and that the hepatocyte differentiates as it goes with biological age closely related to cell differentiation. In other words, the acinus may be represented by a tube with two orifices, one for cell inflow situated at the portal tract rim and the other for cell outflow, at the terminal hepatic vein with hepatocytes streaming through the tube in an orderly fashion. In normal liver, cell proliferation is suggested as the only driving force of this flow with each mitosis associated

with displacement of the cells by one cell location and the greater the cell production, the faster the flow and vice versa (Zajicek et al., 1991). Thus, the microscopic section of the liver "displays an instantaneous image of a tissue in flux" (Schwartz-Arad et al., 1989). Schwartz-Arad et al. (1989) further suggest that:

> throughout its life the hepatocyte traverses three acinus zones; in each it is engaged in different metabolic activity. When young it performs among other functions gluconeogenesis, which is found in zone 1 hepatocytes (i.e. periportal), and when old it turns into a zone 3 cell (i.e., pericentral), with a pronounced glycolitic make up. The three zones thus represent differentiation stages of the hepatocyte, and since they differ by their distance from the origin, e.g. zone 2 (i.e., midzonal) is more distant than zone 1, again, hepatocyte differentiation is proportional to its distance.

Chen et al. (1995) report that:

> Hepatocytes are a heterogeneous population that are composed of cells expressing different patterns of genes. For example, gamma-glutamyl transpeptidase and genes related to gluconeogenesis are expressed preferential in periportal hepatocytes, whereas enzymes related to glycolysis are more abundant in the centrilobular area. Glutamine synthetase is expressed in a small number of hepatocytes surrounding the central veins. Most cytochrome p450 enzymes are expressed or induced preferentially in centrilobular hepatocytes relative to periportal hepatocytes.

Along with changes in metabolic function, Vielhauer et al. (2001) reported that there is evidence of zonal differences in carcinogen DNA effects and, also, chemical-specific differences for DNA repair enzyme and that enhanced DNA repair is a general feature of many carcinogenic states including the enzymes that repair alkylating agents but also oxidative repair. As part of this process of differentiation and as livers age, the hepatocyte changes and increases its ploidy with polyploid cells predominant in zone 2 of the acinus (Schwartz-Arad et al., 1989). The reported decrease in DNA absorbance in zone 3 may be due to: (1) a decline in chromatin affinity to the dye; (2) cell death; and (3) DNA exit from intact cells and Zajicek and Schwartz-Arad (1990) suggest that the fewer metabolic demands in Zone 3, under normal conditions, causes the cell to "deamplify" its genes and for DNA excess to leak out cells adjacent to the terminal hepatic vein or to be eliminated by apoptosis reflecting cell death. Thus, the three acinus zones represent differentiation states of one and the same hepatocyte, which increase ploidy as functional demands change. Zajicek and Schwartz-Arad (1990) also report that nuclear size is generally proportional to DNA content and that as DNA accumulates, the nucleus enlarges. This has import for histopathological descriptions of hepatocellular hypertrophy and attendant nuclear changes after toxic insult as well.

The gene amplification associated with polyploidy is manifested by DNA accumulation that involves the entire genome (Zajicek and Schwartz-Arad, 1990). Polyploidization is always attended by the intensification of the transcription and translation and in rat liver the amino acid label and activity of many enzymes increases proportionately to their ploidy. "Individual chromosomes of a tetraploid genome of a hepatocyte reduplicate in the same sequence as in a diploid one. In this case the properties of the chromosomes evidently remain unchanged and polyploidy only means doubling the indexes of the diploid genome" (Brodsky and Uryvaeva, 1977). Polyploidy will be manifested in the liver by either increases in the number of chromosomes per nucleus in an individual cell or by the appearance of two nuclei in a single cell. Most cell polyploidization occurs in youth with mitotic polyploidization occurring predominantly from 2 to 3 weeks postnatally and increases with age in mice (Brodsky and Uryvaeva, 1977). Hepatocytes progress through a modified or polyploidizing cell cycle, which contains gaps and S-phases, but proceeds without cytokinesis. The result is the formation of the first polyploidy cell, which is binucleated with diploid nuclei and has increased cell ploidy but not cell number. The subsequent proliferation of binucleated hepatocytes occurs with a fusion of mitotic nuclei during metaphase that gives rise to mononucleated cells with higher levels of ploidy. Thus, during normal liver ontogenesis, a polyploidizing cell cycle without cytokinesis alternates with a mitotic cycle of binucleated cells and results in progressive and irreversible increases in either cell or nuclear ploidy (Brodsky and Uryvaeva, 1977).

Polyploidization of the liver occurs during maturation in rodents, and therefore, experimental paradigms that treat or examine rodent liver during that period should take into consideration the normally changing baseline of polyploidy in the liver. The development of polyploidy has been correlated in rodents to correspond with maturation. Brodsky and Uryvaeva (Brodsky and Uryvaeva, 1977) report that it is cells with diploid nuclei that proliferate in young mice, but that among the newly formed cells, the percentage of those with tetraploid nuclei is high. By 1 month, most mice (CBA/C57BL mice) already have a polyploid parenchyma, but binucleated cells with diploid nuclei predominate. In adult mice, the ploidy class with the highest percentage of hepatocytes was the 4n X 2 class. The intensive proliferation of diploid hepatocytes occurs only in baby mice during the first 2 weeks of life and then toward 1 month, the diploid cells cease to maintain themselves and transform into polyploid cells. In aged animals, the parenchyma retains only 0.02% of the diploid cells of the newborn animal. While the weight of the liver increases almost 30 times within 2 years, the number of cells increase much less than the weight or mean ploidy. Hence, the postnatal growth of the liver parenchyma is due to cell polyploidization (Brodsky and Uryvaeva, 1977). In male Wistar rats, fetal hepatocytes (22 days gestation) were reported to be 85.3% diploid (2n) and 7.4% polyploid (4n + 8n) cells with 7.3% of cells in S-phase (S1 and S2). By 1 month of age (25-day-old suckling rats) there were 92.9% diploid and 2.5% polyploid; at 2 months, 47.5% diploid and 50.9%

polyploid; at 6 months, 29.1% diploid and 69.6% polyploid; and by 8 months, 11.1% diploid and 87.3% polyploidy (Sanz et al., 1996). However, mouse and rat differ in their polyploidization.

> In the mouse, which has a higher degree of polyploidy than the rats, the scheme of polyploidization differs in that each cell class, including mononucleate cells, forms from the preceding one without being supplemented by self-maintenance. Each cell class is regarded as the cell clone and it is implied that the cells of each class have the same mitotic history and originate from diploid initiator cells with similar properties. In this model 1 reproduction would give a 2n × 2 cell, the second reproduction a 4n cell, and third reproduction a 4n X 2 cell all coming from an originator diploid cell (Brodsky and Uryvaeva, 1977).

The cell polyploidy is most extensive in mouse liver, but also common for rat and humans livers. The livers of young and aged mice differ considerably in the ploidy of the parenchymal cells, but still perform fundamentally the same functions. In some mammals, such as the mouse, rats, dog and human, the liver is formed of polyploid hepatocytes. In others, for example, guinea pig and cats, the same functions are performed by diploid cells (Brodsky and Uryvaeva, 1977). One obvious consequence of polyploidization is enlargement of the cells. The volume of the nucleus and cytoplasm usually increases proportionately to the increase in the number of chromosome sets with polyploidy reducing the surface/volume ratio. The labeling of tritium doubles with the doubling of the number of chromosomes in the hepatocyte nucleus (Brodsky and Uryvaeva, 1977). Kudryavtsev et al. (1993) have reported that the average levels of cell and nuclear ploidy are relatively lower in humans than in rodent, but the pattern of hepatocyte polyploidization is similar, and at maturity and especially during aging, the rate of hepatocyte polyploidization increases with elderly individuals having binucleated and polyploid hepatocytes constituting about one-half of liver parenchyma. Gramantieri et al. (1996) report that in adult human liver, a certain degree of polyploidization is physiological; the polyploidy compartment (average 33% of the total hepatocytes) includes both mononucleated (28%) and binucleated (72%) cells and the average percentage of binucleated cells in the total hepatocyte population is 24% (Melchiorri et al., 1994).

Historically, aging in human liver has been characterized by fewer and larger hepatocytes, increased nuclear polyploidy, and a higher index of binucleate hepatocytes (Popper, 1986), but Schmucker (2005) notes that data concerning the effect of aging on hepatocyte volume in rodent and humans are in conflict with some showing increases in volume to be unchanged and to increase by 25% by age 60 by others in humans. The irreversibility of hepatocyte polyploidy has been used in efforts to identify the origin of tumor progenitor cells (diploid vs. polyploidy) (see Section E.3.1.8, below). The associations with polyploidy and disease have been an active area of study in cancer mode-of-action studies (see Sections E.3.1.4 and E.3.3.1, below).

Not only are polyploid cells most abundant in zone 2 of the liver acinus and increase in number with age, but polyploid cells have been reported to be more abundant following a number of toxic insults and exposure to chemical carcinogens. Wanson et al. (1980) reported that one of the earliest lesions obtained in the liver after *N*-nitrosomorpholine treatment development of hypertrophic parenchymal cells presenting a high degree of ploidy. Gupta (2000) reports hepatic polyploidy is often encountered in the presence of liver disease and that for animals and people, polyploidy is observed during advancement of liver injury due to cirrhosis or other chronic liver disease (often described as large-cell dysplasia referring to nuclear and cytoplasmic enlargement, nuclear pleomorphisms, and multinucleation and probably representing increased prevalence of polyploidy cells) and in old animals with toxic liver injury and impaired recovery. Gorla et al. (2001) report that weaning and commencement of feeding, compensatory liver hypertrophy following partial hepatectomy, toxin and drug-induced liver disease, and administration of specific growth factors and hormones may induce hepatic polyploidy. They go on to state that "although liver growth control has long been studied, whether the replication potential of polyploidy hepatocytes is altered remains unresolved, in part, owing to difficulties in distinguishing between cellular DNA synthesis and generation of daughter cells." Following carbon tetrachloride intoxication, the liver ploidy rises and more cells become binucleate (Zajicek et al., 1989). Minamishima et al. (2002) report that in 8–12-week-old female mice before partial hepatectomy, there were 78.6% 2C, 19.1% 4C, and 2.3% 8C cells but 7 days after, there were 42.0% 2C, 49.1% 4C, and 9.0% 8C. Zajicek et al. (1991) describe how hepatocyte streaming is affected after the rapid hepatocyte DNA synthesis that occurs after the mitogenic stimulus of a partial hepatectomy. These data are of relevance to findings of increased DNA synthesis and liver weight gain following toxic insults and disease states. Zajicek et al. (1991) suggest that following a mitogenic stimulus, not all DNA synthesizing cells do divide but accumulate newly formed DNA and turn polyploid (i.e., during the first 3 days after partial hepatectomy in rats 50% of synthesized DNA was accumulated) and that since the acinus increased 15% and cell density declined 10%, overall cell mass increased 5%. However, cell influx rose 1,300%. "In order to accommodate all these cells, the 'acinus-tube' ought to swell 13-fold, while in reality it increased only 5%" and that on day 3 "the liver remnant did not even double in its size." Zajicek et al. (1991) conclude that apparently "cells were eliminated very rapidly, and may have even been sloughed off, since the number of apoptotic bodies was very low" and therefore, "partial hepatectomy triggers two processes: an acute process lasting about a week marked by massive and rapid cell turnover during which most newly formed hepatocytes are eliminated, probably sloughed off into the sinusoids; and a second more protracted process which served for liver mass restoration mainly by forming new acini." Thus, a mitogenic stimulus may induce increased ploidy and increased cell number as a result of increased DNA synthesis, and many of the rapidly expanding number of cells resulting from

such stimulation are purged, and therefore, do not participate in subsequent disease states of the liver.

Zajicek et al. (1989) note that the accumulation of DNA rather than proliferation of hepatocytes "should be considered when evaluating the labeling index of hepatocytes labeled with tritiated thymidine" as the labeling index, defined as the proportion of labeled cells, can serve as a proliferation estimate only if it is assumed that a synthesizing cell will ultimately divide. In tissues, such as the liver, "where cells also accumulate DNA, proliferation estimates based on this index may fail" (Zajicek et al., 1989). The tendency to accumulate DNA is also accompanied by a decreasing probability of a cell to proliferate, since young hepatocytes generally divide after synthesizing DNA, while older cells prefer instead to accumulate DNA. However, polyploidy per se does not preclude cells from dividing (Zajicek et al., 1989). The ploidy level achieved by the cell, no matter how high, does not, in itself, prevent it from going through the next mitotic cycle and the reproduction of hepatocytes in the ploidy classes of 8n and 8n X 2 is common phenomenon (Brodsky and Uryvaeva, 1977). However, along with a reduced capacity to proliferate, Sigal et al. (1999) report that the onset of polyploidy increases the probability of cell death. The proliferative potentials of hepatocytes depend not only on their ploidy, but also on the age of the animals, with liver restoration occurring more slowly in aged animals after partial hepatectomy (Brodsky and Uryvaeva, 1977). Species differences in the ability of hepatocytes to proliferate and respond to a mitogenic stimulus have also been documented (see Section E.3.2, below). The importance of the issues of cellular proliferation vs. DNA accumulation and the differences in ability to respond to a mitogenic stimulus becomes apparent as identification of the cellular targets of toxicity (i.e., diploid vs. polyploidy) and the role of proliferation in proposed modes of action are brought forth. Polyploidization, as discussed above, has been associated with a number of types of toxic injury, disease states, and carcinogenesis by a variety of agents.

E.1.2. Effects of Environment and Age: Variability of Response

The extent of polyploidization of the liver not only changes with age, but structural and functional changes, as well as environmental factors (e.g., polypharmacy), also affect the vulnerability of the liver to toxic insult. In a recent review by Schmucker (2005), several of these factors are discussed. Schmucker (2005) reports that approximately 13% of the population of the United States is over the age of 65 years, that the number will increase substantially over the next 50 years, and that increased age is associated with an overall decline in health and vitality contributing to the consumption of nearly 40% of all drugs by the elderly. Schmucker (2005) estimates that 65% of this population is medicated and many are on polypharmacy regimes with a major consequence of a marked increase in the incidence of adverse drug reactions (ADRs) (i.e., males and females exhibit three- and fourfold increases in ADRs, respectively, when 20- and 60-year-old groups are compared). The percentage of deaths

attributed to liver diseases dramatically increases in humans beyond the age of 45 years with data from California demonstrating a fourfold increase in liver disease-related mortality in both men and women between the ages of 45 and 85 years (Siegel and Kasmin, 1997). Furthermore, Schmucker (2005) cites statistics from the U.S. Department of Health and Human Services to illustrate a loss in potential lifespan prior to 75 years of age due to liver disease (i.e., liver disease reduced lifespan to a greater extent than colorectal and prostatic cancers, to a similar extent as chronic obstructive pulmonary disease, and nearly as much as HIV). Thus, the elderly are predisposed to liver disease.

As stated above, the presence of high polyploidy cell in normal adults, nuclear polyploidization with age, and increase in the mean nuclear volume have been reported in people. Watanabe et al. (1978) reported the results from a cytophotometrical analysis of 35 cases of sudden death including 22 persons over 60 years of age that revealed that although the nuclear size of most hepatocytes in a senile liver remains unchanged, there was an increase in cells with larger nuclei. Variations in both cellular area and nucleocytoplasmic ratio were also analyzed in the study, but the binuclearity of hepatocytes was not considered. No cases with a clinical history of liver disease were included. Common changes in senile liver were reported to include atrophy, fatty metamorphosis of hepatocytes, and occasional collapse of cellular cords in the centrilobular area, slight cellular infiltration and proliferation of Kupffer cells in sinusoids, and elongation of Glisson's triads with slight to moderate fibrosis in association with round cell infiltration. Furthermore, cells with giant nuclei, with each containing two or more prominent nucleoli, and binuclear cells were increased. There was a decrease in diploid populations with age and an increase in tetraploid population and a tendency of polyploidy cells with higher values than hexaploids with age. Cells with greater nuclear size and cellular sizes were observed in livers with greater degrees of atrophy.

Schmucker notes that one of the most documented age-related changes in the liver is a decline in organ volume but also cites a decrease in functional hepatocytes and that other studies have suggested that the size or volume of the liver lobule increases as a function of increasing age. Data are cited for rats suggesting sinusoidal perfusion rate in the rat liver remains stable throughout the lifespan (Vollmar et al., 2002) but evidence in humans shows age-related shifts in the hepatic microcirculation attributable to changes in the sinusoidal endothelium (McLean et al., 2003) (i.e., a 60% thickening of the endothelial cell lining and an 80% decline in the number of endothelial cell fenestrations, or pores, with increasing age in humans) that are similar in baboon liver (Cogger et al., 2003). Such changes could impair sinusoidal blood flow and hepatic perfusion, and the uptake of macromolecules such as lipoproteins from the blood. Schmucker reports that there is a consensus that hepatic volume and blood flow decline with increasing age in humans but that the effects of aging on hepatocyte structure are less clear. In rats, the volume of individual hepatocytes was reported to increase by 60% during development and maturation,

but subsequently decline during senescence yielding hepatocytes of equivalent volumes in senescent and very young animals (Schmucker, 2005).

The smooth surfaced endoplasmic reticulum (SER), which is the site of a variety of enzymes involved in steroid, xenobiotic, lipid, and carbohydrate metabolism, also demonstrated a marked age-related decline rat hepatocytes (Schmucker et al., 1978; Schumucker et al., 1977). Schmucker also notes that several studies have reported that the older rodents have less effective protection against oxidative injury in comparison to the young animals, age-related decline in DNA base excision repair, and increases in the level of oxidatively damaged DNA in the livers of senescent animals in comparison to young animals. Age-related increases in the expression an activity of stress-induced transcription factors (i.e., increased NF-κB binding activity but not expression) were also noted, but that the importance of changes in gene expression to the role of oxidative stress in the aging process remains unsolved. An age-related decline in the proliferative response of rat hepatocytes to growth factors following partial hepatectomy was noted, but despite a slower rate of hepatic regeneration, older livers eventually achieved their original volume with the mechanism responsible for the age-related decline in the posthepatectomy hepatocyte proliferative response unidentified.

As with other tissues, telomere length has been identified as a critical factor in cellular aging with the sequential shortening of telomeres to be a normal process that occurs during cell replication (see Sections E.3.1.1 and E.3.1.5, below). An association in telomere length and strain susceptibility for carcinogenesis in mice has been raised. Herrera et al. (1999) examined susceptibility to disease with telomere shortening in mice. However, this study only cites shorter telomeres for C57BL6 mice in comparison to mixed C57BL6/129sv mice. The actual data are not in this paper and no other strains are cited. Of the differing cell types examined, Takubo and Kaminishi (2001) report that hepatocytes exhibited the next fastest rate of telomere shortening despite being relatively long-lived cells raising the question of whether or not there are correlations between age, hepatocyte telomere length, and the incidence of liver disease (Schmucker, 2005). Aikata et al. (2000) and Takubo et al. (2001) report that the mean telomere length in healthy livers is approximately 10 kilobase (kb) pairs at 80 years of age and these hepatocytes retain their proliferative capacity but that in diseased livers of elderly subjects was approximately 5 kb pairs. Thus, short telomere length may compromise hepatic regeneration and contribute to a poor prognosis in liver disease or as a donor liver (Schmucker, 2005).

Schmucker (2005) reports that interindividual variability in Phase I drug metabolism was so large in human liver microsomes, particularly among older subjects, that the determination of any statistically significant age or gender-related differences were precluded. In fact, Schmucker (2001) notes that "the most remarkable characteristic of liver function in the elderly is the increase in interindividual variability, a feature that may obscure age-related differences." Schumer notes that The National Institute on Aging estimates that only 15% of individuals aged over 65 years exhibit no disease or disability with this percentage diminishing to 11 and 5% for

men and women respectively over 80 years. Thus, the large variability in response and the presence of age-related increases in pharmacological exposures and disease processes are important considerations in predicting potential risk from environmental exposures.

E.2. CHARACTERIZATION OF HAZARD FROM TCE STUDIES

The 2001 Draft assessment of the health risk assessment of TCE (U.S. EPA, 2001) extensively cited the review article by Bull (2000) to describe the liver toxicity associated with TCE exposure in rodent models. Most of the attention has been paid to the study of TCE metabolites, rather than the parent compound, and the review of the TCE studies by Bull (2000) was cursory. In addition, gavage exposure to TCE has been associated with a significant occurrence of gavage-related accidental deaths and vehicle effects, and TCE exposure through drinking water has been reported to decrease palatability and drinking water consumption, and to have significant loss of TCE through volatilization, thus further limiting the TCE database.

In its review of the draft assessment, U.S. EPA's Science Advisory regarding this topic suggested that in its revision, the studies of TCE should be more fully described and characterized, especially those studies considered to be key for the hazard assessment of TCE. Although the database for studies of the parent compound is somewhat limited, a careful review of the rodent studies involving TCE can bring to light the consistency of observations across these studies, and help inform many of the questions regarding potential modes of action of TCE toxicity in the liver. Such information can inform current mode-of-action hypothesis (e.g., such as PPARα activation) as well. Accordingly, the primary acute, subchronic, and chronic studies of TCE will be described and examined in detail below with comments on consistency, major conclusions, and the limitations and uncertainties in their design and conduct. Since all chronic studies were conducted primarily with the goal of ascertaining carcinogenicity, their descriptions focus on that endpoint, however, any noncancer endpoints described by the studies are described as well. For details regarding evidence of hepatotoxicity in humans and associations with increased risk of HCC, please refer to Sections 4.5.1 and 4.5.2. Some of the earlier studies with TCE were contaminated with epichlorhydrin and are discussed in Sections 4.6 and 4.7 of the TCE assessment document.

E.2.1. Acute Toxicity Studies

A number of acute studies have been undertaken to describe the early changes in the liver after TCE administration with the majority using the gavage route of administration. Some have been detailed examinations, while others have reported primarily liver weight changes as a marker of TCE-response. The matching and recording of age, but especially initial and final body weight for control and treatment groups, is of particular importance for studies using liver weight gain as a measure of TCE-response as difference in these parameters affect TCE-induced liver weight gain. Most data are for exposures of at least 10 days.

E.2.1.1. Soni et al. (1998)

Soni et al. (1998) administered TCE in corn oil to male Sprague-Dawley rats (200–250 g, 8–10 weeks old) i.p. at exposure levels of 250, 500, 1,250, and 2,500 mg/kg. Groups (4–6 animals per group) were sacrificed at 0, 6, 12, 24, 36, 48, 72, and 96 hours after administration of TCE or corn oil. Using this paradigm only 50% of rats survived the 2,400 mg/kg i.p. TCE administration with all deaths occurring between days 1 and 3 after TCE administration. Tritiated thymidine was also administered i.p. to rats 2 hours prior to euthanasia. Light microscopic sections of the central lobe in 3–4 sections were examined for each animal. The grading scheme reported by the authors was: 0, no necrosis; +1 minimal, defined as only occasional necrotic cells in any lobule; +2, mild, defined as less than one-third of the lobular structure affected; +3, moderate, defined as between one-third and two-thirds of the lobular structure affected; and +4 severe, defined as greater than two-thirds of the lobular structure affected. At the 2,500 mg/kg dose, histopathology data were obtained for the surviving rats (50%). Lethality studies were done separately in groups of 10 rats. The survival in the groups of rats administered TCE and sacrificed from 0 to 96 hours was given as 30% mortality at 48 hours and 50% mortality by 72 hours.

The authors report that controls and 0-hour groups did not show signs of tissue injury or abnormality. The authors only report a single number with one significant figure for each group of animals with no means or SDs provided. In terms of the extent of necrosis there was no difference between the 250 and 500 mg/kg/treated dose groups though 96 hours with a single +1 given as the maximal amount of hepatocellular necrosis (minimal as defined by occasional necrotic cells in any lobule). At the 1,250 mg/kg dose, the maximal score was achieved 24 hours after TCE administration and was reported as simply +2 (mild, defined as less than one-third of lobular structure affected). The level of necrosis was reported to diminish to a score of 0 by 72 hours after 250 mg/kg TCE with no decrease at 500 mg/kg. At 1,250 mg/kg, the extent of necrosis was reported to diminish from +2 to +1 by 72 hours after administration. At the 2,500 mg/kg dose (LD_{50} for this route) by 48 hours, the surviving rats were reported to have a score of +4 (severe as defined by greater than two thirds of the lobular structure affected). The authors report that:

> The necrosed cells were concentrated mostly in the midzonal areas and the cells around central vein area were unaffected. Extensive necrosis was observed between 24 and 48 hours for both 1250 and 2500 mg/kg groups. Injury was maximal in the group receiving 2500 mg/kg between 36 and 48 hours as evidenced by severe midzonal necrosis, vacuolization, and congestion. Infiltration of polymorphonuclear cell was evident at this time as a mechanism for cleaning dead cells and tissue debris from the lobules. At the highest dose, the injury also started to spread toward the centrilobular areas. At the highest dose, 30 and 50% lethality was observed at 48 and 72 h, respectively. After 48 h, the

number of necrotic cells decreased and the number of mitotic cells increased. The groups receiving 500 and 1250 mg/kg TCE showed relatively higher mitotic activity as evidenced by cells in metaphase compared to other groups.

The authors do not give a quantitative estimate or indication as to the magnitude of the number of cells going through mitosis. Although there was variability in the number of animals dying at 1,250 mg/kg through this route of exposure, no indication of variability in response within these treatment groups was given by the authors in regard to extent of histopathological changes. The authors do not comment on the manner of death using this paradigm or of the effects of i.p. administration regarding potential peritonitis and inflammation.

TCE hepatotoxicity was "assessed by measuring plasma" SDH and ALT after TCE administration with vehicle treated control groups reported to induce no increases in these enzymes. Plasma SDH levels were reported to increase in a linear fashion after 250, 500, and 1,250 mg TCE/kg i.p. administration by 6 hours (i.e., ~3-, 10.5-, 22-, and 24.5-fold in comparison to controls from 250, 500, 1,250, and 2,500 mg/kg TCE, respectively) with little difference between the 1,250 and 250 mg/kg dose. By 12 hours the 250, 500, and 1,250 mg/kg levels had diminished to levels similar to that of the 250 mg/kg dose at 6 hours. The 2,500 mg/kg levels was somewhat diminished from its 6-hour level. By 24 hours after TCE exposure by the i.p. route of administration, all doses were similar to that of the 250-mg/kg-TCE 6-hour level. This pattern was reported to be similar for 5-, 36-, 48-, 72-, and 96-hour time points as well. The results presented were the means and SE for four rats per group. The authors did not indicate which rats were selected for these results from the 4–6 that were exposed in each group. Thus, only SDH levels showed dose-dependence in results at the 6-hour time point, and such increases did not parallel the patterns reported for hepatocellular necrosis from histopathological examination of liver tissues.

For ALT, the pattern of plasma concentrations after i.p. TCE administration differed both from that of SDH and from liver histopathology. Plasma ALT levels were reported to increase in a nonlinear fashion and to a much smaller extent than SDH (i.e., ~2.7-, 1.9-, 2.1-, and 4.0-fold of controls from 250, 500, 1,250, and 2,500 mg/kg TCE, respectively). The patterns for 12, 24, 36, 48, 72, and 96 hours were similar to that of the 6-hour exposure and did not show a dose-response. The authors injected carbon tetrachloride (2.5.mL/kg) into a separate group of rats and then incubated the resulting plasma with unbuffered TCA (TCA; 0, 200, 600, or 600 nmol) with decreases in enzyme activity in vitro at the two higher concentrations. It is not clear whether in vitro unbuffered TCA concentrations of this magnitude, which could precipitate proteins and render the enzymes inactive, are relevant to the patterns observed in the in vivo data. The extent of extinguishing of SDH and ALT activity at the two highest TCA levels in vitro were the same, suggestive of the generalized in vitro pH effect. However, the enzyme activity levels after TCE exposure had different patterns, suggesting that in vitro TCA results are not representative of the

in vivo TCE results. Neither ALT nor SDH levels corresponded to time course or dose-response reported for the histopathology of the liver presented in this study.

Tritiated thymidine results from isolated nuclei in the liver did not show a pattern consistent with either the histopathology or enzyme results. These results were for whole-liver homogenates and were not separated by nuclear size or cell origin. Tritiated thymidine incorporation was assumed by the authors to represent liver regeneration. There was no difference between treated and control animals at 6 hours after i.p. TCE exposure and only a decrease (~50% decrease) in thymidine incorporation after 12 hours of the 2,500 mg/kg TCE exposure level. By 24 hours, there was 5.6- and 2.8-fold tritiated thymidine incorporation at the 500 and 1,250 mg/kg TCE levels, with the 250 and 2,500 mg/kg levels similar to controls. For 36, 48, and 72 hours after i.p. TCE exposure, there continued to be no dose-response and no consistent pattern with enzyme or histopathological lesion patterns. The authors presented "area under the curve" data for tritiated thymidine incorporation for 0–95 hours, which did not include control values. There was a slight elevation at 500 mg/kg TCE and a slight decrease at 2,500 mg/kg from the 250 mg/kg TCE levels. Again, these data did not fit either histopathology or enzyme patterns and also can include the contribution of nonparenchymal cell nuclei as well as changes in ploidy.

The use of an i.p. route of administration is difficult to compare to oral and inhalation routes of exposure given that peritonitis and direct contact with TCE and corn oil with liver surfaces may alter results. Whereas Soni et al. (1998) report the LD_{50} to be 2,500 mg/kg TCE via i.p. administration, both Elcombe et al. (1985) and Melnick et al. (1987) do not report lethality from TCE administered for 10 days at 1,500 mg/kg in corn oil, or up to 4,800 mg/kg-day for 10 days in encapsulated feed. Also, TCE administered via gavage or oral administration through feed will enter the liver through the circulation with periportal areas of the liver the first areas exposed with the entire liver exposed in a fashion dependent on blood concentration levels. However, with i.p. administration, the absorption and distribution pattern of TCE will differ. The lack of concordance with measures of liver toxicity from this study and the lack concordance of patterns and dose-response relationships of toxicity reported from other more environmentally and physiologically relevant routes of exposure make the relevance of these results questionable.

E.2.1.2. Soni et al. (1999)

A similar paradigm and the same results were reported for Soni et al. (1999), in which hepatocellular necrosis, tritiated thymidine incorporation, and in vitro inhibition of SDH and ALT data were presented along with dose-response studies with ally alcohol and a mixture of TCE, thioacetamide, allyl alcohol, and chloroform. The same issues with interpretation present for Soni et al. (1998) also apply to this study as well.

E.2.1.3. Okino et al. (1991)

This study treated adult Wistar male rats (8 weeks of age) with TCE after being on a liquid diet for 3 weeks and either untreated or pretreated with phenobarbital or ethanol. TCE exposure was at 8,000 ppm for 2 hours, 2,000 or 8,000 ppm for 2 hours, and 500 or 2,000 ppm for 8 hours. Each group contained five rats. Livers from rats, that were not pretreated with either ethanol or phenobarbital, were reported to show only a few necrotic hepatocytes around the central vein at 6 and 22 hours after 2 hours of 8,000 ppm TCE exposure. At increased lengths and/or concentrations of TCE exposure, the frequencies of necrotic hepatocytes in the centrilobular area were reported to be increased, but the number of necrotic hepatocytes was still relatively low (out of ~150 hepatocytes the percentages of necrotic pericentral hepatocytes were 0.2 ± 0.4, 0.3 ± 0.4, 2.7 ± 1.0, 0.2 ± 0.4, and 3.5 ± 0.4% for control, 2,000 ppm TCE for 2 hours, 8,000 ppm TCE for 2 hours, 500 ppm TCE for 8 hours, and 2,000 ppm TCE for 8 hours, respectively).

"Ballooned" hepatocytes were reported to be zero for controls and all TCE treatments with the exception of 0.3 ± 0.6% ballooned midzonal hepatocytes after 8,000 ppm TCE for 2 hours of exposure. Microsomal protein (mg/g/liver) was increased with TCE exposure concentration and duration, but not reported to be statistically significant (i.e., mg/g/liver microsomal protein was 21.2 ± 4.3, 22.0 ± 1.5, 25.9 ± 1.3, 23.3 ± 0.8, and 24.1 ± 1.0 for control, 2,000 ppm TCE for 2 hours, 8,000 ppm TCE for 2 hours, 500 ppm TCE for 8 hours, and 2,000 ppm TCE for 8 hours, respectively).

The metabolic rate of TCE was reported to be increased after exposures over 2,000 ppm TCE (i.e., metabolic rate of TCE in nmol/g/liver/minute was 29.5 ± 5.7, 51.3 ± 6.0, 63.1 ± 16.0, 37.3 ± 3.3, and 69.5 ± 4.3 for control, 2,000 ppm TCE for 2 hours, 8,000 ppm TCE for 2 hours, 500 ppm TCE for 8 hours, and 2,000 ppm TCE for 8 hours, respectively). However, the CYP content of the liver was not reported to increase with TCE exposure concentration or duration.

The liver/body weight ratios were reported to increase with all TCE exposures except 500 ppm for 8 hours (i.e., the liver/body weight ratio was 3.18 ± 0.15, 3.35 ± 0.10, 3.39 ± 0.20, 3.15 ± 0.10, and 3.57 ± 0.14% for control, 2,000 ppm TCE for 2 hours, 8,000 ppm TCE for 2 hours, 500 ppm TCE for 8 hours, and 2,000 ppm TCE for 8 hours, respectively). These values represent 1.05-, 0.99-, 1.06-, and 1.12-fold of control in the 2,000 ppm TCE for 2 hours, 8,000 ppm TCE for 2 hours, 500 ppm TCE for 8 hours, and 2,000 ppm TCE for 8 hours treatment groups, respectively. A statistically significant difference observed after 8 hours of 2,000 ppm TCE exposure. Initial body weights and those 22 hours after cessation of exposure were not reported, which may have affected liver weight gain. However, these data suggest that TCE-related increases in metabolism and liver weight occurred as early as 22 hours after exposures of this magnitude from 2 to 8 hours of TCE with little concurrent hepatic necrosis.

Ethanol and phenobarbital pretreatment were reported to enhance TCE toxicity. In ethanol-treated rats, a few necrotic hepatocytes were reported to be around the central vein along

with hepatocellular swelling without pyknotic nuclei at 6 hours after TCE exposure with no pathological findings in the midzonal or periportal areas. At 22 hours, centrilobular hepatocytes were reported to have a few necrotic hepatocytes and cell infiltrations around the central vein, but midzonal areas were reported to have ballooned hepatocytes with pyknotic nuclei frequently accompanied by cell infiltrations. In phenobarbital-treated rats 6 hours after TCE exposure, centrilobular hepatocytes showed prenecrotic changes with no pathological changes reported to be observed in the periportal areas. By 22 hours, zonal necrosis was reported in centrilobular areas or in the transition zone between centrilobular and periportal areas. Treatment with phenobarbital or ethanol induced hepatocellular necrosis primarily in centrilobular areas with phenobarbital having a greater effect (89.1 ± 8.5% centrilobular necrosis) at the higher dose and shorter exposure duration (8,000 ppm × 2 hours) with ethanol having a greater effect (16.8 ± 5.3% centrilobular necrosis) at the lower concentration and longer duration of exposure (2,000 ppm × 8 hours).

E.2.1.4. Nunes et al. (2001)

This study was focused on the effects of TCE and lead co-exposure but treated male 75-day-old Sprague-Dawley rats with 2,000 mg/kg TCE for 7 days via corn-oil gavage (n = 10). The rats ranged in weight from 293 to 330 g (~12%) at the beginning of treatment and were pretreated with corn oil for 9 days prior to TCE exposure. TCE was reported to be 99.9% pure. Although the methods section states that rats were exposed to TCE for 7 days, Table 1 of the study reports that TCE exposure was for 9 days. The beginning body weights were not reported specifically for control and treatment groups, but the body weights at the end of exposure were reported to be 342 ± 18 g for control rats and 323 ± 3 g for TCE-exposed rats, and that difference (~6%) to be statistically significant. Because beginning body weights were not reported, it is difficult to distinguish whether differences in body weight after TCE treatment were treatment-related or reflected differences in initial body weights. The liver weights were reported to be 12.7 ± 1.0 g in control rats and 14.0 ± 0.8 g for TCE treated rats with the percent liver/body weight ratios of 3.7 and 4.3%, respectively. The increase in percent liver/body weight ratio represents 1.16-fold of control and was reported to be statistically significant. However, difference in initial body weight could have affected the magnitude of difference in liver weight between control and treatment groups. The authors report no gross pathological changes in rats gavaged with corn oil or with corn oil plus TCE, but observed that one animal in each group had slightly discolored brown kidneys. Histological examinations of "selected tissues" were reported to show an increased incidence of chronic inflammation in the arterial wall of lungs from TCE-dosed animals. There were no descriptions of liver histology given in this report for TCE-exposed animals or corn-oil controls.

E.2.1.5. Tao et al. (2000)

The focus of this study was to assess the effects of methionine on methylation and expression of c-Jun and c-Myc in mouse liver after 5 days of exposure to TCE (1,000 mg/kg in corn oil) and its metabolites. Female 8-week-old $B6C3F_1$ mice (n = 4–6) were administered TCE ("molecular biology or HPLC grade") for 5 days with and without methionine (300 mg/kg i.p.). Data regarding percent liver/body weight was presented as a figure. Of note is the decrease in liver/body weight ratio by methionine treatment alone (~4.6% liver/body weight for control and ~4.0% liver/body weight for control mice with methionine or ~13% difference in liver/body weight ratios between these groups). Neither initial body weights nor body weights after exposure were reported by the authors, so the reported effects of treatment could have reflected differences in initial body weights of the mice. TCE exposure was reported to increase the percent liver/body weight ratio to ~5.8% without methionine and to increase percent liver/body weight ratio to ~5.7% with methionine treatment. These values represent 1.26-fold of control levels from TCE exposure without methionine and 1.43-fold of control from TCE exposure with methionine. The number of animals examined was reported to be 4–6 per group. The authors reported the differences between TCE treated animals and their respective controls to be statistically significant, but did not examine the differences between controls with and without methionine. There were no descriptions of liver histology given in this report for TCE-exposed animals or corn-oil controls.

E.2.1.6. Tucker et al. (1982)

This study describes acute LD_{50}, and 5- and 14-day studies of TCE in a 10% emulphor solution administered by gavage. Screening-level subchronic drinking water experiments with TCE dissolved in 1% emulphor in mice were also conducted but with little detail reported. The authors did describe the strains used (CD-1 and ICR outbred albino) and that they were "weanling mice," but the ages of the mice and their weights were not given. The TCE was described as containing 0.004% diisopropylamine as the preservative and that the stabilizer had not been found carcinogenic or overtly toxic. The authors report that "the highest concentration a mouse would receive during these studies is 0.03 mg/kg/day." The main results are basically an LD_{50} study and a short-term study with limited reporting for 4- and 6-month studies of TCE exposure. Importantly, the authors documented the loss of TCE from drinking water solutions (<20% of the TCE was lost during the 3 or 4 days in the water bottles at 1.0, 2.5, and 5.0 mg/mL concentrations, but in the case of 0.1 mg/mL, up to 45% was lost over a 4-day period). The authors also report that high doses of TCE in drinking water reduced palatability to such an extent that water consumption by the mice was significantly decreased.

The LD_{50} with 95% confidence were reported to be 2,443 mg/kg (1,839–3,779 mg/kg) for female mice and 2,402 mg/kg (2,065–2,771 mg/kg) for male mice. However, the number of mice used in each dosing group was not given by the authors. The deaths occurred within

24 hours of TCE administration with no animals recovering from the initial anesthetic effect of TCE dying during the 14-day observation period. The authors reported that the only gross pathology observed was hyperemia of the stomach of mice dying from lethal doses of TCE, and that mice killed at 14 days showed no gross pathology.

In a separate experiment, male CD-1 mice were exposed to TCE by daily gavage for 14 days at 240 and 24 mg/kg. These two doses did not cause treatment-related deaths and body weight and "most" organ weights were reported by the authors to not be significantly affected but the data were not shown. The only effect noted was increased liver weight, which appeared to be dose dependent but was reported to be significant only at the higher dose. The only significant difference found in hematology was a 5% lower hematocrit in the higher dose group. The number of animals tested in this experiment was not given by the authors.

Male CD-1 mice (n = 11) were given TCE via gavage for 5 days (0.73 g/kg TCE twice on day 0, 1.46 g/kg twice on day 1, 2.91 g/kg twice on day 3, and 1.46 g/kg TCE on days 4 and 5) with only 4 of 11 mice treated with TCE surviving.

In a subchronic study, male and female CD-1 mice received TCE in drinking water at concentrations of 0, 0.1, 1.0, 2.5, and 5 mg/mL in 1% emulphor, and a naïve group received deionized water. There were 140 animals of each sex in the naïve group and in each treatment group, except for 260 mice in the vehicle groups. Thirty mice of each sex and treatment were selected for recording body weights for 6 months. The method of "selection" was not given by the authors. These mice were weighed twice weekly and fluid consumption was measured by weighing the six corresponding water bottles. The authors reported that male mice at the two highest doses of TCE consumed 41 and 66 mL/kg-day less fluid over the 6 months of the study than mice consuming vehicle only and that this same decreased consumption was also seen in the high dose (5 mg/mL) females. They report that weight gain was not affected except at the high dose (5mg/mL) and even though the weight gain for both sexes was lower than the vehicle control group, it was not statistically significant. However, these data were not shown. The authors report that gross pathological examinations performed on mice killed at 4 and 6 months were unremarkable and that a number of mice from all of the dosing regimens had liver abnormalities, such as pale, spotty, or granular livers. They report that 2 of 58 males at 4 months, and 11 of 59 mice at 6 months had granular livers and obvious fatty infiltration, and that mice of both sexes were affected. Animals in the naïve and vehicle groups were reported to infrequently have pale or spotty livers, but exhibit no other observable abnormalities. No quantitation or more detailed descriptions of the incidence of or severity of effects were given in this report.

The average body weight of male mice receiving the highest dose of TCE was reported to be 10% lower at 4 months and 11% lower at 6 months with body weights of female mice at the highest dose also significantly lower. Enlarged livers (as percentage of body weight) were observed after both durations of exposure in males at the three highest doses and in females at

the highest dose. In the 4-month study, brain weights of treated females were significantly increased when compared to vehicle control. However, the authors state:

> This increase is apparently because the values for the vehicle group were low, because the naïve group was also significantly increased when compared to vehicle control. A significant increase in kidney weight occurred at the highest dose in males at 6 months and in females, after both 4 and 6 months of TCE exposure. Urinalysis indicated elevated protein and ketone levels in high-dose females and the two highest dose males after 6 months of exposure (data not shown).

The authors describe differences in hematology to include:

> a decreased erythrocyte count in the high dose males at 4 and 6 months (13% and 16%, respectively); decreased leukocyte counts, particularly in the females at 4 months and altered coagulation values consisting of increased fibrinogen in males at both times and shortened prothrombin time in females at 6 months (data not shown). No treatment-related effects were detected on the types of white cells in peripheral blood.

It must be noted that effects reported from this study may have also been related to decreased water consumption, this study did not include any light microscopic evaluation, and that most of the results described are for data not shown. However, this study does illustrate the difficulties involved in trying to conduct studies of TCE in drinking water, that the LD_{50} values for TCE are relatively high, and that liver weight increases were observed with TCE exposure as early as a few weeks and increased liver weight were sustained through the 6-month study period.

E.2.1.7. Goldsworthy and Popp (1987)

The focus of this study was peroxisomal proliferation activity after exposure to a number of chlorinated solvents. In this study 1,000 mg/kg TCE (99+% epoxide stabilizer free) was administered to male F-344 rats (170–200 g or ~10% difference) and $B6C3F_1$ (20–25 g or ~20% difference) mice for 10 days in corn oil via gavage. The ages of the animals were not given. The TCE-exposed animals were studied in two experiments (experiments #1 and #3). In experiment #2, corn oil and methyl cellulose vehicles were compared. Animals were killed 24 hours after the last exposure. The authors did not show data on body weight, but stated that the administration of test agents (except WY-14,643 to rats which demonstrated no body weight gain) to rats and mice for 10 days "had little or no effect on body weight gain." Thus, differences in initial body weight between treatment and control groups, which could have affected the magnitude of TCE-induced liver weight gain, were not reported. The liver/body weight ratios in corn oil gavaged rats were reported to be 3.68 ± 0.06 and 4.52 ± 0.08% after

TCE treatment, which represented 1.22-fold of control (n = 5). Cyanide-(CN-)insensitive palmitoyl CoA[12] oxidation (PCO) was reported to be 1.8-fold increased after TCE treatment in this same group. In $B6C3F_1$ mice the liver/body weight ratio in corn oil gavaged mice was reported to be 4.55 ± 0.13 and 6.83 ± 0.13% after TCE treatment which represented 1.50-fold of control (n = 7). CN-insensitive PCO activity was reported to be 6.25-fold of control after TCE treatment in this same group. The authors report no effect of vehicle on PCO activity, but do not show the data nor discuss any effects of vehicle on liver weight gain. Similarly, the results for experiment #3 were not shown nor liver weight discussed with the exception of PCO activity reported to be 2.39-fold of control in rat liver and 6.25-fold of control for mouse liver after TCE exposure. The number of animals examined in Experiment #3 was not given by the authors or the variation between enzyme activities. However, there appeared to be a difference in PCO activity in experiments #1 and #3 in rats. There were no descriptions of liver histology given in this report for TCE-exposed animals or corn-oil controls.

E.2.1.8. Elcombe et al. (1985)

In this study, preservative-free TCE was given via gavage to rats and mice for 10 consecutive days with a focus on changes in liver weight, structure, and hepatocellular proliferation induced by TCE. Male Alderley Park rats (Wistar derived) (180–230 g), male Osborne-Mendel rats (240–280 g), and male $B6C3F_1$ or male Alderley Park Mice (Swiss) weighing 30–35 g were administered 99.9% pure TCE dissolved in corn oil via gavage. The ages of the animals were not given by the authors. The animals were exposed to 0, 500, 1,000, or 1,500 mg/kg body weight TCE for 10 consecutive days. The number of mice and rats varied widely between experiments and treatment groups and between various analyses. In some experiments, animals were injected with tritiated thymidine approximately 24 hours following the final dose of TCE and killed 1 hour later. The number of hepatocytes undergoing mitosis was identified in 25 random high-power fields (X40) for each animal with 5,000 hepatocyte per animal examined. There was no indication by the authors that zonal differences in mitotic index were analyzed. Sections of the liver were examined by light and electron microscopy by conventional staining techniques. Tissues selected for electron microscopy included central vein and portal tract so that zonal differences could be elucidated. Morphometric analysis of peroxisomes was performed "according to general principles of Weibel et al. (1964) on electronphotomicrographs from pericentral hepatocytes." DNA content of samples and peroxisomal enzyme activities were determined in homogenized liver (catalase and PCO activity).

The authors reported that TCE treatment had no significant effect on body-weight gain in either strain of rat or mouse during the 10-day exposure period. However, marked increases (up

[12]CoA = coenzyme A.

to 175% of control value) in the percent liver/body weight ratio were observed in TCE-treated mice. Smaller increases (up to 130% of control) in relative liver weight were observed in TCE-treated rats. No significant effects of TCE on hepatic water content were seen, so the liver weight did not represent increased water retention.

An interesting feature of this study was that it was conducted in treatment blocks at separate times with separate control groups of mice for each experimental block. Therefore, there were three control groups of $B6C3F_1$ mice (n = 10 for each control group) and three control groups for Alderley Park (n = 9–10 for each control group) mice that were studied concurrently with each TCE treatment group. However, the percent liver/body weight ratios were not the same between the respective control groups. There was no indication from the authors as to how controls were selected or matched with their respective experimental groups. The authors did not give liver weights for the animals, so the actual changes in liver weights were not given. The body weights of the control and treated animals were also not given by the authors. Therefore, if there were differences in body weight between the control groups or treatment groups, the liver/body weight ratios could also have been affected by such differences. The percentage increase over control could also have been affected by what control group each treatment group was compared to. There was a difference in the mean percent liver/body weight ratio in the control groups, which ranged from 4.32 to 4.59% in the $B6C3F_1$ mice (~6% difference) and from 5.12 to 5.44% in the Alderley Park mice (~6% difference). The difference in average percent liver/body weight ratio for untreated mice between the two strains was ~16%. Because the ages of the mice were not given, the apparent differences between strains may have been due to both age or to strain.

After TCE exposure, the mean percent liver/body weight ratios were reported to be 5.53% for 500 mg/kg, 6.50% for 1,000 mg/kg, and 6.74% for 1,500 mg/kg TCE-exposed $B6C3F_1$ mice. This resulted in 1.20-, 1.50-, and 1.47-fold values of control in percent liver weight/body weight for $B6C3F_1$ mice. For Alderley Park mice, the percent liver/body weight ratios were reported to be 7.31, 8.50, and 9.54% for 500, 1,000, and 1,500 mg/kg TCE treatment, respectively. This resulted in 1.43-, 1.56-, and 1.75-fold of control values. Thus, there appeared to be more of a consistent dose-related increase in liver/body weight ratios in the Alderley Park mice than the $B6C3F_1$ mice after TCE treatment. However, the variability in control values may have distorted the dose-response relationship in the $B6C3F_1$ mice. The SDs for liver/body weight ratio were as much as 0.52% for the treated $B6C3F_1$ mice and 0.91% for the Alderley Park treated mice. In regard to the correspondence of the magnitude of the TCE-induced increases in percent liver/body weight with the magnitude of difference in TCE exposure concentrations, in the $B6C3F_1$, mice the increases were similar (approximately twofold) between the 500 and 1,000 mg/kg TCE exposure groups. For the Alderley Park mice, the increases in TCE exposure concentrations were slightly less than the magnitude of increases in percent liver/body ratios between all of the concentrations (i.e., ~1.3-fold of control vs. 2-fold for 500

and 1,000 mg/kg TCE dose and 1.3-fold of control vs. 1.5-fold for the 1,000 and 1,500 mg/kg TCE dose).

The DNA content of the liver varied greatly between control animal groups. For $B6C3F_1$ mice it ranged from 2.71 to 2.91 mg/g liver. For Alderley Park mice, it ranged from 1.57 to 2.76 mg/g liver. The authors do not discuss this large variability in baseline levels of DNA content. The DNA content in $B6C3F_1$ mice was mildly depressed by TCE treatment in a nondose-dependent manner. DNA concentration decrease from control ranged from 20 to 25% between all three TCE exposure levels in $B6C3F_1$ mice. For Alderley Park mice there was also nondose related decrease in DNA content from controls that ranged from 18 to 34%. Thus, the extent of decrease in DNA content of the liver from TCE treatment in $B6C3F_1$ mice was similar to the variability between control groups. The lack of dose-response for apparent treatment-related effects in $B6C3F_1$ mice and especially in the Alderley Park mice was confounded by the large variability in the control animals. The changes in liver weight after TCE exposure for the AP mice did not correlate with changes in DNA content further, raising doubt about the validity of the DNA content measures. However, a small difference in DNA content due to TCE treatment in all groups was reported for both strains and this is consistent with hepatocellular hypertrophy.

The reported results for incorporation of tritiated thymidine in liver DNA showed large variation in control groups and SDs that were especially evident in the Alderley Park mice. For $B6C3F_1$ mice, mean control levels were reported to range from 5,559 to 7,767 dpm/mg DNA with SDs ranging from 1,268 to 1,645 dpm/mg DNA. In Alderley Park mice, mean control levels were reported to range from 6,680 to 10,460 dpm/mg DNA with SDs ranging from 308 to 5,235 dpm/mg DNA. For $B6C3F_1$ mice, TCE treatment was reported to induce an increase in tritiated thymidine incorporation with a very large SD, indicating large variation between animals. For the 500 mg/kg TCE treatment group, the values were reported as 12,334 ± 4,038, for the 1,000 mg/kg TCE treatment group, 21,909 ± 13,386, and for the 1,500 mg/kg treatment TCE group, 26,583 ± 10,797 dpm/mg DNA. In Alderley Park mice, TCE treatment was reported to give an increase in tritiated thymidine incorporation also with a very large SD. For 500 mg/kg TCE, the values were reported as 19,315 ± 12,280; for 1,000 mg/kg, TCE 21,197 ± 8,126; and for 1,500 mg/kg TCE, 38,370 ± 13,961. As a percentage of concurrent control, the increase in tritiated thymidine was reported to be 2.11-, 2.82-, and 4.78-fold of control in $B6C3F_1$ mice, and 2.09-, 2.03-, and 5.74-fold of control in Alderley Park mice. Accordingly, the change in tritiated thymidine incorporation did show a treatment related increase but not a dose-response.

Similar to the DNA content of the liver, the large variability in measurements between control groups and variability between animals limit quantitative interpretation of these data. The increase in tritiated thymidine, seen most consistently only at the highest exposure level in both strains of mice, could have resulted from either a change in ploidy of the hepatocytes or cell number. However, the large change in volume in the liver (75%) in the Alderley Park mice,

could not have resulted from only a fourfold of control in cell proliferation even if all tritiated thymidine incorporation had resulted from changes in hepatocellular proliferation. As mentioned in Section E.1.1 above, the baseline level of hepatocellular proliferation in mature control mice is very low and represents a very small percentage of hepatocytes.

In the experiments with male rats, the same issues discussed above, associated with the experimental design, applied to the rat experiments with the additional concern that the numbers of animals examined varied greatly (i.e., 6–10) between the treatment groups. In Osborne-Mendel rats, the control liver/body weight ratio was reported to vary from 4.26 to 4.36% with the SDs varying between 0.22 and 0.27%. For the Alderley Park rats, the liver/body weight ratios were reported to vary between 4.76 and 4.96% (in control groups) with SDs varying between 0.24 and 0.47%. TCE treatment was reported to induce a dose-related increase in liver/body weight ratio in Osborne-Mendel rats with mean values of 5.16, 5.35, and 5.53% in 500, 1,000, and 1,500 mg/kg TCE treated groups, respectively. This resulted in 1.18-, 1.26-, and 1.30-fold values of control. In Alderley Park rats, TCE treatment was reported to result in increased liver weights of 5.45, 5.83, and 5.65% for 500, 1,000, and 1,500 mg/kg TCE respectively. This resulted in 1.14-, 1.17-, and 1.17-fold values of control. Again, the variability in control values may have distorted the nature of the dose-response relationships in Alderley Park rats. TCE treatment was reported to result in SDs that ranged from 0.31 to 0.48% for Osborne-Mendel rats and from 0.24 to 0.38% for Alderley Park rats. What is clear from these experiments is that TCE exposure was associated with increased liver/body weight in rats.

The reported mean hepatic DNA concentrations and SDs varied greatly in control rat liver as it did in mice. The variation in DNA concentration in the liver varied more between control groups than the changes induced by TCE treatment. For Osborne-Mendel rats, the mean control levels of mg DNA/g liver were reported to range from 1.99 to 2.63 mg DNA/liver with SDs varying from 0.17 to 0.33 mg DNA/g. For Alderley Park rats, the mean control levels of mg DNA/g liver were reported to be 2.12–3.16 mg DNA/g with SD ranging from 0.06 to 1.04 mg DNA/g. TCE treatment decreased the liver DNA concentration in all treatment groups. For Osborne-Mendel rats, the decrease ranged from 8 to 13% from concurrent control values and for Alderley Park rats the decrease ranged from 8 to 17%. There was no apparent dose response in the decreases in DNA content, with all TCE treatment levels giving a similar decrease from controls and the same limitations discussed above for the mouse data apply here. The magnitude of increases in liver/body ratios shown by TCE treatment were not correlated with the changes in DNA content. However, as with the mouse data, the small differences in DNA content due to TCE treatment in all groups and in both strains were consistent with hepatocellular hypertrophy.

Incorporation of tritiated thymidine was reported to be even more variable between control groups of rats than it was for mice and was reported to be especially variable between control groups (i.e., 2.7-fold difference between control groups within strain) and differed between the strains (average of 2.5-fold between strains). For Osborne-Mendel rats, the mean

control levels were reported to range from 13,315 to 33,125 dpm/mg DNA, while for Alderley Park rats, tritiated thymidine incorporation ranged from 26,613 to 69,331 dpm/mg DNA for controls. The SDs were also very large (i.e., for control groups of Osborne-Mendel rats, they were reported to range from 8,159 to 13,581 dpm/mg DNA, while for Alderley Park rats, they ranged from 9,992 to 45,789 dpm/mg DNA). TCE treatment was reported to induce increases over controls of 110, 118, and 106% for 500, 1,000, and 1,500 mg/kg TCE-exposed groups, respectively, in Osborne-Mendel rats with large SDs for these treatment groups as well. In Alderley Park rats, the increases over controls were reported to be 206, 140, and 105% for 500, 1,000, and 1,500 mg/kg TCE, respectively. In general, these data do indicate that TCE treatment appeared to give a mild increase in tritiated thymidine incorporation but the lack of dose-response can be attributable to the highly variable measurements of tritiated thymidine incorporation in control animal groups. The variation in the number of animals examined between groups and small numbers of animals examined additionally decrease the likelihood of being able to discern the magnitude of difference between species- or strain-related effects for this parameter. Again, given the very low level of hepatocyte turnover in control rats, this does not represent a large population of cells in the liver that may be undergoing proliferation and cannot be separated from changes in ploidy.

The authors report that the reversibility of these phenomena was examined after the administration of TCE to Alderley Park mice for 10 consecutive days. Effects upon liver weight, DNA concentration, and tritiated thymidine incorporation 24 and 48 hours after the last dose of TCE were reported to be still apparent. However, 6 days following the last dose of TCE, all of these parameters were reported to return to control values with the authors not showing the data to support this assertion. Thus, cessation of TCE exposure would have resulted in a 75% reduction in liver weight by one week in mice exposed to the highest TCE concentration.

Analyses of hepatic peroxisomal enzyme activities were reported for catalase and β-oxidation (PCO activity) following administration of TCE to $B6C3F_1$ mice and Alderley Park rats exposed to 1,000 mg/kg TCE for 10 days. The authors only used five control and five exposed animals for these tests. An 8-fold of control value for PCO activity and a 1.5-fold of control value for catalase activity were reported for $B6C3F_1$ mice exposed to 1,000 mg/kg TCE. In the Alderley Park rats, no significant changed occurred. It is unclear which mice or rats were selected from the previous experiments for these analyses and what role selection bias may have played in these results. The reduced number of animals chosen for this analysis also reduces the power of the analysis to detect a change. In rats, there was a reported 13% increase in PCO; however, the variation between the TCE-treated rats was more than double that of the control animals in this group and the other limitations described above limit the ability to detect a response. There was no discussion given by the authors as to why only one dose was tested in half of the animals exposed to TCE or why the strain with the lowest liver weight change due to TCE exposure was chosen as the strain to test for peroxisomal proliferative activity.

The authors provided a description of the histopathology at the light microscopy level in $B6C3F_1$ mice, Alderley Park mice, Osborne-Mendel rats, and Alderley Park rats, but did not provide a quantitative analysis or specific information regarding the variability of response between animals within groups. There appeared to be 20 animals examined in the 1,000 mg/kg TCE exposed group of $B6C3F_1$ mice but no explanation as to why there were only 10 animals examined in analyses for liver weight changes, DNA concentration, and tritiated thymidine incorporation. There was no indication by the authors regarding how many rats were examined by light microscopy.

Apart from a few inflammatory foci in occasional animals, hematoxylin and eosin (H&E) section from $B6C3F_1$ control mice were reported to show no abnormalities. The authors suggest that this is a normal finding in the livers of mice kept under "non-SPF conditions." A stain for neutral lipid was reported to not be included routinely in these studies, but subsequent electron microscopic examination of lipid was reported to show increases in the livers of corn-oil treated control animals. The individual fat droplets were described as "generally extremely fine and are not therefore detectable in conventionally process H&E stained sections, since both glycogen and lipid are removed during this procedure." Thus, this study documents effects of using corn oil gavage in background levels of lipid accumulation in the liver.

The finding of little evidence of gross hepatotoxicity in TCE-treated mice was reported, even at a dose of 1,500 mg/kg. Specifically,

> Of 19 animals examined receiving 1500 mg/kg body weight TCE, only 6 showed any evidence of hepatocyte necrosis, and this pathology was restricted to single small foci or isolated single cells, frequently occurring in a subcapsular location. Examination of 20 animals receiving 1000 mg/kg body wt TCE demonstrated no hepatocyte necrosis. Of 20 animals examined receiving 500 mg/kg body wt TCE, 1 showed necrosis of single isolated hepatocytes; however, this change was not a treatment-related finding.

TCE-treated mice were reported to show:

> a change in staining characteristic of the hepatocytes immediately adjacent to the central vein of the hepatocyte lobules, giving rise to a marked 'patchiness' of the liver sections. Often this change consisted of increased eosinophilia of the central cells. There was some evidence of cell hypertrophy in the centrilobular regions. These changes were evident in most of the TCE treated animals, but there was a dose-related trend, relatively few of the 500 mg/kg animals being affected, while the majority of the 1,500 mg/kg animals showed central change. No other significant abnormalities were seen in the liver of TCE treated mice compared to controls apart from occasional mitotic figures and the appearance of isolated nuclei with an unusual chromatin pattern. This pattern generally consisted of a course granular appearance with a prominent rim of chromatin around the

periphery of the nucleus. These nuclei may have been in the very early stages of mitosis. Similar changes were not seen in control mice.

The authors briefly commented on the findings in the Alderley Park mice stating that:

> H& E sections from Alderley Park mice gave similar results as for $B6C3F_1$ mice. No evidence of hepatotoxicity was seen at a dose of 500 mg/kg body wt TCE. However, a few animals at the higher doses showed some necrosis and other degenerative changes. This change was very mild in nature, being restricted to isolated necrotic cells or small foci, frequently in subcapsular position. Hypertrophy and increased eosinophilia were also noticed in the centrilobular regions at higher doses.

Thus, from the brief description given by the authors, the centrilobular region is identified as the location of hepatocellular hypertrophy due to TCE exposure in mice, and for it to be dose-related with little evidence of accompanying hepatotoxicity.

The description of histopathology for rats was even more abbreviated than for the mouse. H& E sections from Osborne-Mendel rats showed that:

> livers from control rats contained large quantities of glycogen and isolated inflammatory foci, but were otherwise normal. The majority of rats receiving 1,500 mg/kg body weight TCE showed slight changes in centrilobular hepatocytes. The hepatocytes were more eosinophilic and contained little glycogen. At lower doses, these effects were less marked and were restricted to fewer animals. No evidence of treatment-related hepatotoxicity (as exemplified by single cell or focal necrosis) was seen in any rat receiving TCE. H& E sections from Alderley Park Rats showed no signs of treatment-related hepatotoxicity after administration of TCE. However, some signs of dose-related increase in centrilobular eosinophilia were noted.

Thus, both mice and rats exhibited pericentral hypertrophy and eosinophilia as noted from the histopathological examination.

The study did report a quantitative analysis of the effects of TCE on the number of mitotic figures in livers of mice. Few if any control mice exhibited mitotic figures. But, the authors report:

> a considerable increase in both the numbers of figures per section was noted after administration of TCE." The numbers of animals examined for mitotic figures ranged from 75 (all control groups were pooled for mice) to 9 in mice, and ranged from 15 animals in control rat groups to as low as 5 animals in the TCE treatment groups. The range of mitotic figures found in 25 high-power fields was reported and is equivalent to the number of mitotic figures per 5,000 hepatocytes examined in random fields.

Thus, the predominance of mitotic figures in any zone of the liver cannot be ascertained.

For $B6C3F_1$ mice, the number of animals with mitotic figures was reported to be 0/75, 3/20, 7/20, and 5/20 for control, 500, 1,000, and 1,500 mg/kg TCE exposed mice, respectively. The range of the number of mitotic figures seen in 5,000 hepatocytes was reported to be 0, 0–1, 0–5, and 0–5 for those same groups with group means of 0, 0.15 ± 0.36, 0.6 ± 1.1, and 0.5 ± 1.2. These results demonstrate a very small and highly variable response due to TCE treatment in $B6C3F_1$ mice in regard to mitosis. Thus, the highest percentage of cells undergoing mitosis within the window of observation would be on average 0.012% with a SD twice that value. The data presented for mitotic figures also indicated no differences in results between 1,000 and 1,500 mg/kg treated $B6C3F_1$ mice in regard to mitotic figure detection. However, the tritiated thymidine incorporation data indicated that thymidine incorporation was approximately twofold greater at 1,500 than 1,000 mg/kg TCE in $B6C3F_1$ mice. For Alderley Park mice, the number of animals with mitotic figures was reported to be 1/15, 0/9, 4/9, and 2/9 for control, 500, 1,000, and 1,500 mg/kg TCE exposed mice. The range of the number of mitotic figures seen in 5,000 hepatocytes was 0–1, 0, 0–2, and 0–1 for those same groups with group means of 0.06 ± 0.25, 0.7 ± 0.9, and 0.2 ± 0.4. These results reveal the detection of, at the most, two mitotic figures in 5,000 hepatocytes for any mouse an any treatment group and no dose-related increased after TCE treatment in Alderley Park mice. Thus, the highest percentage of cells with a mitotic figure would be on average 0.014% with a SD twice that value. The small number of animals examined reduces the power of the experiment to draw any conclusions as to a dose-response.

Similar to the $B6C3F_1$ mice, there did not appear to be concordance between mitotic figure detection and thymidine incorporation for Alderley Park mice. Thymidine incorporation showed a 2-fold increase over control for 500 and 1,000 mg/kg TCE and a 5.7-fold increase for 1,500 mg/kg TCE treated animals. However, in regard to mitotic figure detection, there were fewer mitotic figures in 500 mg/kg TCE treated mice than controls, and fewer animals with mitotic figures and fewer numbers of figures in the 1,500 mg/kg dose than the 1,000 mg/kg exposed group. The inconsistencies between mitotic index data and thymidine incorporation data in both strains of mice suggest that either thymidine incorporation is representative of only DNA synthesis and not mitosis, an indication of changes in ploidy rather than proliferation, or that this experimental design is incapable of discerning the magnitude of these changes accurately. Data from both mouse strains show very little, if any, hepatocyte proliferation due to TCE exposure with the mitotic figure index data having that advantage of being specific for hepatocytes and to not to also include nonparenchymal cells or inflammatory cells in the liver.

The results for rats were similar to those for mice and even more limited by the varying and low number of animals examined. For Osborne-Mendel rats, the numbers of animals with mitotic figures were reported to be 8/15, 2/9, 0/7, and 0/6 for control, 500, 1,000, and 1,500 mg/kg TCE exposed rats groups, respectively, with the respective ranges of the number of mitotic figures seen in 5,000 hepatocytes to be 0–8, 0–3, 0, and 0. The group means were 1.5 ±

2.0, 0.4 ± 1.0, 0, and 0 for these groups. It would appear from these results that there are fewer mitotic figures after TCE treatment with the highest percentage of cells undergoing mitosis to be on average 0.03% in control rats. However, thymidine incorporation studies show a modest increase at all treatment levels over controls in Osborne-Mendel rats rather than a decrease from controls. For Alderley Park rats, the numbers of animals with mitotic figures were reported to be 13/15, 5/9, 9/9, and 4/9 for control, 500, 1,000, and 1,500 mg/kg TCE exposed rat groups with the ranges of the number of mitotic figures seen in 5,000 hepatocytes to be 0–26, 0–5, 1–7, and 0–9. The group means were 7.2 ± 4.7, 1.6 ± 4.3, 3.8 ± 3.4, and 1.8 ± 2.9 for these groups.

It would appear that there are fewer mitotic figures after TCE treatment with the highest percentage of cells to an average of 0.14% in control rats. However, thymidine incorporation studies show twofold greater level at 500 mg/kg TCE than for control animals and a 40 and 5% increase at 1,000 and 1,500 mg/kg TCE exposure groups, respectively. Similar to the results reported in mice, results in both rat strains show an inconsistency in mitotic index and thymidine incorporation. The control rats appear to have a much greater mitotic index than any of the mouse groups (treated or untreated) or the TCE-treatment groups. However, it is the mice that were exhibiting the largest increased in liver weight after TCE exposure. By either thymidine incorporation or mitosis, these data do provide a consistent result that at 10 days of exposure, very little sustained hepatocellular proliferation is occurring in either mouse or rat and neither is correlated well with the concurrent changes in liver weight observed from TCE exposure.

This study provided a qualitative discussion and quantitative analysis of structural changes using electron microscopy. The qualitative discussion was limited and included statements about increased observances without quantitative data shown other than the morphometric analysis. The authors reported that:

> the ultrastructure of control mouse liver was essentially normal, although mild dilatation of RER and SER was a frequent finding. Lipid droplets were also usually present in the cell cytoplasm. The ultrastructural changes seen in mouse liver following administration of up to 1,500 mg/kg body wt TCE for 10 days were essentially similar in the $B6C3F_1$ mouse and the Alderley Park mouse. The most notable change in both strains of mouse was a dramatic increase in the number of peroxisomes. This change was only apparent in the cells immediately surrounding the central veins. Peroxisome proliferation was not noticeable in periportal cells. The induced peroxisomes were generally small and very electron dense and frequently lacked the characteristic nucleoid core found in peroxisomes of control livers.

The authors conclude that:

> morphometric analysis showed evidence of a dose-related response, peroxisomal induction appearing to reach a maximum at 1,000 mg/kg in $B6C3F_1$ mice…Lipid was increased in the livers of treated mice at all doses and was present both as

free droplets in the cytoplasm and as liposomes (small lipid droplets in ER cisternae). The centrilobular cell, which showed the greatest increase in numbers of peroxisomes, showed no evidence of this lipid accumulation: fatty change was more prominent in those cells away from the central vein (i.e., zone 2 of the liver acinus). Accumulation of lipid, particularly in liposomes, was less marked in Alderley Park mouse than in B6C3F$_1$ mouse. Mild proliferation of smooth endoplasmic reticulum was seen in both strains and both rough and smooth endoplasmic reticulum was generally more dilated than in control mice.

Electron microscopic results for rat liver were reported

to show similar changes in Osborne-Mendel and Alderley Park rat treated with TCE…Rats receiving either 1,000 or 1,500 mg/kg TCE for 10 days generally showed mild proliferation of SER in centrilobular hepatocytes. The cisternae of RER were frequently dilated, giving rise to a rather disorganized appearance in contrast to the parallel stacks seen in control livers, although no detachment of ribosomes was evident. The SER was also dilated. In contrast to mice, peroxisomes were only very slightly and not significantly, increased in the liver of TCE –treated rats. Morphometric analysis confirmed this observation, with the volume density of peroxisomes in the cytoplasm of centrilobular hepatocytes being only slightly increased in rats of both strains receiving 1,000 or 1,500 mg/kg body wt TCE…Lipid droplets were occasionally increased in some livers obtained from rats receiving TCE, but the degree of fatty change generally appeared similar to that found in control rats receiving corn oil. There were no changes in membrane –bound liposomes, other organelles, or Golgi condensing vesicles. Centrilobular glycogen was somewhat depleted in male rats receiving 1,500 mg/kg TCE. Periportal cells were ultrastructurally normal in all rats.

For the morphometric analysis, the number of mice examined ranged from seven in the control group to eight in the 1,500 mg/kg TCE exposed group. The authors did not indicate which control animals were used for the morphometric analysis from the 75 animals examined for mitotic index, the 20 examined by light microscopy, or the 30 mice used as concurrent controls in the liver weight, DNA concentration, and tritiated thymidine incorporation studies. The authors stated that morphometry was performed on three randomly selected photomicrographs from each of three randomly selected pericentral hepatocytes for each animal (i.e., nine photomicrographs per animal). A mean value representing the exposure group was reported with the variability between photomicrographs per animal or the variation between animals unclear. The morphometric analysis did not examine all treatment groups (e.g., only the control and 500 mg/kg TCE group were examined in Alderley Park mice).

The percent cytoplasmic volume of the peroxisomal compartment (mean ± SD) was reported to be 0.6 ± 0.6% for controls, 4.8 ± 3.3% for 500 mg/kg TCE, 6.7 ± 1.9% for 1,000 mg/kg TCE, and 6.4 ± 2.5% for 1,500 mg/kg TCE in B6C3F$_1$ mice. In Alderley Park mice, only 12 control and 12,500 mg/kg TCE exposed mice were examined and, similarly, their

selection criteria was not given. The percent cytoplasmic volume of the peroxisomal compartment was 1.2 ± 0.4% for control and 4.7 ± 2.8% for 500 mg/kg TCE exposed mice.

For Osborne-Mendel rats, control rats (n = 9) were reported to have a percent cytoplasmic volume of the peroxisomal compartment of 1.8 ± 0.4%; 1,000 mg/kg TCE (n = 5), 2.3 ± 1.6%, and 1,500 mg/kg exposed rats (n = 7), 2.3 ± 2.0%. For Alderley Park rats, only two groups were examined (control and 1,000 mg/kg TCE exposure). The percent cytoplasmic volume of the peroxisomal compartment for control rats (n = 15) was reported to be 1.8 ± 0.8% and for 1,000 mg/kg TCE (n = 16), 2.4 ± 1.2%. The varying numbers of animals examined, the varying and inconsistent number of treatment groups examined, the limited number of photomicrographs per animal, and the potential selection bias for animals examined make quantitative conclusions regarding this analysis difficult. Although control levels differed by a factor of 2 between the two strains of mice examined, as well as the number of control animals examined (7 vs. 12), it appears that the 500-mg/kg TCE-exposed $B6C3F_1$ and Alderley Park mice had similar percentages of peroxisomal compartment in the pericentral cells examined (~4.8%). There also appeared to be little difference between 1,000 mg/kg TCE treated Osborne-Mendel and Alderley Park rats for this parameter (~2.4%). Although few animals were examined, there was little difference reported between 500, 1,000, and 1,500 mg/kg TCE exposure groups in regard to percentages of peroxisomal compartment in $B6C3F_1$ mice (4.8–6.7%). For the few rats of the Osborne-Mendel strain examined, there also did not appear to be a difference between 1,000 and 1,500 mg/kg TCE exposure for this parameter (2.3%).

Based on peroxisome compartment volume data, one would expect there to be little difference between TCE exposure groups in mice or rats in regard to enzyme activity or other "associated events." However, such comparisons are difficult due to limited power to detect differences and the possibility of bias in selection of animals in differing assays. For the $B6C3F_1$ mice, only 5 animals per group were examined for enzyme analysis, 7–8 animals for morphometric analysis, 75 animals in control, and 20 animals in 1,000 mg/kg TCE-exposed groups for mitotic figure identification, and 10 animals per group for thymidine incorporation. Since only a few animals were tested for enzyme activity, the comparison between peroxisomal compartment volume and that parameter is very limited. There was a reported 47% increase in catalase activity between control (n = 5) and 1,000 mg/kg TCE exposed $B6C3F_1$ mice (n = 5) and a 7.8-fold increase in PCO activity. The percent peroxisome compartment was reported to be 10.6-fold greater (0.6 vs. 6.4%). However, the $B6C3F_1$ control percent volume of peroxisomal compartment was reported to be half that of the Alderley Park mouse control. An accurate determination of the quantitative differences in peroxisomal proliferation would be dependent on an accurate and stable control value. For Alderley Park rats, there was an 8% decrease in catalase activity between control (n = 5) and 1,000 mg/kg TCE exposed rats (n = 5), and a 13% increase in PCO activity. The percent peroxisome compartment was reported to be 33% greater in the TCE-exposed than control group. Thus, for the very limited data that were

available to compare peroxisomal compartment volume with enzyme activity, there was consistency in result.

However, were such increases in peroxisomes associated with other events reported in this study? Mouse peroxisome proliferation associated enzyme activities in $B6C3F_1$ mice at 1,000 mg/kg TCE were reported to be 8-fold over control values in mice after 10 days of treatment. However, this increase in activity was not accompanied by a similar increase in thymidine incorporation (2.8-fold of control) or concordant with increases in mitotic figures (7/20 mice having any mitotic figures at all with a range of 0–5 and a mean of 0.014% of cells undergoing mitosis for 1,000 mg/kg TCE vs. 0 for control).

Although results reported in the rat showed discordance between thymidine incorporation and detection of mitotic figures, there was also discordance with these indices and those for peroxisomal proliferation. In comparison to controls, there was a reported 13% increase in PCO activity in Alderley Park rats exposed to 1,000 mg/kg TCE, a group mean of mitotic figures half that in the TCE treated animals vs. controls, and increase in thymidine incorporation of 40%. Thus, these results are not consistent with TCE induction of peroxisome enzyme activity to be correlated with hepatocellular proliferation by either mitotic index or thymidine incorporation. Thymidine incorporation in liver DNA seen with TCE exposure also did not correlate with mitotic index activity in hepatocytes and suggests that this parameter may be a reflection of polyploidization rather than hepatocyte proliferation. More importantly, these data show that hepatocyte proliferation, indicated by either measure, is confined to a very small population of cells in the liver after 10 days of TCE exposure. Hepatocellular hypertrophy in the centrilobular region appears to be responsible for the liver weight gains seen in both rats and mice rather than increases in cell number. These results at 10 days do not preclude the possibility that a greater level of hepatocyte proliferation did not occur earlier and then had subsided by 10 days, as is characteristic of many mitogens. Thymidine incorporation represents the status of the liver at one time point rather than over a period of whole week, and thus, would not capture the earlier bouts of proliferation. However, there is no evidence of a sustained proliferative response, as measured at the 10-day time period, in hepatocytes in response to TCE indicated from these data.

In regards to weight gain, although the volume of the peroxisomal compartment was reported to be similar at 500 mg/kg TCE in $B6C3F_1$ and Alderley Park mice (4.3%), the liver weight/body weight gain in comparison to control was 20% higher in $B6C3F_1$ mice vs. 43% higher in Alderley Park mice after 10 days of exposure. The liver/body weight ratio was 5.53% in the $B6C3F_1$ mice and 7.31% in the Alderley Park mice at 500 mg/kg TCE for 10 days.

Similarly, although the peroxisomal compartment was similar at 1,000 mg/kg TCE in Osborne-Mendel (2.3%) and Alderley Park rats (2.4%), the liver weight/body weight gain was 26% in Osborne-Mendel rats but 17% in Alderley Park rats at this level of TCE exposure. The liver/body weight ratio was 5.35% in the Osborne-Mendel rats and 5.83% in the Alderley Park mice at 1,000 mg/kg TCE for 10 days. Although there are several limitations regarding the

quantitative interpretation of the data, as discussed above, the data suggest that liver weight and weight gain after TCE treatment was not just a function of peroxisome proliferation. This study does clearly demonstrate TCE-induced changes at the lowest level tested in several parameters without toxicity and without evidence of regenerative hyperplasia or sustained hepatocellular proliferation. In regards to susceptibility to liver cancer induction in more susceptible ($B6C3F_1$) vs. less susceptible (Alderley Park/Swiss) strains of mice (Maltoni et al., 1988), there was a greater baseline level of liver weight/body weight ratio change, a greater baseline level of thymidine incorporation as well as greater responses for those endpoints due to TCE exposure in the "less susceptible" strain. However, both strains showed a hepatocarcinogenic response to TCE induction and the limitations of being able to make quantitative conclusions regarding species and strain susceptibility TCE toxicity from this study have been described in detail above.

E.2.1.9. Dees and Travis (1993)

The focus of this study was to evaluate the nature of DNA synthesis induced by TCE exposure in mice. The mitotic rate of liver cells was extrapolated using tritiated thymidine uptake into DNA of male and female mice treated with HPLC grade (99 + pure) TCE. Male and female hybrid $B6C3F_1$ mice 8 weeks of age (male mice weighed 24–27 g [~12% difference] and females weighing 18–21 g [~4% difference]) were dosed orally by gavage for 10 days with 100, 250, 500, and 1,000 mg/kg body weight TCE in corn oil (n = 4 per treatment group). Sixteen hours after the last daily dose of TCE, mice received tritiated thymidine and were sacrificed 6 hours later. Hepatic DNA was extracted from whole liver and standard histopathology was also performed. Hepatic DNA content and cellular distributions were also determined for thymidine uptake using autoradiography of tissue sections. Tritiated thymidine incorporation into DNA was determined by microscopic observations of autoradiography slides and reported as positive cells per 100 (200× power) fields.

Changes in the treatment groups were reported to:

> include an increase in eosinophilic cytoplasmic staining of hepatocytes located near central veins, accompanied by loss of cytoplasmic vacuolization. Intermediate zones appeared normal and no changes were noted in portal triad areas. Male and female mice given 1,000 mg/kg body weight TCE exhibited apoptosis located near central veins. No evidence of cellular proliferation was seen in the portal areas. No evidence of increased lipofuscin was seen in liver sections from male and female mice treated with TCE. Evaluation of cell death in male and female mice receiving TCE was performed by enumerating apoptosis.

The apoptosis "did not appear to be in proportion to the applied TCE dose given to male or female mice." The mean number of apoptosis per 100 (400×) fields in each group of 4 animals (male mice) was 0, 0, 0, 1, and 8 for control, 100, 250, 500, and 1,000 mg/kg TCE

treated groups, respectively. Variations in number of apoptosis between mice were not given by the authors. Feulgen stain was <1 for all doses except for 9 at 1,000 mg/kg.

Mitotic figure were reported to be:

> frequently seen in liver sections from both male and female mice treated with TCE. Dividing cells were most often found in the intermediate zone and resembled mature hepatocytes. Incorporation of the radiolabel into cells located near the portal triad areas was rare. In general, mitotic figures were very rare, but when found they were usually located in the intermediate zone. Little or no incorporation of label was seen in areas near the bile duct epithelia or in areas close to the portal triad.

No quantitative description of mitotic index was reported by the authors, but this description is consistent with there being replication of mature hepatocytes induced by TCE.

The distribution of tritiated thymidine was given for specific cell types in the livers of five animals per treatment group and radiolabel was reported to be predominantly associated with perisinusoidal cell in control mice. The authors state that the label was more often found in cells resembling mature hepatocytes. The mean number of labeled cells in autoradiographs per 100 (200× power) fields was reported to be ~125 and ~150 labeled perisinusoidal cells in controls male and female mice, respectively. The authors do not give any SDs for the female perisinusoidal data except for the 1,000-mg/kg exposure group. For mature hepatocytes, the mean baseline level of cell labeling for control male and female mice were reported to be ~65 and ~90 labeled cells, respectively. Although the baseline levels of hepatocyte labeling were reported to differ between male and female mice, the mean peak level of labeling was similar at ~250 labeled cells for male and female mice treated with TCE. In male mouse liver, the number of labeled cells increased approximately twofold of control levels after 500 and 1,000 mg/kg TCE and in female mouse liver increased approximately fourfold of control levels after 250, 500, and 1,000 mg/kg TCE over their respective control levels.

Incorporation of tritiated thymidine into DNA extracted from whole liver in male and female mice was reported to be significantly elevated after TCE treatment but, unlike the autoradiographic data, there was no difference between genders and the mean peak level of tritiated thymidine incorporation occurred at 250 mg/kg TCE treatment and remained constant for the 500 and 1,000 mg/kg treated groups. Increased thymidine incorporation into DNA extracted from liver of male and female mice were reported to show a very large SD with TCE treatment (e.g., at 100 mg/kg TCE exposure, male mice had a mean of ~130 dpm tritiated thymidine/μg DNA with the upper bound of the SD to be 225 dpm). The increased thymidine incorporation peaked at a level that was a little <2-fold of control level. Thus, for both male and female mice both autoradiographs and total hepatic DNA were reported to show that male and

female mice had similar peaks of increased thymidine incorporation after TCE exposure that reached a plateau at the 250 mg/kg TCE exposure level and did not increase with increasing exposure concentration. These data also indicate a very small population undergoing mitosis due to TCE exposure after 10 days of exposure. If higher levels of hepatocyte replication had occurred earlier, such levels were not sustained by 10 days of TCE exposure. More importantly, these data suggest that tritiated thymidine levels were targeted to mature hepatocytes and in areas of the liver where greater levels of polyploidization occur. The ages and weights of the mice were described by these authors, unlike Elcombe et al. (1985), and a different strain was used. However, these results are consistent with those of Elcombe in regard to the magnitude of thymidine incorporation induced by TCE treatment and the lack of a dose response once a relative low level of exposure has been exceeded.

The total liver DNA content of male and female mice treated with TCE were also determined with the total micrograms DNA/g liver reported to be ~4 µg/g for female control mice and ~2 µg/g for male control mice. Although not statistically significant, the total DNA concentration dropped from ~4 to ~3 at 100 mg/kg through 1,000 mg/kg exposure to TCE in female mice. For male mice, the total DNA rose slightly in the 250- and 500-mg/kg groups to ~3 µg/g and was similar to control levels at the 100 and 1,000 mg/kg TCE treatment groups. The SD in male mice was very large and the number of animals small making quantitative judgments regarding this parameter difficult. The slight decrease reported for female mice would be consistent with the results of Elcombe et al. (1985) who describe a slight decrease in hepatic DNA in male mice. However, the reported slight increase in hepatic DNA in male mice in this study is not consistent. Given the small number of animals and the large deviations for female and male mice in the TCE treated groups, this study may not have had the sensitivity to detect slight decreases reported by Elcombe et al. (1985).

In regard to clinical evaluation and weight analyses, both male and female mice given TCE were reported "to appear clinically ill. These mice showed reduced activity and failed to groom. Control mice showed no adverse effects. Female mice were markedly more affected by TCE than their male counterparts. Several deaths of female mice occurred during the course of the TCE treatment regimen." The authors do not give cause of deaths but state that two female mice died in the group receiving 250 mg/kg TCE and one in the group receiving 1,000 mg/kg during the gavage regimen of the female mice. This appears to be similar gavage error or "accidental death" reported in NTP studies chronic studies of TCE (see below).

The authors report:

> no significant difference in the absolute body weight of male and female mice were noted in control groups. Body weight gain in female and males mice treated with TCE was not significantly different from that of control mice. Liver weights in male mice given 500 or 1,000 mg/kg and corrected for total body weight were

significantly elevated. The corrected liver weights of female mice increase proportionally with the applied dose of TCE.

For male mice, liver weights were reported to be 1.40 ± 0.16, 1.38 ± 1.23, 1.48 ± 0.09, 1.61 ± 0.07, and 1.63 ± 0.11 g for control, 100, 250, 500, and 1,000 mg/kg TCE in male mice (n = 5), respectively. Body weights were smaller for the 100 mg/kg TCE treatment group although not statistically significant. The liver weights after treatment had a much larger reported SD (1.23 g for 100 mg/kg group vs. <0.16 for all other groups). The percent liver/body weight ratios were reported to be 5.40, 5.41, 5.42, 5.71, and 6.34% for the same groups in male mice. This represents 1.06- and 1.17-fold of control at the 500 and 1,000 mg/kg dose. The authors report a statistically significant increase in percent liver/body weight ratio only for the 500 mg/kg (i.e., 1.06-fold of control) and 1,000 mg/kg (i.e., 1.17-fold of control) TCE exposure groups.

The results for female mice liver weights were reported in Table III of the paper, which was mistakenly labeled as for male mice. The reported values for liver weight were 1.03 ± 0.07, 1.05 ± 0.10, 1.15 ± 0.98, 1.21 ± 0.18, and 1.34 ± 0.08 g for control, 100, 250, 500, and 1,000 mg/kg TCE in female mice (n = 5, except for 250 and 1,000 mg/kg groups), respectively. The percent liver/body weight ratios were 5.26, 5.44, 5.68, 6.24, and 6.57% for the same groups. These values represent 1.03-, 1.08-, 1.19-, and 1.25-fold of controls in percent liver/body weight. The magnitude of increase in TCE-induced percent liver/body weight ratio in female mice is reflective of the magnitude of the difference in dose up to 1,000 mg/kg where it is slightly lower. The female mice were reported to have statistically significant increases in percent liver/body ratios at the lowest dose tested (100 mg/kg TCE) after 10 days of TCE exposure that also increased proportionately with dose. Male mice were not reported to have a significant increase in percent liver/body weight until 500 mg/kg TCE but a statistically significant increase in liver weight at 250 mg/kg TCE. Male mice had a much larger variation in initial body weight than did female mice (range of means of 24.86–27.84 g between groups for males or ~11% difference and range of means of 19.48–20.27 g for females or ~4%), which may contribute to an apparent lack of effect for a parameter that is dependent on body weight. Only five mice were used in each group so the power to detect a change was relatively small.

The results from this experiment are consistent with those of Elcombe et al. (1985) in showing a slight increase in thymidine incorporation (approximately twofold of control) and mitotic figures that are rare after TCE exposure. This study also records a lack of apoptosis with TCE treatment except at the highest exposure level (i.e., 1,000 mg/kg). The increases in liver weight induced by TCE were reported to be dose-related, especially in female mice where baseline body weights were more consistent. However, the incorporation of tritiated thymidine reached a plateau at 250 mg/kg TCE in the DNA of both genders of mice. This study specifically identified where thymidine incorporation and mitotic figures were occurring in

TCE-treated livers and noted that the mature hepatocyte that appeared to be primarily affected, as well as in the portion of the liver where mature hepatocytes with higher ploidy are found. The authors note that the "lack of thymidine incorporation in the periportal area, where the liver stem cells are reside," suggesting that the mature hepatocyte is the target of TCE effects on DNA synthesis. This finding is consistent with a change in ploidy accompanying hepatocellular hypertrophy and not just cell proliferation after 10 days of TCE exposure. Like Elcombe et al. (1985), these data represent "a snapshot in time," which does not show whether increased cell proliferation may have happened at an earlier time point and then subsided by 10 days. However, like Elcombe et al. (1985), it suggests that sustained proliferation is not a feature of TCE exposure and that the level of DNA synthesis (which is very low in quiescent control liver) is increased in a small population of hepatocytes due to TCE exposure that is not dose-dependent (only twofold increase over control in animals exposed from 250 to 1,000 mg/kg TCE). In regards to toxicity, no evidence of increased lipid peroxidation in TCE-treated animals was reported using histopathologic sections stained to enhance observation of lipofuscin. No necrosis is noted by these authors and the deaths in female mice are likely due to gavage error.

E.2.1.10. Nakajima et al. (2000)

This study focused on the effect of TCE treatment on PPARα-null mice in terms of peroxisome proliferation but also included information on differences in liver weight between null and wild-type mice, as well as gender-related effects. SV129 wild-type and PPARα-null mice (10 weeks of age) were treated with corn oil or 750 mg/kg TCE in corn oil daily for 2 weeks via gavage (n = 6 per group). A small portion of the liver was removed for histopathological examination but the lobe used was not specified by the authors. Liver peroxisome proliferation was reported to be evaluated morphologically using 3,3'-diamino-benzidine (DAB) staining of sections and electron photomicroscopy to detect the volume density of peroxisomes (percent of cytoplasm) in 15 micrographs of the pericentral area per liver. A number of β-oxidation enzymes and P450s were analyzed by immunoblot of liver homogenates.

The final body weights, liver weights, and percent liver/body weight ratios were reported for all treatment groups. For male mice, vehicle treated PPARα-null mice had slightly lower mean body weights (24.5 ± 1.8 vs. 25.4 ± 1.9 g [SD]), slightly larger liver weights (1.14 ± 0.13 vs. 1.05 ± 0.15 g or ~9%), and slightly higher percent liver/body weight ratios (4.12 ± 0.32 vs. 4.10 ± 0.37%) than wild-type mice. The mean values for final body weights of the groups of mice in this study were reported and were similar which, as demonstrated by the inhalation studies by Kjellstrand et al. (1983b) (see Section E.2.2.5), is particularly important for determining the effects of TCE treatment on percent liver/body weight ratios. For both groups of male mice, 2 weeks of TCE treatment significantly increased both liver weight and percent liver/body weight ratios. For male wild-type mice, the increase in percent liver/body weight was

1.50-fold of vehicle control and for male PPARα-null mice the increase was 1.26-fold of control after 2 weeks of TCE treatment.

For female mice, vehicle-treated PPARα-null mice had slightly higher mean body weights (22.7 ± 2.1 vs. 22.4 ± 2.0 g), slightly larger liver weights (0.98 ± 0.15 vs. 0.95 ± 0.14 g or ~3%), and slightly higher percent liver/body weight ratios (4.32 ± 0.35 vs. 4.24 ± 0.41%) than wild-type mice. For both groups of female mice, 2 weeks of TCE treatment significantly increased percent liver/body weight ratios. For liver weights, there was a reporting error for PPARα-null female treated with TCE so that liver weight changes due to TCE treatment cannot be determined for this group. For female wild-type mice, the increase in percent liver/body weight was 1.24-fold of vehicle control and for female PPARα-null mice, the increase was 1.26-fold of control after 2 weeks of TCE treatment.

Thus, for both wild-type and PPARα-null mice, TCE exposure resulted in increased percent liver/body weight over controls that was statistically significant after 2 weeks of gavage exposure using corn oil as the vehicle. For male mice, there was a greater TCE-induced increase in percent liver/body weight in wild-type than PPARα-null mice (1.50- vs. 1.26-fold of control) that was statistically significant, but for female mice, the induction of increased liver weight was statistically increased but the same in wild-type and PPARα-null mice (i.e., both were ~1.25-fold of control). These date indicate that TCE-induced increases in mouse liver weight were not dependent on a functional PPARα receptor in female mice and suggest that some portion may be in male mice.

In regard to light and electron microscopic results, the numbers of peroxisomes in hepatocytes of wild-type mice were reported to be increased, especially in the pericentral area of the hepatic lobule, to a similar extent in both males and females (15 micrographs, n = 4 mice). TCE exposure was reported to increase the volume density of peroxisomes twofold of control in the pericentral area with no evident change in peroxisomes in the periportal areas, but data were not shown for that area of the liver lobule. In contrast, no increase in peroxisomes was reported to be observed in PPARα-null mice. Therefore, increases in liver weight observed in PPARα-null mice after TCE treatment did not result from peroxisome proliferation. Similarly, the small twofold increase in peroxisome volume from 2 to 4% of cytoplasmic volume in the pericentral area of the liver lobule in wild-type mice could not have been responsible for the 50% increase liver weight observed in male wild-type mice.

Although no difference was reported between male and female wild-type mice in regard to TCE-induced peroxisome proliferation in wild-type mice, the levels of hepatic enzymes associated with peroxisomes (acyl-CoA [AOX], peroxisomal bifunctional protein [PH], peroxisomal thiolase [PT], very long chain acyl-CoA synthetase, and D-type peroxisomal bifunctional protein [DBF], cytosolic enzyme [cytosolic thioesterase II (CTEII)], mitochondrial enzymes [mitochondrial trifunctional protein α subunits α and β(TPα and TPβ)], and microsomal enzymes [CYP 4A1 (CYP4A1)]) as measured by immunoblot analysis were significantly

elevated in male wild-type mice (n = 4) by a factor of ~2–3, but except for a slight elevation in PH and PT, were reported to not be elevated in female wild-type mice (n = 4). The magnitude of increase in peroxisomal enzymes was similar to that of peroxisomal volume in male mice. No TCE-induced increases in any of these enzymes were reported in male or female PPARα-null mice by the authors. For CYP4A1, an enzyme reported to be induced by peroxisomal proliferators, TCE exposure resulted in a much lower amount in female than male wild-type mice (i.e., 2% of the level induced by TCE in males). However, the expression of catalase was reported to be "nearly constant in all samples" (at most ~30% change), which the authors suggested resulted from induction by TCE that was independent of PPARα. The basis for selection of four mice for this comparison out of the six studied per group was not given by the authors. A comparison of control wild-type and PPARα-null mice showed that in males background levels of the enzymes examined were generally similar except for DBF in which the null mice had values ~50% of the wild-type controls. A similar decrease was reported for female PPARα-null mice. With regard to gender differences in wild-type mice, females had similar values as males with the exceptions of TPα, TPβ, and CYP2E1, which were in untreated female wild-type mice at a 3.06-, 2.38-, and 1.63-fold for l TPα, TPβ, and CYP2E1 levels over males, respectively. Female PPARα-null mice had increases of 2.50-, 1.54-, and 2.07-fold over male wild-type mice.

With regard to the induction of TCE metabolizing enzymes (CYP1A2, CYP2E1, and ALDH), CYP1A2 was reported to be decreased by TCE treatment of both male and female wild-type mice but liver CYP2E1 reported to be increased in male mice and constant in female mice which resulted in similar expression level in both genders after TCE treatment. There was no gender difference in ALDH activity reported after TCE exposure and activity was reported to be independent of PPARα. The authors concluded that TCE metabolizing abilities of the liver of male and female mice were similar, and therefore, poor induction of peroxisomal related enzymes was not due to gender-related differences in TCE metabolism.

To investigate whether the a gender-related difference peroxisomal enzymes after TCE exposure was due to a lower levels of PPARα and RXRα receptors, western blotting was employed (n = 3). The level of PPARα protein was reported to be increased in both male wild-type mice with less induction in females (control vs. TCE, 1.00 ± 0.20 vs. 2.17 ± 0.24 in males and 0.95 ± 0.25 vs. 1.44 ± 0.09 in females) after TCE treatment. The hepatic level of RXRα was also reported to be increased in the same manner as PPARα (control vs. TCE, 1.00 ± 0.33 vs. 1.92 ± 0.04 in males 0.81 ± 0.16 vs. 1.14 ± 0.10 in females). Northern blot analysis of hepatic PPARα mRNA was reported to show greater TCE induction in male (2.6-fold of control) than in female (1.5-fold of control) wild-type mice. Thus, males appeared to have higher induction of the two receptor proteins as well as a greater response in peroxisomal enzymes and CYP4A1, even though TCE-induced increases in peroxisomal volume was similar between male and female mice. The increased response in males for induction of the two receptor proteins is

consistent with liver weight data that shows some portion of the induction of increased liver weight response in male mice using this paradigm may be due to gender-specific differences in PPARα response. However, as noted below (see Section E.2.2), corn oil vehicle has liver effects alone, especially in the male liver, that have also been associated with PPARα responses.

E.2.1.11. Berman et al. (1995)

This study included TCE in a suite of compounds used to compare endpoints for toxicological screening methods. Female F344 rats of 77 days of age (n = 8 per group) were administered TCE in corn oil for 1 day (0, 150, 500, 1,500, or 5,000 mg/kg-day) or for 14 days (0, 50, 150, 500, or 1,500 mg/kg-day). Blood samples were taken 24 hours after the last dose and livers were weighed and H&E sections were examined for evidence of parenchymal cell degeneration, necrosis, or hypertrophy. No details were provided by the authors for the extent or severity of the liver affects by histopathological examination. The serum chemistry analysis included LDH, ALP, ALT, AST, total bilirubin, creatine, and BUN. The starting and ending body weights of the animals or the absolute liver weights were not reported by the authors.

The results of a multivariate analysis were reported to show a lowest effective dose of 1,500 mg/kg after 1 day of TCE exposure and 150 mg/kg after 14 days of TCE exposure that was statistically significant. Liver weight and liver weight changes were not reported by the authors but the percent liver to body weight ratios were. For the two control groups, there was a difference in percent liver/body weight of ~8% (3.43 ± 0.74% for the 1-day control group and 3.16 ± 0.41% for the 14-day control group, mean ± SEM). For the 1-day groups, only the 5,000 mg/kg group was reported to show a statistically significant difference in percent liver/body weight between control and TCE treatment (i.e., ~1.08-fold increase). Hepatocellular necrosis was noted to occur in the 1,500 and 5,000 mg/kg groups in 6/7 and 6/8 female rats, respectively, but not to occur in lower doses. The extent of necrosis was not noted by the authors for the two groups exhibiting a response after 1 day of exposure. Serum enzymes indicative of liver necrosis were not presented and because only positive results were presented in the paper, were presumed to be negative. Therefore, the extent of necrosis was not of a magnitude to affect serum enzyme markers of cellular leakage.

After 14 days of TCE exposure, there was a dose-related increase reported for percent liver/body weight ratios that was statistically significant at all TCE dose levels although the multivariate analysis indicated the lowest effective dose to be 150 mg/kg. The percent liver/body weight ratio was 3.16 ± 0.41, 3.38 ± 0.56, 3.49 ± 0.69, 3.82 ± 0.76, and 4.47 ± 0.66% for control, 50, 150, 500, and 1,500 mg/kg TCE exposure levels, respectively, after 14 days of exposure. No hepatocellular necrosis was reported at any dose and hepatocellular hypertrophy was reported only at the 1,500 mg/kg dose and in all rats. These rat liver weights were 1.07-, 1.10-, 1.21-, and 1.41-fold of controls for the 50, 150, 500, and 1,500 mg/kg TCE dose groups, respectively. The 7% increase in liver weight at the 50 mg/kg dose was approximately the same

difference between the two control groups for days 1 and 14 treatments. Without the data for starting and final body weights and an examination of whether the control animals had similar body weight, it is impossible to discern whether the reported effects at the low dose of TCE were also reflected differences between the control groups. No serum enzyme levels changes were reported after 14 days of exposure to TCE for any group.

The authors note that their study provided evidence of liver effects at lower levels than other studies citing Elcombe et al. (1985) and Goldsworthy and Popp (1987). They suggest that the differences in sensitivity to TCE between their results and those of these two studies may reflect differences in strain or gender of the rats examined. However, they did not study male rats of this strain concurrently so that differences in gender may have reflected differences between experiments. The increase in liver weight without reporting increases in hepatocellular hypertrophy as well as the lack of necrosis as low doses is consistent with the results of Melnick et al. (1987) in male Fischer rats given TCE orally (see Section E.2.1.12).

E.2.1.12. Melnick et al. (1987)

The focus of this study was to assess microencapsulation as a way to expose rodents to substances such as TCE that have issues related to volatilization in drinking water or apparent gavage-related deaths. In this study, liver weight changes, extent of focalized necrosis, and indicators of peroxisome proliferation were reported as metrics of TCE toxicity. TCE (99+ %) was encapsulated in gelatin-sorbitol microcapsules and was 44.1% TCE w/w. The TCE microcapsules were administered to male F344 rats (6-week-old and weighing between 89 and 92 g or ~3% difference) in the diet (0, 0.55, 1.10, 2.21, and 4.42% TCE in the diet) for 14 days. The number of animals in each group was 10. A parallel group of animals was administered TCE in corn oil gavage for 14 consecutive days (corn oil control, 0.6, 1.2, and 2.8 g/kg-day TCE). The dosage levels of TCE in the gavage study were reported to be "adjusted 5 times during the 14-day" treatment period to be similar to the dosage levels of TCE in the feed study. The TWA dosage levels of TCE in the feed study were reported to be 0.6, 1.3, 2.2, and 4.8 g/kg-day.

There was less food consumption reported in the 2.2 and 4.8 g/kg-day dose feed groups, which the authors attribute to either palatability or toxicity. There were no deaths in any of the groups treated with microencapsulated TCE while, similar to many other gavage studies of TCE reported in the literature, there were four deaths in the high-dose gavage group. Mean body weight gains of the two highest dose groups of the feed study and of the highest dose group of the gavage study were reported to be significantly lower than the mean body weight gains of the respective control groups (i.e., ~22 and ~35% reduction at 2.2 and 4.8 g/kg-day in the feed study, respectively, and ~33% reduction at 2.8 g/kg-day TCE in the gavage study).

After 14 days of treatment, liver weights were reported to be 8.1 ± 0.8, 8.4 ± 0.8, 9.5 ± 0.5, 10.1 ± 1.2, 8.9 ± 1.3, and 7.4 ± 0.5 g for untreated control, placebo control, 0.6, 1.3, 2.2, and

4.8 g/kg TCE exposed feed groups, respectively. The corresponding percent liver/body weight ratios were reported to be 5.2 ± 0.3, 5.3 ± 0.2, 6.0 ± 0.3, 6.5 ± 0.5, 7.0 ± 0.9, and 7.1 ± 0.5% for untreated control, placebo control, 0.6, 1.3, 2.2, and 4.8 g/kg TCE exposed groups, respectively. The increased percent liver/body weight ratio represents 1.13-, 1.23-, 1.32-, and 1.34-fold of placebo controls, respectively.

For the gavage experiment, after 14 days of treatment, liver weights were reported to be 7.1 ± 1.3, 9.3 ± 1.2, 9.1 ± 0.9, and 7.7 ± 0.4 g for corn oil control, 0.6, 1.2, and 2.8 g/kg TCE exposed groups, respectively. The corresponding percent liver/body weight ratios were reported to be 5.0 ± 0.4, 6.0 ± 0.4, 6.1 ± 0.3, and 7.3 ± 0.5% for corn oil control, 0.6, 1.2, and 2.8 g/kg TCE exposed groups, respectively. The percent liver/body weight ratios represent 1.20-, 1.22-, and 1.46-fold of corn oil controls, respectively. The 2.8 g/kg TCE gavage results are reflective of the 6 surviving animals in the group rather than 10 animals in the rest of the groups. There was no explanation given by the authors for the lower liver weights in the control gavage group than the placebo control in the feed group (i.e., 20% difference), although the initial and final body weights appeared to be similar. The decreased body weights in the feed and gavage study are reflective if TCE systemic toxicity and appeared to affect the TCE-induced liver weight increases in those groups.

The authors reported that the only treatment-related lesion observed microscopically in rats from either dosed-feed or gavage groups was individual cell necrosis of the liver with the frequency and severity of this lesion similar at each dosage levels of TCE administered microencapsulated in the feed or in corn oil. Using a scale of minimal = 1–3 necrotic hepatocytes/10 microscopic 200× fields, mild = 4–7 necrotic hepatocytes/10 microscopic 200× fields, and moderate = 8–12 necrotic hepatocytes/10 microscopic 200× fields, the frequency of lesion was 0–1/10 for controls, 2/10 for 0.6 and 1.3 g/kg, and 9/10 for 2.2 and 4.8 g/kg feed groups. The mean severity was reported to be 0.0–0.1 for controls, 0.3–0.4 for 0.6 and 1.3 g/kg, and 2.0–2.5 for 2.2 and 4.8 g/kg feed groups. For the corn oil gavage study, the corn oil control and 0.6 g/kg groups were reported to have a frequency of 0 lesions/10 animals; the 1.2 g/kg group had a frequency of 1/10 animals, while the 2.8 g/kg group had a frequency of 5/6 animals. The mean severity score was reported to be 0 for the control and 0.6 g/kg groups, 0.1 for the 1.2 g/kg groups, and 1.8 for the remaining six animals in the 2.8 g/kg group. The individual cell necrosis was reported to be randomly distributed throughout the liver lobule with the change to not be accompanied by an inflammatory response. The authors also report that there was no histologic evidence of cellular hypertrophy or edema in hepatic parenchymal cells. Thus, although there appeared to be TCE-treatment-related increases in focal necrosis after 14 days of exposure, the extent was, even at the highest doses, mild and involved few hepatocytes.

Microsomal NADPH cytochrome c-reductase was reported to be elevated in the 2.2 and 4.8 g/kg feed groups and in the 1.2 and 2.8 g/kg gavage groups. CYP levels were reported to be elevated only in the two highest dose groups of the feed study. The authors reported a dose-

related increase in peroxisome PCO and catalase activities in liver homogenates from rats treated with TCE microcapsules or by gavage, and that treatment with corn oil alone, but not placebo capsules, caused a slight increase in PCO activity.

After 14 days of treatment, PCO activities were reported to be 270 ± 12, 242 ± 17, 298 ± 64, 424 ± 55, 651 ± 148, and 999 ± 266 nmol hydrogen peroxide (H_2O_2) produced/minute/g liver for untreated control, placebo control, 0.6, 1.3, 2.2, and 4.8 g/kg TCE exposed feed groups, respectively. This represents 1.23-, 1.75-, 2.69-, and 4.13-fold of placebo controls, respectively. After 14 days of treatment, catalase activities were reported to be 8.49 ± 0.81, 7.98 ± 1.62, 8.49 ± 1.92, 8.59 ± 1.31, 13.03 ± 2.01, and 15.76 ± 1.11 nmol H_2O_2 produced/minute/g liver for untreated control, placebo control, 0.6, 1.3, 2.2, and 4.8 g/kg TCE exposed groups, respectively. This represents 1.06-, 1.07-, 1.63-, and 1.97-fold of placebo controls, respectively. Thus, although reported to be dose related, only the two highest exposure levels of TCE increased catalase activity and to a smaller extent than PCO activity in microencapsulated TCE fed rats.

For the gavage experiment, after 14 days of treatment, PCO activities were reported to be 318 ± 27, 369 ± 26, 413 ± 40, and 1,002 ± 271 nmol H_2O_2 produced/minute/g liver for corn oil control, 0.6, 1.2, and 2.8 g/kg TCE exposed groups, respectively. This represents 1.16-, 1.29-, and 3.15-fold of corn oil controls. After 14 days of treatment, catalase activities were reported to be 8.59 ± 0.91, 10.10 ± 1.82, 12.83 ± 3.43, and 13.54 ± 2.32 nmol H_2O_2 produced/minute/g liver for corn oil control, 0.6, 1.2, and 2.8 g/kg TCE exposed groups, respectively. This represents 1.18-, 1.49-, and 1.58-fold of corn oil controls. As stated by the authors, the corn oil vehicle appeared to elevate catalase activities and PCO activities.

In regard to dose-response, liver and body weight were affected by decreased body weight gain in the higher dosed animals in this experiment (i.e., 2.2 g/kg-day TCE exposure and above) and by gavage related deaths in the highest-dosed group. The lower liver weight in the gavage control group also may have affected the determination of the magnitude of TCE-related liver weight gain at that dose. At the two doses, below which body weight gain was affected, there appeared to be an approximately 20% increase in percent liver/body weight ratio in the gavage study and a 13 and 23% weight increase in the feed study.

The extent of PCO activity appeared to increase more steeply with dose in the feed study than did liver weight gain (i.e., a 1.23-fold of liver/body weight ratio at 1.3 g/kg-day corresponded with a 1.75-fold PCO activity over control). At the two highest doses in the feed study, the increase in PCO activity was 2.69- and 4.13-fold of control, but the increase in liver weight was not more than 34%. For the gavage study, there was also a steeper increase in PCO activity than liver weight gain. For catalase activity, the increase was slightly less than that of liver/body weight ratio percent for the two doses that did not decrease body weight gain in the feed study. In the gavage study, they were about the same. In regard to what the cause of liver weight gain was, the authors report that there was no histologic evidence of cellular hypertrophy or edema in hepatic parenchymal cells and do not describe indicators of hepatocellular

proliferation or increased polyploidy. Accordingly, the cause of liver weight gain after TCE exposure in this paradigm is not readily apparent.

E.2.1.13. Laughter et al. (2004)

Although the focus of the study was an exploration of potential modes of action for TCE effects through macroarray transcript profiling (see Section E.3.1.2 for discussions of limitations of this approach and especially the need for phenotypic anchoring, Section E.3.4.1.3 for use of PPARα knockout mice, and Section E.3.4.2.2 for discussion of genetic profiling data for TCE), information was reported regarding changes in the liver weight of PPARα-null mouse and their background strains. SV129 wild-type and PPARα-null male mice (9 ± 1.5 weeks of age) were treated with three daily doses of TCE in 0.1% methyl cellulose for either 3 days or 3 weeks (n = 4–5/group). Thus, this paradigm does not use corn oil, which has been noted to affect toxicity (see Section E.2.2 below), but is not comparable to other paradigms that administer the total dose in one daily gavage administration rather than to give the same cumulative dose but in three daily doses of lower concentration. The initial or final body weights of the mice were not reported. Thus, the effects of systemic toxicity from TCE exposure on body weight and the influence of differences in initial body weight on percent liver/body weight determinations cannot be made.

For the 3-day study, mice were administered 1,500 mg/kg TCE or vehicle control. For the 3-week study, mice were administered 0, 10, 50, 125, 500, 1,000, or 1,500 mg/kg TCE 5 days/week except for 4 days/week on the last week of the experiment. In a separate study, mice were given TCA or DCA at 0.25, 0.5, 1, or 2 g/L (pH ~7) in the drinking water for 7 days. For each animal, a block of the left, anterior right, and median liver lobes was reported to be fixed in formalin with five sections stained for H&E and examined by light microscopy. The remaining liver samples were combined and used as homogenates for transcript arrays. In the 3-week study, bromodeoxyuridine (BrdU) was administered via miniosmotic pump on day 1 of week 3 and sections of the liver assessed for BrdU incorporation in at least 1,000 cells per animal in 10–15 fields.

Although initial body weights, final body weights, and the liver weights were not reported, the percent liver/body ratios were. In the 3-day study, control wild-type and PPARα-null mice were reported to have similar percent liver/body weight ratios of ~4.5%. These animals were ~10 weeks of age upon sacrifice. However, at the end of the 3-week experiment, the percent liver/body weight ratios were increased in the PPARα-null male mice and were 5.1%. There was also a slight difference in the percent liver/body weight ratios in the 1-week study (4.3 ± 0.4 vs. 4.6 ± 0.2% for wild-type and PPARα-null mice, respectively). These results are consistent with an increasing baseline of hepatic steatosis with age in the PPARα-null mice and increase in liver weight.

In the 3-day study, the mean report for the percent liver/body ratio was 1.4-fold of the wild type animals tested with TCE in comparison to the control level. In the PPARα-null mice, there was a 1.07-fold of control level reported by the authors to not be statistically significant. However, given the low number of animals tested (the authors give only that four to five animals were tested per group without identification as to which groups had four animals and which had five), the ability of this study to discern a statistically significant difference is limited.

In the 3-week study, wild-type mice exposed to various concentrations of TCE had percent liver/body weights that were within ~2% of control values except for the 1,000 mg/kg and 1,500 mg/kg groups that were ~1.18- and 1.30-fold of control levels, respectively. For the PPARα-null mice exposed to TCE for 3 weeks, the variability in percent liver/body weight was greater than that of the wild-type mice in most of the groups. The baseline level percent liver/body weight was 1.16-fold in the PPARα-null mice in comparison to wild-type mice. At the 1,500 mg/kg TCE exposure level, percent liver/body weights were not recorded because of the death of the null mice at this level. The authors reported that at the 1,500 mg/kg level, all PPARα-null mice were moribund and had to be removed from the study. However, at the 1,000 mg/kg TCE exposure level, there was a 1.10-fold of control percent liver/body weight value that was reported to not be statistically significant. As noted above, the power of the study was limited due to low numbers of animals and increased variability in the null mice groups. The percent liver/body weight reported in this study was actually greater in the null mice than the wild-type male mice at the 1,000 mg/kg TCE exposure level (5.6 ± 0.4 vs. 5.2 ± 0.5%, for null and wild-type mice, respectively).

Thus, at 1 and 3 weeks, TCE appeared to induce increases in liver weight in PPARα-null mice, although not reaching statistical significance in this study, with concurrent background of increased liver weight reported in the knockout mice. At 1,000 mg/kg TCE exposure for 3 weeks, percent liver/body weight was reported to be 1.18-fold in wild-type and 1.10-fold in null mice of control values. As discussed above, Nakajima et al. (2000) reported statistically significant increased liver weight in both wild-type and PPARα-null mice after 2 weeks of exposure with less TCE-induced liver weight increases in the knockout mice (see Section E.2.1.10). They also used more mice, carefully matched to weights of their mice, and used a single dose of TCE each day with corn oil gavage.

The authors noted that inspection of the livers and kidneys of the moribund null mice, who were removed from the 3-week study, “did not reveal any overt signs of toxicity in this dose group that would lead to morbidity” but did not show the data and did not indicate when the animals were affected and removed. For the wild-type mice exposed to the same concentration (1,500 mg/kg) but whose survival was not affected by TCE exposure, the authors reported that these mice exhibited mild granuloma formation with calcification or mild hepatocyte degeneration, but gave no other details or quantitative information as to the extent of the lesions or what parts of the liver lobule were affected. The authors noted that “wild-type mice

administered 1000 and 1500 mg/kg exhibited centrilobular hypertrophy" and that "the mice in the other groups did not exhibit any gross pathological changes after TCE exposure." Thus, the hepatocellular hypertrophy reported in this study for TCE appeared to be correlated with increases in percent liver/body weight in wild-type mice. In regard to the PPARα-null mice, the authors stated that "differences in the liver to body weights in the control PPARα-null mice [between Study 1 and 2 the 3-day and 3-week studies] were noted and may be due to differences in the degree of steatosis that commonly occurs in this strain." Further mention of the background pathology due to knockout of the PPARα was not discussed. The increased percent liver/body weight reported between control and 1,000 mg/kg TCE exposed mice (5.1 vs. 5.6%) was not accompanied by any discussion of pathological changes that could have accounted for the change.

Direct comparisons of the effects of TCE, DCA, and TCA cannot be made from this study as they were not studied for similar durations of exposure. However, while TCE induced increased in percent liver/body weight ratios after 3 days and 3 weeks of exposure in wild-type mice at the highest dose levels, for TCA exposure, percent liver/body weight after 1 week exposure in drinking water was slightly elevated at all dose levels with no dose-response (~10% increase), and for DCA exposure in drinking water, a similar elevation in percent liver/body weight was also reported for the 0.25, 0.5, and 1.0 g/L dose levels (~11%) and that was increased at the 2.0 g/L level by ~25% reaching statistical significance. The authors interpret these data to show no TCA-related changes in wild-type mice but the limited power of the study makes quantitative conclusions difficult.

For PPARα-null mice, there was a slight decrease in percent liver/body weight between control and TCA treated mice at the doses tested (~2%). For DCA-treated mice, all treatment levels of DCA were reported to induce a higher percent liver/body weight ratio of at least ~5% with a 13% increase at the 2.0 g/L level. Again, the limited power of the study and the lack of data for TCE at similar durations of exposure as those studied for TCA and DCA makes quantitative conclusions difficult and comparisons between the chemicals difficult. However, the pattern of increased percent liver/body weight appears to be more similar between TCE and DCA than TCA in both wild-type and PPARα-null mice.

In terms of histological description of effects, the authors note that "livers from the 2 g/L DCA-treated wild-type and PPARα-null mice had hepatocyte cytoplasmic rarefication probably due to an increase in glycogen accumulation." However, no special procedures of staining were performed to validate the assumption in this experiment. No other pathological descriptions of the DCA treatment groups were provided. In regard to TCA, the authors noted that "the livers from wild-type but not PPARα-null mice exposed to 2.0g/L TCA exhibited centrilobular hepatocyte hypertrophy." No quantitative estimate of this effect was given and although the extent of increase of percent liver/body weight was similar for all dose levels of TCA, there is no indication from the study that lower concentrations of TCA also increased hepatocellular

hypertrophy or why there was no concurrent increase in liver weight at the highest dose of TCA in which hepatocellular hypertrophy was reported. Thus, reports of hepatocellular hypertrophy for DCA and TCA in the 1-week study were not correlated with changes in percent liver/body weight.

For control animals, BrdU incorporation in the last week of the 3-week study was reported to be at a higher baseline level in PPARα-null mice than wild-type mice (~2.5-fold). For wild-type mice the authors reported a statistically significant increase at 500 and 1,000 mg/kg TCE at levels of ~1 and ~4.5% hepatocytes incorporating the label after 5 days of BrdU incorporation. Whether this measure of DNA synthesis is representative of cellular proliferation or of polyploidization was not examined by the authors. Even at 1,000 mg/kg TCE, the percent of cells that had incorporated BrdU was <5% of hepatocytes in wild-type mice. The magnitude percent liver/body weight ratio change at this exposure level was fourfold greater than that of hepatocytes undergoing DNA synthesis (16% increase in percent liver/body weight ratio vs. 4% increase in DNA synthesis). The ~1% of hepatocytes undergoing DNA synthesis at the 500 mg/kg TCE level, reported to be statistically significant by the authors, was not correlated with a concurrent increase in percent liver/body weight ratio. Thus, TCE-induced changes in liver weight were not correlated with increases in DNA synthesis in wild-type mice after 3 weeks of TCE exposure.

For PPARα-null mice, there was an approximately threefold of control value for the percent of hepatocytes undergoing DNA synthesis at the 1,000 mg/kg TCE exposure level. The higher baseline level in the null mouse, large variability in response at this exposure level, and low power of this experimental design limited the ability to detect statistical significance of this effect, although the level was greater than that reported for the 500 mg/kg TCE exposure in wild-type mice that was statistically significant. Thus, TCE appeared to induce an increase in DNA synthesis in PPARα-null mice, albeit at a lower level than wild-type mice. However, the ~2% increase in percent of hepatocytes undergoing DNA synthesis during the 3rd week of a 3-week exposure to 1,000 mg/kg TCE in PPARα-null mice was insufficient to account for the ~10% observed increase in liver weight. For wild-type and PPARα-null mice, the magnitude of TCE-induced increases in liver weight were four- to fivefold higher than that of increases in DNA-synthesis under this paradigm and in both types of mice, a relatively small portion of hepatocytes were undergoing DNA synthesis during the last week of a 3-week exposure duration. Whether the increases in liver weight could have resulted from an early burst of DNA synthesis as well as whether the DNA synthesis results reported here represents either proliferation or polyploidization, cannot be determined from this experiment. Because of the differences in exposure protocol (i.e., use of three daily doses in methylcellulose rather than one dose in corn oil), the time course of the transient increase in DNA synthesis reported cannot be assumed to be the same for this experiment and others.

Not only were PPARα-null mice different than wild-type mice in terms of background levels of liver weights, and hepatic steatosis, but this study also reported that background levels of PCO activity to be highly variable and, in some instances, different between wild-type and null mice. There was reported to be approximately sixfold PCO activity in PPARα-null control mice in comparison to wild-type control mice in the 1-week DCA/TCA experiment (~0.15 vs. 0.85 units of activity/g protein). However, in the same figure, a second set of data are reported for control mice for comparison to WY-14,643 treatment in which PCO activity was slightly decreased in PPARα-null control mice vs. wild-type controls (~0.40 vs. 0.65 units of activity/g protein). In the experimental design description of the paper, WY-14,643 treatment and a separate control were not described as part of the 1-week DCA/TCA experiment. For the only experiment in which PCO activity was compared between wild-type and PPARα-null mice exposed to TCE (i.e., 3-day exposure study), there was a reported increased over the control value of ~2.5-fold that was reported to be statistically significant at 1,500 mg/kg TCE (1.5 vs. 0.60 units of activity/g protein). For control mice in the 3-day TCE experiment, there was an increase in this activity in PPARα-null mice in comparison to wild-type mice (~0.60 vs. 0.35 units of activity/g protein). While not statistically significant, there appeared to be a slight increase in PCO activity after 1,500 mg/kg TCE exposure for 3 days in PPARα-null mice of ~30%. However, as noted above, the background levels of this enzyme activity varied widely between the experiments with not only values for control animals varying as much as sixfold (i.e., for PPARα-null mice), but also for WY-14,643 administration. There was a 6.6-fold difference in PCO results for WY-14,643 in PPARα-null mice at the same concentration of WY-14,643 in the 3-day and 1-week experiment, and a 1.44-fold difference in results in wild-type mice in these two data sets.

E.2.1.14. Ramdhan et al. (2008)

Ramdhan et al. (2008) examined the role of CYP2E1 in TCE-induced hepatotoxicity, using CYP2E1 +/+ (wild-type) and CYP2E1 -/- (null) Sv/129 male mice (6/group) that were exposed for 7 days to 0, 1,000, or 2,000 ppm TCE by inhalation for 8 hours/day. The exposure concentrations are noted by the authors to be much higher than occupational exposures and to have increased liver toxicity after 8 hours of exposure as measured by plasma AST levels. To put this exposure concentration into perspective, the Kjellstrand et al. (1983a; 1983b) inhalation studies for 30 days showed that these levels were well above the 150-ppm exposure levels in male mice that induced systemic toxicity. Nunes also reported hepatic necrosis up to 4% in rats at 2,000 ppm for just 8 hours not 7 days. AST and ALT were measured at sacrifice. Histological changes were scored using a qualitative scale of 0 = no necrosis, 1 = minimal as defined as only occasional necrotic cells in any lobule, 2 = mild as defined as less than one-third of the lobule structure affected, 3 = moderate as defined as between one-third and two-thirds of the lobule structure affected, and 4 = severe defined as greater than two-thirds of the lobule structure

affected. Real-time polymerase chain reaction (PCR) was reported for mRNA encoding a number of receptors and proteins. Total RNA and Western Blot analysis was obtained from whole-liver homogenates. The changes in mRNA expression were reported as means for six mice per group after normalization to a level of β-actin mRNA expression and were shown relative to the control level in the CYP2E1 wild-type mice.

The deletion of the CYP2E1 gene in the null mouse had profound effects on liver weight. The body were was significantly increased in control CYP2E1 -/- mice in comparison to wild-type controls (24.48 ± 1.44 g for null mice vs. 23.66 ± 2.44 g, m ± SD). This represents a 3.5% increase over wild-type mice. However, the liver weight was reported in the CYP2E1 -/- mice to be 1.32-fold of that of CYP2E1 +/+ mice (1.45 ± 0.10 g vs. 1.10 ± 0.14 g). The percent liver/body weight ratio was 5.47 vs. 4.63% or 1.18-fold of wild-type control for the null mice.

The authors report that 1,000 and 2,000 ppm TCE treatment did induce a statistically significant change body weight for null or wild-type mice. However, there was an increase in body weight in the wild-type mice (i.e., 23.66 ± 2.44, 24.52 ± 1.17, and 24.99 ± 1.78 for control, 1,000, and 2,000 ppm groups, respectively) and an increase in the variability in response in the null mice (i.e., 24.48 ± 1.44, 24.55 ± 2.26, and 24.99 ± 4.05, for control, 1,000, and 2,000 ppm exposure groups, respectively). The percent liver/body weight was 5.47 ± 0.23, 5.51 ± 0.27, and 5.58 ± 0.70% for control, 1,000, and 2,000 ppm the CYP2E1 -/- mice, respectively. The percent liver/body weight was 4.63 ± 0.13, 6.62 ± 0.40, and 7.24 ± 0.84% for control, 1,000, and 2,000 ppm wild-type mice, respectively. Therefore, while there appeared to be little difference in the TCE and control exposures for percent liver/body weights in the CYP2E1 -/- mice (2%), there was a 1.56-fold of control level after 2,000 ppm in the wild-type mice after 7 days of inhalation exposure.

The authors reported that "in general, the urinary TCE level in CYP2E1 -/- mice was less than half that in CYP2E1 +/+ mice: urinary TCA levels in the former were about one-fourth those in the latter." Of note is the large variability in urinary TCE detected in the 2,000-ppm TCE exposed wild-type mice, especially after day 4, and that, in general, the amount of TCE in the urine appeared to be greatest after the 1st day of exposure and steadily declined between 1 and 7 days (i.e., ~45% decline at 2,000 ppm and a ~70% decline at 1,000 ppm) in the wild-type mice. The amount of TCE in the urine was proportional to the difference in dose at days 1 and 5 (i.e., a twofold difference in dose resulted in a twofold difference in TCE detected in the urine). As the detection of TCE in the urine declined with time, the amount of TCA was reported to steadily increase between days 1 and 7 (e.g., from ~3 mg TCA after the 1st day to ~5.5 mg after 7 days after 2,000 ppm exposure in wild-type mice). However, unlike TCE, there was a much smaller differences in response between the two TCE exposure levels (i.e., a 12–44% or 1.12–1.44-fold difference in TCA levels in the urine at days 1–7 for exposure concentrations that differ by a factor of 2). This could be indicative of saturation in metabolism and TCA clearance into urine at these high concentrations levels. The authors note that their results suggest that the

metabolism of TCE in both null and wild-type mice may have reached saturation at 1,000 ppm TCE.

For ALT and AST activities in CYP2E1 -/- or CYP2E1 +/+ mice, both liver enzymes were significantly elevated only at the 2,000 ppm level in CYP2E1 +/+ mice. Although the increases in excreted TCA in the urine differed by only ~33% between the 1,000 and 2,000 ppm levels, liver enzyme levels in plasma differed by a much greater extent after 7 days exposure between the 1,000 and 2,000 ppm groups of CYP2E1 +/+ mice (i.e., 1.26- and 1.83-fold of control [ALT] and 1.40- and 2.20-fold of control [AST] for 1,000 and 2,000 ppm TCE exposure levels, respectively). The authors reported a correlation between plasma ALT and both TCE (r = 0.7331) and TCA (r = 0.8169) levels but do not report details of what data were included in the correlation (i.e., were data from CYP2E1 +/+ mice combined with those of the CYP2E1 -/- mice and were control values included with treated values?).

The authors show photomicrograph of a section of liver from control CYP2E1 +/+ and CYP2E1 -/- mice and describe the histological structure of the liver to appear normal. This raises the question as to the cause of the hepatomegaly for the CYP2E1 mice in which the liver weight was increased by a third.

The qualitative scoring for each of the six animals per group showed that none of the CYP2E1 -/- control or treated mice showed evidence of necrosis. For the CYP2E1 +/+ mice, there was no necrosis reported in the control mice and in three of six mice treated with 1,000 ppm TCE. Of the three mice that were reported to have necrosis, the score was reported as 1–2 for two mice and 1 for the third. It is not clear what a score of 1–2 represented given the criteria for each score given by the authors, which defined a score of 1 as minimal and 2 as mild. For the 2,000 ppm TCE-exposed mice, all mice were reported to have at least minimal necrosis (i.e., four mice were reported to have scores of 1–2, one mouse a score of 3, and one mouse a score of 1).

What is clear from the histopathology data are that there appeared to be great heterogeneity of response between the six animals in each TCE-exposure group in CYP2E1 +/+ mice and that there was a greater necrotic response in the 2,000 ppm exposed mice than the 1,000 ppm mice. These results are consistent with the liver enzyme data but not consistent with the small difference between the 1,000 and 2,000 ppm exposure groups for TCA content in urine and, by analogy, metabolism of TCE to TCA. A strength of this study is that it reports the histological data for each animal so that the heterogeneity of liver response can be observed (e.g., the extent of liver necrosis was reported to range from only occasional necrotic cells in any lobule to between one-third and two-thirds of the lobular structure affected after 2,000 ppm TCE exposure for 7 days). Immunohistochemical analysis was reported to show that CYP2E1 was expressed mainly around the centrilobular area in CYP2E1 +/+ mice where necrotic changes were observed after TCE treatment.

Given the large variability in response within the liver after TCE exposure in CYP2E1 mice, phenotypic anchoring becomes especially important for the interpretation of mRNA expression studies (see Sections E.1.1 and E.3.1.2 for macroarray transcript profiling limitations and the need for phenotypic anchoring). However, the data for mRNA expression of PPARα, peroxisomal bifunctional protein (hydratase+3-hydroxyacyl-CoA dehydrogenase),very long chain acyl-CoA dehydrogenase (VLCAD), CYP4A10, NFκB (p65, P50, P52), and IκBα was reported at the means ± SD for six mice per group and represented total liver homogenates. A strength of the study was that they did not pool their RNA and can show means and SDs between treatment groups. The low numbers of animals tested, however, limits the ability to detect statistically significance of the response. By reporting the means, differences in the responses within dose groups was limited and reflected differential response and involvement for different portions of the liver lobule and for the responses of the heterogeneous group of liver cells populating the liver.

The authors reported that they normalized values to the level of β-actin mRNA in the same preparation with a value of 1 assigned as the mean from each control group. The values for mRNA and protein expression reported in the figures appeared to have all been normalized to the control values for the CYP2E1 -/- mice. Although all of the CYP2E1 -/- control values were reported as a value of 1, the control values for the CYP2E1+/+ mice differed with the greatest difference being presented for the CYP4A10-mRNA (i.e., the control level of CYP4A10 mRNA was approximately threefold higher in the CYP2E1+/+ mice than the CYP2E1 -/- mice). Further characterization of the CYP2E1 mouse model was not provided by the authors.

The mean expression of PPARα mRNA was reported slightly reduced after TCE treatment in CYP2E1 -/- mice (i.e., 0.72- and 0.78-fold of control after 1,000 and 2,000 ppm TCE exposure, respectively). The CYP2E1 -/- mice had a higher baseline of PPARα mRNA expression than the CYP2E1+/+ mice (i.e., the control level of the CYP2E1 -/- mice was 1.5-fold of the CYP2E1+/+ mice). After TCE exposure, the CYP2E1 +/+ had a similar increase in PPARα mRNA (~2.3-fold) at both 1,000 and 2,000 ppm TCE. Thus, without the presence of CYP2E1, there did not appear to be increased PPARα mRNA expression. For PPARα protein expression, there was a similar pattern with ~1.6-fold of control levels of protein in the CYP2E1 -/- mice after both 1,000 and 2,000 ppm TCE exposures.

In the CYP2E1 +/+ mice, the control level of PPARα protein was reported to be ~1.5-fold of the CYP2E1 -/- control level. Thus, while the mRNA expression was less, the protein level was greater. After TCE treatment, there was a 2.9-fold of control level of protein at 1,000 ppm TCE and a 3.1-fold of control level of protein at 2,000 ppm. Thus, the magnitude of mRNA increase was similar to that of protein expression for PPARα in CYP2E1 +/+ mice. The magnitude of both was threefold or less over control after TCE exposure. This pattern was similar to that of TCA concentration formed in the liver where there was very little difference between the 1,000 and 2,000 ppm exposure groups in CYP2E1 +/+ mice. However, this pattern

was not consistent with the liver enzyme and histopathology of the liver that showed a much greater response after 2,000 ppm exposure than 1,000 ppm TCE. In addition, where the mean enzyme markers of liver injury and individual animals displayed marked heterogeneity in response to TCE exposure, there was a much smaller degree of variability in the mean mRNA expression and protein levels of PPARα.

For peroxisomal bifunctional protein, there was a greater increase after 1,000 ppm TCE-treated exposure than after 2,000 ppm TCE-treatment for both the CYP2E1 -/- and CYP2E1 +/+ mice (i.e., there was a 2:1 ratio of mRNA expression in the 1,000 vs. 2,000 ppm exposed groups). The CYP2E1 +/+ mice had a much greater response than the CYP2E1 -/- mice (i.e., the CYP2E1 -/- mice had a 2-fold of control and the CYP2E1 +/+ mice had a 7.8-fold of control level after 1,000 ppm TCE treatment). For peroxisomal bifunctional protein expression, the magnitude of protein induction after TCE exposure was much greater than the magnitude of increase in mRNA expression. In the CYP2E1 -/- mice, 1,000 ppm TCE exposure resulted in a 6.9-fold of control level of protein, while the 2,000 ppm TCE group had a 2.3-fold level. CYP2E1 +/+ mice had a ~50% higher control level than CYP2E1 mice and after TCE exposure, the level of peroxisomal bifunctional protein expression was 44-fold of control at 1,000 ppm TCE and 40-fold of control at 2,000 ppm. Thus, CYP2E1 -/- mice were reported to have less mRNA expression and peroxisomal bifunctional protein formed than CYP2E1 +/+ mice after TCE exposure. However, there appeared to be more mRNA expression after 1,000 than 2,000 ppm TCE in both groups and protein expression in the CYP2E1 -/- mice. After 2,000 ppm TCE, there was similar peroxisomal bifunctional protein expression between the 1,000 and 2,000 ppm TCE treated CYP2E1 +/+ mice. Again, this pattern was more similar to that of TCA detection in the urine—not that of liver injury.

For VLCAD, the expression of mRNA was similar between control and treated CYP2E1 -/- mice. For CYP2E1 +/+ mice, the control level of VLCAD mRNA expression was half that of the CYP2E1 -/- mice. After 1,000 ppm TCE, the mRNA level was 3.7-fold of control and after 2,000 ppm TCE the mRNA level was 3.1-fold of control. For VLCAD, protein expression was 1.8-fold of control after 1,000 ppm and 1.6-fold of control after 2,000 ppm in CYP2E1 -/- mice. The control level of VLCAD protein in CYP2E1 +/+ mice appeared to be 1.2-fold control CYP2E1 -/- mice. After 1,000-ppm TCE treatment, the CYP2E1 -/- mice were reported to have 3.8-fold of control VLCAD protein levels and after 2,000-ppm TCE treatment, 3.9-fold of control protein levels. Thus, although showing no increase in mRNA, there was an increase in VLCAD protein levels that was similar between the two TCE exposure groups in CYP2E1 -/- mice. Both VLCAD mRNA and protein levels were greater in CYP2E1 +/+ mice than CYP2E1 -/- mice after TCE exposure. This was not the case for peroxisomal bifunctional protein. The magnitudes of TCE-induced increases in mRNA and protein increases were similar between the 1,000 and 2,000 ppm TCE exposure concentrations, a pattern more similar to TCA detection in the urine but not that of liver injury.

Finally, for CYP4A10 mRNA expression, there was an increase in expression after TCE treatment of threefold for 1,000 ppm and fivefold after 2,000 ppm in CYP2E1 -/- mice. Thus, although the enzyme assumed to be primarily responsible for TCE metabolism to TCA was missing, there was still a response for the mRNA of this enzyme commonly associated with PPARα activation. Of note is that urinary concentrations of TCA were not zero after TCE exposure in CYP2E1 -/- mice. Both 1,000 and 2,000 ppm TCE exposure resulted in ~0.44 mg TCA after 1 day or about 15–22% of that observed in CYP2E1 +/+ mice. Thus, some metabolism of TCE to TCA is taking place in the null mice, albeit at a reduced rate. For CYP2E1 +/+ mice, 1,000 ppm TCE resulted in an 8.3-fold of control level of CYP4A10 mRNA and 2,000 ppm TCE resulted in a 9.3-fold of control level.

The authors did not perform an analysis of CYP4A10 protein. The authors state that "in particular, the mRNA levels of microsomal enzyme CYP4A10 significantly increased in CYP2E1+/+ mice after TCE exposure in a dose-dependent manner." However, the twofold difference in TCE exposure concentrations did not result in a similar difference in response as shown above. Both resulted in approximately ninefold of control response in CYP2E1 +/+ mice. As with PPARα, peroxisomal bifunctional protein, and VLCAD, the response was more similar to that of TCA detection in the urine and not measured of hepatic toxicity. These data show that CYP2E1 metabolism of TCE is important in the manifestation of TCE liver toxicity; however, data suggest that effects other than TCA concentration and indicators of PPARα are responsible for acute hepatotoxicity resulting from very high concentrations of TCE.

The NFκB family and IκBα were also examined for mRNA and protein expression. These cell signaling molecules are involved in inflammation and carcinogenesis and are discussed in Sections E.3.3.3.3 and E.3.4.1.4. Given that presence of hepatocellular necrosis in some of the CYP2E1 +/+ mice to varying degrees, inflammatory cytokines and cell signaling pathways would be expected to be activated. The authors reported that:

> overall, TCE exposure did not significantly increase the expression of p65 and p50 mRNAs in either CYP2E1+/+ or CYP2E1 -/- mice… However, p52 mRNA expression significantly increased in the 2,000 ppm group of CYP2E1+/+ mice, and correlation analysis showed that a significant positive relationship existed between the expression of NFκB p52 mRNA and plasma ALT activity.., while no correlation was seen between NFκB p64 or p50 and ALT activity (data not shown).

The authors also note that TCE treatments "did not increase the expression of TNFR1 and TNFR2 mRNA in CYP2E1+/+ and CYP2E1 -/- mice (data not shown)."

A more detailed examination of the data reveals that there was a similar increases in p65, p50, and p52 mRNA expression increases with TCE treatment in CYP2E1 +/+ mice at both TCE exposure levels. However, only p52 levels for the 2,000 ppm exposed mice were reported to be

statistically significant (see comment above about the statistical power of the experimental design and variability between animals). For 1,000 ppm TCE exposure, the levels of p65, p50, and p52 mRNA expression were 1.5-, 1.8-, and 2.0-fold of control. For 2,000 ppm TCE, the levels of p65, p50, and p52 mRNA expression were 1.8-, 1.8-, and 2.1-fold of control. Thus, there was generally a similar response in all of these indicators of NFκB mRNA expression in CYP2E1 +/+ mice that was mild with little to no difference between the 1,000 and 2,000 ppm TCE exposure levels. For IκBα mRNA expression, there was no difference between control and treatment groups for either type of mice. For CYP2E1 -/- mice, there appeared to be a ~50% decrease in P52 mRNA expression in mice treated with both exposure concentrations of TCE. The authors plotted the relationship between p52 mRNA and plasma ALT concentration for both CYP2E1 -/- and CYP2E1 +/+ mice together and claimed that the correlation coefficient (r = 0.5075) was significant. However, of note is that none of the CYP2E1 -/- mice were reported to have either hepatic necrosis or significant increases in ALT detection.

For protein expression, the authors showed results for p50 and p42 proteins. The control CYP2E1 -/- mice appeared to have a slightly lower level of p50 protein expression (~30%) with a much larger increase in p52 protein expression (i.e., 2.1-fold) than CYP2E1 +/+ mice. There appeared to be a 2-fold increase in p50 protein expression after both 1,000 and 2,000 ppm TCE exposures in the CYP2E1 +/+ mice and a similar increase in p52 protein levels (i.e., 1.9- and 2.5-fold of control for 1,000- and 2,000-ppm TCE exposures, respectively). Thus, the magnitude of mRNA and protein levels were similar for p50 and p52 in CYP2E1 +/+ mice and there was no difference between the 1,000 and 2,000 ppm treatments. For the CYP2E1 -/- mice, there was a modest increase in p50 protein after TCE exposure (1.1- and 1.3-fold of control for 1,000 and 2,000 ppm respectively) and a slight decrease in p52 protein (0.76- and 0.79-fold of control). There was little evidence that the patterns of either expression or protein production of NFκB family and IκBα corresponded to the markers of hepatic toxicity or that they exhibited a dose-response. The authors note that although he expression of p50 protein increased in CYP2E1 +/+ mice, "the relationship between p50 protein and ALT levels was not significant (data not shown)." For TNFR1, there appeared to be less protein expression in the CYP2E1 +/+ mice than the CYP2E1 -/- mice (i.e., the null mice levels were 1.8-fold of the wild-type mice levels). Treatment with TCE resulted in mild decrease of protein levels in the CYP2E1 -/- mice and a 1.4- and 1.7-fold of control level in the CYP2E1 +/+ mice for 1,000 and 2,000 ppm levels, respectively. For p65, although TCE treatment-related effects were reported, of note is that the levels of protein were 2.4 higher in the CYP2E1 +/+ mice than the CYP2E1 -/- mice. Thus, protein levels of the NFκB family appeared to have been altered in the knockout mice. Also, as noted in Section E.3.4.1.4, the origin of the NF-κB is crucial as to its effect in the liver and the results of this report are for whole-liver homogenates that contain parenchymal as well as nonparenchymal cell and have been drawn from liver that are heterogeneous in the magnitude of hepatic necrosis. The authors suggest that "TCA may act as a defense against hepatotoxicity

cause by TCE-delivered reactive metabolite(s) via PPARα in CYP2E1+/+ mice." However, the data from this do not support such an assertion.

E.2.1.15. Ramdhan et al. (2010)

Ramdhan et al. (2010) examined the role of mouse and human PPARα in TCE-induced hepatic steatosis and toxicity using male wild type, PPARα-null and PPARα-null mice with human PPARα inserted (hPPARα) (Cheung et al., 2004) on Sv/129 male mice (6/group), which were exposed for 7 days to 0, 1,000, or 2,000 ppm TCE by inhalation for 8 hours/day. This was a similar paradigm as that used in Ramdhan et al. with results between wild type mice directly comparable. The expression of human PPARα cDNA in the humanized mice was limited to hepatocytes under the control of tetracycline regulatory system.

Plasma aminotransferase activities (AST and ALT) were measured in plasma as well as triglycerides. Hepatic triglyceride levels were measured as well. Urinary metabolites were measured similarly to Ramdhan et al. (2008). Hepatic steatosis was identified based on the presence of vacuoles consistent with lipid accumulation and classified as microvesicular steatosis if the nucleus remained in the center of the hepatocyte. Hepatocyte proliferation was classified based on the presence of large hepatocytes with prominent eosinophilic cytoplasm. Histopathology findings were scored in 20 randomly selected 200x microscopic fields per section with steatotic scores of 0–3: none, mild 5–44% of parenchymal involvement of steatosis), moderate (33–66%), or severe (>66%). Necrotic cells were scored as 0–4: no necrosis, minimal (only occasional necrotic cells in any lobule), mild (<one-third of the lobular structure affected), moderate (one-third to two-thirds of lobular structure affected), or severe (>two-thirds of the lobular structure affected). Hepatocyte proliferation was scored as 0 (absent) or 1 (present).

Real-time PCR analysis was performed on total RNA from whole liver. Western Blot analysis was also performed on whole liver (derived from both hepatocytes and non-parenchymal cells) for NFκB, p65, p50, p52, and PPARα.

Significant differences were observed among control mice for each genotype. The mean body weight of hPPARα mice was 14 and 8.5% less than wild type mouse and PPARα-null mice, respectively. The mean liver weight of hPPARα mice was 11% less than PPARα-null mice and the liver/body weight ratio of PPARα-null mice was 11% higher than wild type mice. TCE, at both 1,000 and 2,000 ppm, significantly increased liver weight in the three mouse lines to a similar extent (i.e., 38 and 49% in wild type mice, 20 and 37% in PPAR-null mice, and 28 and 32% in hPPARα mice). The increases were not statistically significant between doses within each strain. Liver/body weight ratios were also significantly increased with TCE exposure at 1,000 and 2,000 ppm relative to controls (i.e., 38 and 43% in wild type mice, 24 and 36% in PPARα-null mice, and 27 and 39% in hPPARα mice, respectively). The difference between 2.000 and 1.000 ppm TCE exposure was statistically significant in PPARα-null mice.

The authors reported no differences in urinary volume by genotype or exposure but did not show the data. TCA and TCOH were detected in all exposed mice with no significant differences between the 1,000 and 2,000 ppm TCE levels. TCA concentrations were reported to be significantly lower and TCOH levels significantly higher in PPARα-null mice relative to wild type mice with no differences in genotype between the sum of total TCA and TCOH concentrations between genotypes.

AST and ALT liver injury biomarkers were reported to vary <10% among control mice of each strain and to be significantly increased in all exposed mice relative to controls (41–74% and 36–79% higher, respectively) with mean levels within each group higher, though not statistically significantly different, with exposure to 2,000 vs. 1,000 ppm TCE.

Higher levels of plasma triglycerides were reported in untreated hPPARα mice than wild-type mice (52%). Significantly higher liver triglyceride levels were reported in untreated hPPARα mice than wild type mice or PPARα-null mice (77 and 30%, respectively) and between untreated PPARα-null mice and wild-type mice (36%). Exposure to 2,000 ppm TCE was reported to induce an even greater difference between the wild type and PPARα-null mice (113%). Exposure to 1,000 ppm TCE was reported to induce greater liver triglyceride level in hPPARα mice (50%) compared to wild type mice as well as 2,000 ppm TCE (87%). There were no significant difference in mean plasma or liver triglyceride levels between the 2,000 and 1,000 ppm TCE treatment groups within each genotype. Hepatic triglyceride levels were reported to be significantly correlated with liver/body weight ratios of all mice used in the study ($r = 0.54$).

Neither necrosis nor inflammatory cells were reported in liver sections from unexposed mice. The authors reported small cytoplasmic vacuoles in sections from unexposed PPARα-null mice and hPPARα mice that resulted in steatosis scores >0. Steatosis was reported to be absent in unexposed wild type mice and significantly increased in exposed vs. unexposed PPARα-null and hPPARα mice. Steatosis scores were reported to be significantly higher in the 2,000 vs. 1,000 ppm TCE exposures to PPARα-null mice. The authors reported steatosis scored to be significantly correlated with liver triglyceride levels of all mice examined in the study ($r = 0.75$). Macrovesicular steatosis was reported to occur more frequently in hPPARα than PPARα-null mice. Necrosis scores were reported to be significantly higher in TCE exposed mice relative to controls in all three genotype mice and to be significantly higher with 2,000 vs. 1,000 ppm TCE exposure in wild type mice and hPPARα mice. Inflammation scores were reported to be significantly higher with exposed group than control with 2,000 ppm TCE exposure than controls for each genotype group with a difference between the 2,000 and 1,000 ppm exposure groups in wild type mice. Hepatocyte proliferation was reported to be significantly increased with 2,000 ppm TCE exposure in wild-type mice, but not in the other genotypes or exposure concentrations. Of note, the criteria for "proliferation" did not employ quantitative methods of

DNA synthesis but phenotypic descriptions of enlarged hepatocytes that may be indicative of polyploidy.

Background expression levels of several genes were reported to differ significantly between strains in control mice. VLCAD, medium chain acyl-CoA dehydrogenase (MCAD), peroxisomal bifunctional protein (hydratase+3-hydroxyacyl-CoA dehydrogenase) (PH), peroxisomal thiolase (PT), diacylflicerol acyltransferase 1 (DGAT1), and p52 mRNA levels were reported to be higher in untreated hPPARα mice than wild type mice and PPARα-null mice. PPARα, proliferation cell nuclear antigen (PCNA), p50, and tumor necrosis factor alpha (TNFα) mRNA levels were reported to be higher in untreated hPPARα mice than PPARα-null mice. VLCAD, PH, and PT mRNA levels were reported to be significantly lower in untreated PPARα-null mice than wild type mice and p50, p52, PPARγ, and TNFα were higher in untreated PPARα-null mice than wild type mice.

Exposure to TCE was reported to not increase the expression of human PPARα mRNA in hPPARα mice but 2,000 ppm TCE exposure did significantly increase mouse PPARα mRNA in wild type mice. PCNA mRNA expression and mRNA expression of VLCAD, MCAD, PH, and PT was increased in TCE exposed vs. control wild type mice and hPPARα mice. More pronounced induction of PH and PT mRNA was reported for exposed wild type mice. Significant differences were not reported in gene expression between 1,000 and 2,000 ppm TCE exposures.

DGAT1 and DGAT2 mRNA was reported to be significantly increased in hPPARα mice exposed to 2,000 ppm TCE and PPARα-null mice exposed to 1,000 and 2,000 ppm TCE in comparison to respective control mice. Exposure to 1,000 and 2,000 ppm TCE was reported to significantly increase PPARγ mRNA in PPARα-null and hPPARα mice. DGAT1 and DGAT2, PPARγ mRNA levels were not changed with TCE exposure in wild type mice.

NFκB p65 mRNA was reported to be significantly increase after TCE exposure in PPARα-null and hPPARα mice but not wild type mice. NFκB p50 mRNA expression was reported to be significantly increased with exposure to TCE in PPARα-null mice only but NFκB p52 and TNFα mRNA expression was increased significantly with exposure in all strains. The authors reported that NFκB p52 mRNA levels were significantly correlated with plasma ALT levels in all mice used in the study ($r = 0.54$).

Protein expression levels were reported to differ between the genotypes of untreated mice. PPARα levels were 10.4 times higher in untreated hPPARα mice than wild type mice. VLCAD, PT, acyl-CoA(ACOX) A, and ACOX B proteins were reported to be significantly higher in untreated hPPARα mice than wild type and PPARα-null mice and NFκB p65 to be lower in hPPARα mice than PPARα-null mice. VLCAD, MCAD, PH, PT, ACOX A, and ACOX B expression was reported to be slightly lower and p65 and p52 expression slightly higher in untreated PPARα-null mice vs. wild type mice.

TCE exposure was reported to increase VLCAD, PH, PT, ACOX A, and ACOX B in wild type and hPPARα mice but not to induce PPARα protein expression. MCAD protein was significantly increased after TCE exposure in hPPARα mice only. PCNA protein was increased in TCE exposed mice in comparison to controls in all strains. NFκB p52 and TNFα proteins were also increased from TCE exposure in all strains but NFκB p50 and p65 proteins were increased in TCE-exposed PPARα-null mice only. 4-Hydroxy-2- nonenal protein (a marker of oxidative stress) was increased by 1,000 ppm TCE exposure in PPARα-null mice and by 2,000 ppm TCE exposure in wild type and hPPARα mice.

The authors reported that they measured hepatic protein expression of CYP2E1 and ALDH2 enzymes and did not observe a significant difference among controls (data not shown) and that TCE exposure did not alter hepatic CYP2E1 expression but did decrease ALDH2 expression to a comparable extent in all mouse lines (data not shown). Thus, changes in urinary TCA levels in the differing strains were not related to changes in expression of these metabolic enzymes.

While the authors of the paper suggested that the increased susceptibility of PPARα-null mice and hPPATα mice to TCE toxicity is indicative of "protection" by having intact and normal PPARα expression in mice, the disturbances they reported in these genotypes without treatment shows that an already compromised animal is more susceptible to additional insult by high levels of TCE exposure. This study provides an extensive set of parameters altered in the PPARα-null and hPPARα mice by such genetic manipulation alone. In particular, insertion of human PPAR in the null mice did not return the mice to a normal state. The authors noted that hepatic triglyceride levels were the highest in untreated hPPARα among the three strains suggesting that human PPARα insertion did not restore proper lipid regulation in the liver. The humanized mice in particular exhibited a ≥10-fold expression of PPAR in an untreated state. Functional differences between the human and rodent versions of PPAR are difficult to ascertain from this study given the large differences in PPAR protein expression between wild type and humanized mice and the presence of human PPAR only in the hepatocytes in this model. The authors noted that the replacement of human PPARα in the humanized mouse may not have been sufficient to prevent steatosis and that the differences in responses between wild type and humanized mice may reflect functional consequences related to the use of an artificial construct of the reinserted gene without normal control elements in addition to or instead of any differences between human or mouse PPARα. They stated that because they used genetically modified mice with underlying dysregulation, and evaluated very high TCE exposures, their findings may not directly reveal the differences in human PPARα function between mice and humans. The increased toxicity from overexpression of human PPARα in this model is also acknowledged as leading to greater background toxicity in unexposed humanized mice.

Responses reported for gene expression are for liver homogenates so that NFκB and TNFα mRNA expression changes could not be distinguished between Kupffer cell or

hepatocytes origin. The authors noted the similarity of TCE induced hepatomegaly in PPARα null mice in this study and that of Nakajima et al. (2000). They noted that TCE induction of PCNA protein (cell proliferation marker) was increased in all three group but using their phenotypic marker of increased cell size of evidence of increased hepatocyte proliferation in wild type mice.

The authors noted differences in this study and their study of similar design (Ramdhan et al., 2008) for gene expression induced by TCE exposure in wild type mice. Differences in TCE-induced effects between the two studies include less pronounced induction of PPARα, more pronounced increases in PH protein and VLCAD mRNA expression, and ALT and AST levels for this study than the previous one for wild type mice. They stated that urinary TCA levels in wild type mice were incorrectly reported by Ramdhan et al. (2008) but have been corrected in this study. They also noted discrepancies in mRNA and protein expression for some genes in this study. Finally, the authors acknowledged that the small number of mice examined in each group limits the power to identify statistically significant biological effects.

E.2.2. Subchronic and Chronic Studies of TCE

For the purposes of this discussion, studies of duration of ≥4 weeks are considered subchronic. Like those of shorter duration, there is variation in the depth of study of liver changes induced by TCE with many of the longer duration studies focused on the induction of liver cancer. Many subchronic studies were conducted a high doses of TCE that caused toxicity with limited reporting of effects. Similar to acute studies, some of the subchronic and chronic studies have detailed examinations of the TCE-induced liver effects while others have reported primarily liver weight changes as a marker of TCE-response. Similar issues also arise with the impact of differences in initial and final body weights between control and treatment groups on the interpretation of liver weight gain as a measure of TCE-response.

For many of the subchronic inhalation studies, issues associated with whole-body exposures make determination of dose levels difficult. For gavage experiments, death from gavage dosing, especially at higher TCE exposures, is a recurring problem and, unlike inhalation exposures, the effects of vehicle can also be at issue for background liver effects. Concerns regarding effects of oil vehicles, especially corn oil, have been raised with Kim et al. (1990a) noting that a large oil bolus will not only produce physiological effects, but alter the absorption, target organ dose, and toxicity of VOCs. Charbonneau et al. (1991) reported that corn oil potentiates liver toxicity from acetone administration that is not related to differences in acetone concentration. Several oral studies, in particular, document that the use of corn oil gavage induces a different pattern of toxicity, especially in male rodents (see Merrick et al., 1989, Section E.2.2.1 below). Several studies listed below report the effects of hepatocellular DNA synthesis and indices of lipid peroxidation (i.e., Channel et al., 1998) are especially subject to background vehicle effects. Rusyn et al. (1999) report that a single dose of dietary corn oil

increases hepatocyte DNA synthesis 24 hours after treatment by ~3.5-fold, activation of NF-κB to a similar extent ~2 hours after treatment almost exclusively in Kupffer cells, a ~3–4-fold increase in hepatocytes after 8 hours, and increased in TNFα mRNA between 8 and 24 hours after a single dose in female rats. In regard to studies that have used the i.p. route of administration, as noted by Kawamoto et al. (1988b) (see Section E.2.2.11), injection of TCE may result in paralytic ileus and peritonitis and that subcutaneous treatment paradigm will result in TCE not immediately being metabolized but retained in the fatty tissue. Wang and Stacey (1990) state that "intraperitoneal injection is not particularly relevant to humans" and that intestinal interactions require consideration in responses such as increase serum bile acid (see Section E.2.6).

E.2.2.1. Merrick et al. (1989)

The focus of this study was the examination of potential differences in toxicity or orally gavaged TCE administered in corn oil an aqueous vehicle in B6C3F$_1$ mice. As reported by Melnick et al. (1987) above, corn oil administration appeared to have an effect on peroxisomal enzyme induction. TCE (99.5% purity) was administered in corn oil or an aqueous solution of 20% Emulphor to 14–17-week-old mice (n = 12/group) at 0, 600, 1,200, and 2,400 mg/kg-day (males) and 0, 450, 900, and 1,800 mg/kg-day (females) 5 times/week for 4 weeks. The authors stated that due to "varying lethality in the study, 10 animals per dose group were randomly selected (where possible) among survivors for histological analysis." Hepatocellular lesions were characterized:

> as a collection of approximately 3–5 necrotic hepatocytes surrounded by macrophages and polymorphonuclear cells and histopathological grading was reported as based on the number of necrotic lesions observed in the tissue sections: 0 = normal; 1 = isolated lesions scattered throughout the section; 2 = one to five scattered clusters of necrotic lesions; 3 = more than five scattered clusters of necrotic lesions; and 4 = clusters of necrotic lesions observed throughout the entire section."

The authors described lipid scoring of each histological section as "0 = no Oil-Red O staining present; 1 = <10% staining; 2 = 10–25% staining; 3 = 25–30% staining; and 4 = ≥50% staining.

The authors reported dose-related increases in lethality in both males and females exposed to TCE in Emulphor with all male animals dying at 2,400 mg/kg-day with 8/12 females dying at 1,800 mg/kg-day. In both males and females, 2/12 animals also died at the next highest dose as well with no unscheduled deaths in control or lowest dose animals. For corn oil gavaged mice, there were 1–2 animals in each TCE treatment groups of male mice that died while there were no unscheduled deaths in female mice.

The authors stated that lethality occurred within the first week after chemical exposure. The authors presented data for final body weight and liver/body weight values for 4 weeks of exposure and listed the number of animals per group to be 10–12 for corn oil gavaged animals. The reduced number of animals in the Emulphor gavaged animals are reflective of lethality and limit the usefulness of this measure at the highest doses (i.e., 1,800 mg/kg-day for female mice). In mice treated with TCE in Emulphor gavage, the final body weight of control male animals appeared to be lower than those that were treated with TCE while for female mice the final body weights were similar between treated and control groups. For male mice treated with Emulphor, body weights were 22.8 ± 0.8, 25.3 ± 0.5, and 24.3 ± 0.4 g for control, 600, and 1,200 mg/kg-day and for female mice body weights were 20.7 ± 0.4, 21.4 ± 0.3, and 20.5 ± 0.3 g for control, 450, and 900 mg/kg-day of TCE.

For percent liver/body weight ratios, male mice were reported to have 5.6 ± 0.2, 6.6 ± 0.1, and 7.2 ± 0.2% for control, 600, and 1,200 mg/kg-day and for female mice were 5.1 ± 0.1, 5.8 ± 0.1, and 6.5 ± 0.2% for control, 450 and 900 mg/kg-day of TCE. These values represent 1.11- and 1.07-fold of control for final body weight in males exposed to 600 and 1,200 mg/kg-day and 1.18- and 1.29-fold of control for percent liver/body weight, respectively. For females, they represent 1.04- and 0.99-fold of control for final body weights in female exposed to 450mg/kg-day and 900 mg/kg-day and 1.14- and 1.27-fold of control for percent liver/body weight, respectively.

In mice treated with corn oil gavage, the final body weight of control male mice was similar to the TCE treatment groups and higher than the control value for male mice given Emulphor vehicle (i.e., 22.8 ± 0.8 g for Emulphor control vs. 24.3 ± 0.6 g for corn oil gavage controls or a difference of ~7%). The final body weights of female mice were reported to be similar between the vehicles and TCE treatment groups. The baseline percent liver/body weight was also lower for the corn oil gavage control male mice (i.e., 5.6% for Emulphor vs. 4.7% for corn oil gavage or a difference of ~19% that was statistically significant). Although the final body weights were similar in the female control groups, the percent liver/body weight was greater in the Emulphor vehicle group (5.1 ± 0.1% in Emulphor vehicle group vs. 4.7 ± 0.1% for corn oil gavage or a difference of ~9%, which was statistically significant). For male mice treated with corn oil, final body weights were 24.3 ± 0.6, 24.3 ± 0.4, 25.2 ± 0.6, and 25.4 ± 0.5 g for control, 600, 1,200, and 2,400 mg/kg-day, and for female mice, body weights were 20.2 ± 0.3, 20.8 ± 0.5, 21.8 ± 0.3, and 22.6 ± 0.3 g for control, 450, 900, and 1,800 mg/kg-day of TCE.

For percent liver/body weight ratios, male mice were reported to have 4.7 ± 0.1, 6.4 ± 0.1, 7.7 ± 0.1, and 8.5 ± 0.2% for control, 600, 1,200, and 2,400 mg/kg-day and for female mice were reported to have 4.7 ± 0.1, 5.5 ± 0.1, 6.0 ± 0.2, and 7.2 ± 0.1% for control, 450, 900, and 1,800 mg/kg-day of TCE. These values represent 1.0-, 1.04-, and 1.04-fold of control for final body weight in males exposed to 600, 1,200, and 2,400 mg/kg-day TCE and 1.36-, 1.64-, and

1.81-fold of control for percent liver/body weight, respectively. For females, they represent 1.03-, 1.08-, and 1.12-fold of control for body weight for 450, 900, and 1,800 mg/kg-day and 1.17-, 1.28-, and 1.53-fold of control for percent liver/body weight, respectively.

Because of premature mortality, the difference in TCE treatment between the highest doses that are vehicle-related cannot be determined. The decreased final body weight and increased percent liver/body weight ratios in the Emulphor control animals make comparisons of the exact magnitude of change in these parameters due to TCE exposure difficult to determine as well as differences between the vehicles. The authors did not present data for age-matched controls, which did not receive vehicle so that the effects of the vehicles cannot be determined (i.e., which vehicle control values were most similar to untreated controls given that there was a difference between the vehicle controls).

A comparison of the percent liver/body weight ratios at comparable doses between the two vehicles shows little difference in TCE-induced liver weight increases in female mice. However, the corn oil vehicle group was reported to have a greater increase in comparison to controls for male mice treated with TCE at the two lower dosage groups. Given that the control values were approximately 19% higher for the Emulphor group, the apparent differences in TCE-dose response may have reflected the differences in the control values rather than TCE exposure. Because controls without vehicle were not examined, it cannot be determined whether the difference in control values was due to vehicle administration or whether a smaller or younger group of animals was studied on one of the control groups. The body weight of the animals was also not reported by the authors at the beginning of the study, so that the impact of initial differences between groups vs. treatment cannot be accurately determined.

Serum enzyme activities for ALT, AST, and LDH (markers of liver toxicity) showed that there was no difference between vehicle groups at comparable TCE exposure levels for male or female mice. Enzyme levels appeared to be elevated in male mice at the higher doses (i.e., 1,200 and 2,400 mg/kg-day for ALT and 2,400 mg/kg-day for AST), with corn oil gavage inducing similar increases in LDH levels at 600, 1,200, and 2,400 mg/kg-day TCE. For ALT and AST, there appeared to be a dose-related increase in male mice with the 2,400 mg/kg-day treatment group having much greater levels than the 1,200 mg/kg-day group. In Emulphor treatment groups there was a similar increase in ALT levels in males treated with 1,200 mg/kg TCE as with those treated with corn oil and those increases were significantly elevated over control levels. For LDH levels, there were similar increase at 1,200 mg/kg-day TCE for male mice treated using either Emulphor or corn oil.

The authors report that visible necrosis was observed in 30–40% of male mice administered TCE in corn oil, but not that there did not appear to be a dose-response (i.e., the score for severity of necrosis was reported to be 0, 4, 3, and 4 for corn oil control, 600, 1,200, and 2,400 mg/kg-day treatment groups from 10 male mice in each group). No information in regard to variation between animals was given by the authors. For male mice given Emulphor

gavage the extent of necrosis was reported to be 0, 0, and 1 for 0, 600, and 1,200 mg/kg-day TCE exposure, respectively. For female mice, the extent of necrosis was reported to be 0 for all control and TCE treatment groups using either vehicle.

Thus, except for LDH levels in male mice exposed to TCE in corn oil, there was not a correlation with the extent of necrosis and the increases in ALT and AST enzyme levels. Similarly, there was an increase in ALT levels in male mice treated with 1,200 mg/kg-day exposure to TCE in Emulphor that did not correspond to increased necrosis.

For Oil-Red O staining, there was a score of 2 in the Emulphor-treated control male and female mice, while 600 mg/kg-day TCE exposure in Emulphor gavaged male mice and 900 mg/kg-day TCE in corn oil gavaged female mice had a score of 0, along with the corn oil gavage controls in male mice. For female control mice treated with corn oil gavage, the staining was reported to have a score of 3. Thus, there did not appear to be a dose-response in Oil-Red oil staining, although the authors claimed that there appeared to be a dose-related increase with TCE exposure.

The authors described lesions produced by TCE exposure as:

> focal and were surrounded by normal parenchymal tissue. Necrotic areas were not localized in any particular regions of the lobule. Lesions consisted of central necrotic cells encompassed by hepatocytes with dark eosinophilic staining cytoplasm, which progressed to normal-appearing cells. Areas of necrosis were accompanied by localized inflammation consisting of macrophages and polymorphonuclear cells.

No specific descriptions of histopathology of mice given Emulphor were provided in terms of effects of the vehicle or TCE treatment. The scores for necrosis were reported to be only a 1 for the 1,200 mg/kg-day concentration of TCE in male mice gavaged with Emulphor, but 3 for male mice given the same concentration of TCE in corn oil. However, enzyme levels of ALT, AST, and LDH were similarly elevated in both treatment groups.

These results do indicate that administration of TCE for 4 weeks via gavage using Emulphor resulted in mortality of all of the male mice and most of the female mice at a dose in corn oil that resulted in few deaths. Not only was there a difference in mortality, but vehicle also affected the extent of necrosis and enzyme release in the liver (i.e., Emulphor vehicle caused mortality as the highest dose of TCE in male and female mice that was not apparent from corn oil gavage, but Emulphor and TCE exposure induced little, if any, focal necrosis in males at concentrations of TCE in corn oil gavage that caused significant focal necrosis). In regard to liver weight and body weight changes, TCE exposure in both vehicles at nonlethal doses induced increased percent liver/body weight changes male and female mice that increased with TCE exposure level. The difference in baseline control levels between the two vehicle groups

(especially in males) make a determination of the quantitative difference that the vehicle had on liver weight gain problematic, although the extent of liver weight increase appeared to be similar between male and female mice given TCE via Emulphor and female mice given TCE via corn oil. In general, enzymatic markers of liver toxicity and results for focal hepatocellular necrosis were not consistent and did not reflect dose-responses in liver weight increases. The extent of necrosis did not correlate with liver weight increases and was not elevated by TCE treatment in female mice treated with TCE in either vehicle, or in male mice treated with Emulphor. There was a reported difference in the extent of necrosis in male mice given TCE via corn oil and female mice given TCE via corn oil, but the necrosis did not appear to have a dose-response in male mice. Female mice given corn oil and male and female mice given TCE in Emulphor had no to negligible necrosis, although they had increased liver weight from TCE exposure.

E.2.2.2. Goel et al. (1992)

The focus of this study was the description of TCE exposure-related changes in mice after 28 days of exposure with regard to TCE-induced pathological and liver weight change. Male Swiss mice (20–22 g body weight or 9% difference) were exposed to 0, 500, 1,000, or 2,000 mg/kg-day TCE (BDH analytical grade) by gavage in groundnut oil (n = 6 per group) 5 days/week for 28 days. The ages of the mice were not given by the authors. Livers were examined for "free -SH contents," total proteins, catalase activity, acid phosphatase activity, and "protein specific for peroxisomal origin of approx, 80 kd."

The authors report no statistically significant change in body weight with TCE treatment but a significant increase in liver weight. Body weight (mean ± SE) was reported to be 32.67 ± 1.54, 31.67 ± 0.61, 33.00 ± 1.48, and 27.80 ± 1.65 g from exposure to oil control, 500, 1,000, and 2,000 mg/kg-day TCE, respectively. There was a 15% decrease in body weight at the highest exposure concentration of TCE that was not statistically significant, but the low number of animals examined limits the power to detect a significant change. The percent relative liver/body weight was reported to be 5.29 ± 0.48, 7.00 ± 0.36, 7.40 ± 0.39, and 7.30 ± 0.48% from exposure to oil control, 500, 1,000, and 2,000 mg/kg-day TCE, respectively. This represents 1.32-, 1.41-, and 1.38-fold of control in percent liver/body weight for 500, 1,000, and 2,000 mg/kg-day TCE, respectively.

The "free –SH content" in μmol –SH/g tissue was reported to be 5.47 ± 0.17, 7.46 ± 0.21, 7.84 ± 0.34, and 7.10 ± 0.34 from exposure to oil control, 500, 1,000, and 2,000 mg/kg-day TCE, respectively. This represents 1.37-, 1.44-, and 1.30-fold of control in –SH/g tissue weight for 500, 1,000, and 2,000 mg/kg-day TCE, respectively. Total protein content in the liver in mg/g tissue was reported to be 170 ± 3, 183 ± 5, 192 ± 7, and 188 ± 3 from exposure to oil control, 500, 1,000, and 2,000 mg/kg-day TCE, respectively. This represents 1.08-, 1.13-, and 1.11-fold of control in total protein content for 500, 1,000, and 2,000 mg/kg-day TCE, respectively. Thus, the increases in liver weight, "free -SH content," and protein content were generally parallel and

all suggest that liver weight increases had reached a plateau at the 1,000 mg/kg-day exposure concentration, perhaps reflecting toxicity at the highest dose as demonstrated by decreased body weight in this study.

The enzyme activities of δ-ALA dehydrogenase ("a key enzyme in heme biosynthesis"), catalase, and acid phosphatase were assayed in liver homogenates. Treatment with TCE decreased δ-ALA dehydrogenase activity to a similar extent at all exposure levels (32–35% reduction). For catalase the activity as units of catalase/mg, protein was reported to be 25.01 ± 1.81, 32.46 ± 2.59, 41.11 ± 5.37, and 33.96 ± 3.00 from exposure to oil control, 500, 1,000, and 2,000 mg/kg-day TCE, respectively. This represents 1.30-, 1.64-, and 1.36-fold in catalase activity for 500, 1,000, and 2,000 mg/kg-day TCE, respectively. The increasing variability in response with TCE exposure concentration is readily apparent from these data as is the decrease at the highest dose, perhaps reflective of toxicity. For acid phosphatase activity in the liver, there was a slight increase (5–11%) with TCE exposure that did not appear to be dose-related.

The authors report that histologically, "the liver exhibits swelling, vacuolization, widespread degeneration/necrosis of hepatocytes as well as marked proliferation of endothelial cells of hepatic sinusoids at 1,000 and 2,000 mg/kg TCE doses." Only one figure is given at the light microscopic level in which it is impossible to distinguish endothelial cells from Kupffer cells and no quantitative measures or proliferation were examined or reported to support the conclusion that endothelial cells are proliferating in response to TCE treatment. Similarly, no quantitation regarding the extent or location of hepatocellular necrosis is given. The presence or absence of inflammatory cells was not noted by the authors. In terms of white blood cell count, the authors noted that it was slightly increased at 500 mg/kg-day but decreased at 1,000 and 2,000 mg/kg-day TCE, perhaps indicating macrophage recruitment from blood to liver and kidney, which was also noted to have pathology at these concentrations of TCE.

E.2.2.3. Kjellstrand et al. (1981b)

This study was conducted in mice, rats, and gerbils and focused on the effects of 150 ppm TCE exposure via inhalation on body and organ weight. No other endpoints other than organ weights were examined in this study and the design of the study is such that quantitative determinations of the magnitude of TCE response are very limited. NMRI mice (weighing ~30 g with age not given), Sprague-Dawley rats (weighing ~200 g with age not given), and Mongolian gerbils (weighing ~60 g with age not given) were exposed to 150-ppm TCE continuously. Mice were exposed for 2, 5, 9, 16, and 30 days with the number of exposed animals and controls in the 2, 5, 9, and 16 days groups being 10. For 30-day treatments, there were two groups of mice containing 20 mice per group and one group containing 12 mice per group. In addition, there was a group of mice (n = 15) exposed to TCE for 30 days and then examined 5 days after cessation of exposure and another group (n = 20) exposed to TCE for 30 days and then examined

30 days after cessation of exposure. For rats, there were three groups exposed to TCE for 30 days, which contained 24, 12, and 10 animals per group. For gerbils, there were three groups exposed to TCE for 30 days, which contained 24, 8, and 8 animals per group. The groups were reported to consist of equal numbers of males and females but for the mice exposed to TCE for 30 days and then examined 5 days later, the number was 10 males and 5 females. Body weights were reported to be recorded before and after the exposure period. However, the authors state "for technical reasons the animals within a group were not individually identified, i.e., we did not know which initial weight in the group corresponded to which final one." They authors stated that this design presented problems in assessing the precision of the estimate. They go on to state that rats and gerbils were partially identifiable as the animals were housed three to a cage and cage averages could be estimated. Not only were mice in one group housed together, but:

> even worse: at the start of the experiment, the mice in M2 [group exposed for 2 days] and M9 [group exposed for 9 days] were housed together, and similarly M5 [group exposed for 5 days] and M16 [group exposed for 16 days]. Thus, we had, e.g., 10 initial weights for exposed female mice in M2 and M9 where we could not identify those 5 that were M2 weights. Owing to this bad design (forced upon us by the lack of exposure units), we could not study weight gains for mice and so we had to make do with an analysis of final weights.

The problems with the design of this study are obvious from the description given by the authors themselves. The authors stated that they assumed that the larger the animal, the larger the weight of its organs so that all organ weights were converted into relative weights as percentage of body weight. The fallacy of this assumption is obvious, especially if there was toxicity that decreased body weight and body fat but at the same time caused increased liver weight, as has been observed in many studies at higher doses of TCE. In fact, Kjellstrand et al. (1983b) reported that a 150 ppm TCE exposure for 30 days does significantly decreases body weight while elevating liver weight in a group of 10 male NMRI mice. Thus, the body weight estimates from this study are inappropriate for comparison to those in studies where body weights were actually measured. The liver/body weight ratios that would be derived from such estimates of body weights would be meaningless.

The group averages for body weight reported for female mice at the beginning of the 30-day exposure varied significantly and ranged from 23.2 to 30.2 g (~24%). For males, the group averages ranged from 27.3 to 31.4 g (~14%). For male mice, there was no weight estimate for the animals that were exposed for 30 days and then examined 30 days after cessation of exposure.

The authors only report relative organ weight at the end of the experiment rather than the liver weights for individual animals. Thus, these values represent extrapolations based on what body weight may have been. For mice that were exposed to TCE for 30 days and examined after

30 days of exposure, male mice were reported to have "relative organ weight" for liver of 4.70 ± 0.10 vs. 4.27 ± 0.13% for controls. However, there were no initial body weights reported for these male mice, and the body weights are extrapolated values. Female mice exposed for 30 days and examined 30 days after cessation of exposure were reported to have "relative organ weights" for liver of 4.42 ± 0.11 vs. 3.62 ± 0.09% for controls. The group average of initial body weights for this group was reported by the authors.

Although the initial body weight for female control mice as a group average was reported to be similar between the female group exposed to 30 days of TCE and sacrificed 30 days later and those exposed for 30 days and sacrificed 5 days later (30.0 vs. 30.8 g), the liver/body weight ratio varied significantly in these controls (4.25 ± 0.19 vs. 3.62 ± 0.09) as did the number of animals studied (5 female mice in the animals sacrificed after 5 days exposure vs. 10 female mice in the group sacrificed after 30 days exposure). In addition, although there were differences between the three groups of mice exposed to TCE for 30 days and then sacrificed immediately, the authors present the data for extrapolated liver/body weight as pooled results between the three groups. In comparison to control values, the authors report 1.14-, 1.35-, 1.58-, 1.47-, and 1.75-fold of control for percent liver/body weight using body weight extrapolated values in male mice at 2, 5, 9, 16, and 30 days of TCE exposure, respectively. For females, they report 1.27-, 1.28-, 1.49-, 1.41-, and 1.74-fold of control at 2, 5, 9, 16, and 30 days of TCE, respectively.

Although the authors combine female and male relative increases in liver weight in a figure, assign error bars around these data point, and attempt to draw assign a time-response curve to it, it is clear that these data, especially for female mice, do not display time-dependent increase in liver/body weight from 5 to 16 days of exposure and that a comparison of results between 5 and 26 animals is very limited in interpretation. Of note is the wide variation in the control values for relative liver/body weight.

For male mice, there did not seem to be a consistent pattern with increasing duration of the experiment, with values of 4.61, 5.15, 5.05, 4.93, and 4.04% for 2-, 5-, 9-, 16-, and 30-day exposure groups. This represented a difference of ~27%. For female mice, the relative liver/body weight was 4.14, 4.58, 4.61, 4.70, and 3.99% for 2-, 5-, 9-, 16-, and 30-day exposure groups. Thus, it appears that the average relative liver/body weight percent was higher in the 5-, 9-, and 16-day treatment group for both genders than that in the 30-day group and was consistent between these days. There is no apparent reason for there to be such large difference between the 16- and 30-day treatment groups due to increasing age of the animals. Of note is that for the control groups paired with animals treated for 30 days and then examined 30 days later, the male mice had increased relative liver/body weights (4.27 vs. 4.04%), but that the females had decreases (3.62 vs. 3.99%). Such variation between controls does not appear to be age or size related, but rather due to variations in measure or extrapolations, which can affect comparisons between treated and untreated groups and add more uncertainty to the estimates. In addition, the

number of mice in the groups exposed to 2–16 days were only 5 animals for each gender in each group, while the number of animals reported in the 30-day exposure group numbered 26 for each gender.

For animals exposed to 30 days and then examined after 5 or 30 days, male mice were reported to have percent liver/body weight 1.26- and 1.10-fold of control after 5 and 30 days cessation of exposure, while female mice were reported to have values of 1.14- and 1.22-fold of control after 5 and 30 days cessation of exposure, respectively. Again, the male mice exposed for 30 days and then examined after 30 days of cessation of exposure did not have reported initial body weights, giving this value a great deal of uncertainty. Thus, while liver weights appeared to increase during 30 days of exposure to TCE and decrease after cessation of exposure in both genders of mice, the magnitudes of the increases and decreases cannot be determined from this experimental design. Of note is that liver weights appeared to still be elevated after 30 days of cessation exposure.

In regard to initial weights, the authors reported that the initial weights of the rats were different in the three experiments they conducted with them and state that "in those 2 where differences were found in females, their initial weights were about 200 g and 220 g, respectively, while the corresponding weights were only about 160 g in that experiment where no differences were found." The differences in initial body weight of the rat groups were significant. In females, group averages were 198, 158, and 224 g, for groups 1, 2, and 3, respectively, and for males, group averages were 222, 166, and 248 g for groups 1, 2, and 3 respectively. This represents as much as a 50% difference in initial body weights between these TCE treatment groups. Control values varied as well with group averages for controls ranging from 167 g for group 2 to 246 g for group 3 at the start of exposure. For female rats, control groups ranged from 158 to 219 g at the start of the experiment.

The number of animals in each group varied greatly as well, making quantitative comparison even more difficult with the numbers varying between 5 and 12 for each gender in rats exposed for 30 days to TCE. The authors pooled the results for these very disparate groups of rats in their reporting of relative organ weights. They reported 1.26- and 1.21-fold of control in male and female rat percent relative liver/body weight after 30 days of TCE exposure. However, as stated above, these estimates are limited in their ability to provide a quantitative estimate of liver weight increase due to TCE.

There were evidently differences between the groups of gerbils in response to TCE with one group reported to have larger weight gain than control and the other two groups reported to not show a difference by the authors. Of the three groups of gerbils, group 1 contained 12 animals per gender but groups 2 and 3 only 4 animals per gender. As with the rat experiments, the initial average weights for the groups varied significantly (30% in females and males). The authors pooled the results for these very disparate groups of gerbils in their reporting of relative organ weights as well. They reported a nearly identical increase in relative

liver/body weight increase for gerbils (1.22-fold of control value in males and 1.25-fold in females) as for the rats after 30 days of TCE exposure. However, similar caveats should be applied in the confidence in this experimental design to determine the magnitudes of response to TCE exposure.

E.2.2.4. Woolhiser et al. (2006)

An unpublished report by Woolhiser et al. (2006) was received by the U.S. EPA to fill the "priority data needed" for the immunotoxicity of TCE as identified by the ATSDR and designed to satisfy U.S. EPA OPPTS 870.7800 Immunotoxicity Test Guidelines. The study was conducted on behalf of the Halogenated Solvents Industry Alliance and has been submitted to the U.S. EPA but not published. Although conducted as an immunotoxicity study, it does contain information regarding liver weight increases in female Sprague-Dawley female rats exposed to 0, 100, 300, and 1,000 ppm TCE for 6 hours/day, 5 days/week for 4 weeks. The rats were 7 weeks of age at the start of the study. The report gives data for body weight and food weight for 16 animals per exposure group and the mean body weights ranged between 181.8 and 185.5 g on the first day of the experiment. Animals were weighed pre-exposure, twice during the first week, and then "at least weekly throughout the study." All rats were immunized with a single i.v. injection of SRBCs via the tail vein at day 25. Liver weights were taken and samples of liver retained "should histopathological examination have been deemed necessary." But, histopathological analysis was not conducted on the liver.

The effect on body weight gain by TCE inhalation exposure was shown by 5 days and continued for 10 days of exposure in the 300 and 1,000 ppm groups. By day 28, the mean body weight for the control group was reported to be 245.7 g, but 234.4, 232.4, and 232.4 g for the 100, 300, and 1,000 ppm groups, respectively. Food consumption was reported to be decreased in the day 1–5 measurement period for the 300 and 1,000 ppm exposure groups and in the 5–10-day measurement period for the 100 ppm group.

Although body weight and food consumption data are available for 16 animals per exposure group, for organ and organ/body weight summary data, the report gives information for only eight rats per group. The report gives individual animal data in its appendix so that the data for the eight animals in each group examined for organ weight changes could be examined separately. The final body weights were reported to be 217.2, 212.4, 203.9, and 206.9 g for the control, 100, 300, and 1,000 ppm exposure groups containing only eight animals. For the 8-animal exposure groups, the mean initial body weights were 186.6, 183.7, 181.6, and 181.9 g for the control, 100, 300, and 1,000 ppm groups. Thus, there was a difference from the initial and final body weight values given for the groups containing 16 rats and those containing 8 rats. The ranges of initial body weights for the eight animals were 169.8–204.3, 162.0–191.2, 169.0–201.5, and 168.2–193.7 g for the control, 100-, 300 -, and 1,000-ppm groups. Thus, the control

group began with a larger mean value and large range of values (20% difference between highest and lowest weight rat) than the other groups.

In terms of the percent liver/body weight ratios, an increase due to TCE exposure is reported in female rats, although body weights were larger in the control group and the two higher exposure groups did not gain body weight to the same extent as controls. The mean percent liver/body weight ratios were 3.23, 3.39, 3.44, and 3.65%, respectively, for the control, 100, 300, and 1,000 ppm exposure groups. This represented 1.05-, 1.07-, and 1.13-fold of control percent liver/body weight changes in the 100, 300, and 1,000 ppm groups. However, the small number of animals and the variation in initial animal weight limit the ability of this study to determine statistically significant increases and the authors report that only the 1,000 ppm group had statistically significant liver weight increases.

E.2.2.5. Kjellstrand et al. (1983b)

This study examined seven strains of mice (wild, C57BL, DBA, B6CBA, A/sn, NZB, and NMRI) after continuous inhalation exposure to 150 ppm TCE for 30 days. "Wild" mice were reported to be composed of "three different strains: 1. Hairless (HR) from the original strain, 2. Swiss (outbred), and 3. Furtype Black Pelage (of unknown strain)." The authors did not state the age of the animals prior to TCE exposure, but stated that weight-matched controls were exposed to air only chambers. The authors stated that "the exposure methods" have been described earlier (Kjellstrand et al., 1980) but the only reference provided was (Kjellstrand et al., 1981b). In both this study (Kjellstrand et al., 1983b) and the 1981 study, animals were continuously exposed with only a few hours of cessation of exposure noted each week, for a change of food and bedding. Under this paradigm, there is the possibility of additional oral exposure to TCE due to grooming and consumption of TCE on food in the chamber.

The study was reported to be composed of two independent experiments with the exception of strain NMRI, which had been studied in Kjellstrand et al. (1983a; 1981b). The number of animals examined in this study ranged from three to six in each treatment group. The authors reported "significant difference between the animals intended for TCE exposure and the matched controls intended for air-exposure were seen in four cases (Table 1)," and stated that the grouping effects developed during the 7-day adaptation period. Premature mortality was attributed to an accident for one TCE-exposed DBA male and fighting to the deaths of two TCE-exposed NZB females and one B6CBA male in each air exposed chamber. Given the small number of animals examined in this study in each group, such losses significantly decrease the power of the study to detect TCE-induced changes. The range of initial body weights between the groups of male mice for all strains was between 18 g (as mean value for the A/sn strain) and 32 g (as mean value for the B6CBA strain) or ~44%. For females, the range of initial body weights between groups for all strains was 15 g (as mean value for the A/sn strain) and 24 g (as mean value for the DBA strain) or ~38%.

Rather than reporting percent liver/body weight ratios or an extrapolated value, as was done in Kjellstrand et al. (1981b), this study only reported actual liver weights for treated and exposed groups at the end of 30 days of exposure. The authors reported final body weight changes in comparison to matched control groups at the end of the exposure periods but not the changes in body weight for individual animals. They reported the results from statistical analyses of the difference in values between TCE and air-exposed groups.

A statistically significant decrease in body weight was reported between TCE-exposed and control mice in experiment 1 of the C57BL male mice (~20% reduction in body weight due to TCE exposure). This group also had a slight but statistically significant difference in body weight at the beginning of exposure, with the control group having a ~5% difference in starting weight. There was also a statistically significant decrease in body weight of 20% reported after TCE exposure in one group of male B6CBA mice that did not have a difference in body weight at the beginning of the experiment between treatment and control groups. One group of female and both groups of male A/sn mice had statistically significant decreases in body weight after TCE exposure (10% for the females, and 22 and 26% decreases in the two male groups) in comparison to untreated mice of the same strain. The magnitude of body weight decrease in this strain after TCE treatment also reflects differences in initial body weight as there were also differences in initial body weight between the two groups of both treated and untreated A/sn males that were statistically significant, 17 and 10% respectively. One group of male NZB mice had a significant increase in body weight after TCE exposure of 14% compared to untreated animals. A female group from the same strain treated with TCE was reported to have a nonsignificant 7% increase in final body weight in comparison to its untreated group. The one group of male NMRI mice (n = 10) in this study was reported to have a statistically significant 12% decrease in body weight compared to controls.

For the groups of animals with reported TCE exposure-related changes in final body weight compared to untreated animals, such body weight changes may also have affected the liver weights changes reported. The authors did not explicitly state that they did not record liver and body weights specifically for each animal, and thus, would be unable to determine liver/body weight ratios for each. However, they did state that the animals were housed 4–6 in each cage and placed in exposure chambers together. The authors only present data for body and liver weights as the means for a cage group in the reporting of their results. While this approach lends more certainty in their measurements than the approach taken by Kjellstrand et al. (1981b) as described above, the relative liver/body weights cannot be determined for individual animals.

It appears that the authors tried to carefully match the body weights of the control and exposed mice at the beginning of the experiment to minimize the effects of initial body weight differences and distinguish the effects of treatment on body weight and liver weight. However, there was no ability to determine liver/body weight ratios and adjust for difference in initial body weight from changes due to TCE exposure. For the groups in which there was no change in

body weight after TCE treatment and in which there was no difference in initial body weight between controls and TCE-exposed groups, the reporting of liver weight changes due to TCE exposure is a clearer reflection of TCE-induced effects and the magnitude of such effects. Nevertheless, the small number of animals examined in each group is still a limitation on the ability to determine the magnitude of such responses and there statistical significance.

In wild-type mice, there were no reported significant differences in the initial and final body weight of male or female mice before or after 30 days of TCE exposure. For these groups there was 1.76- and 1.80-fold of control values for liver weight in groups 1 and 2 for female mice, and for males 1.84- and 1.62-fold of control values for groups 1 and 2, respectively. For DBA mice, there were no reported significant differences in the initial and final body weight of male or female mice before or after 30 days of TCE exposure. For DBA mice, there was 1.87- and 1.88-fold of control for liver weight in groups 1 and 2 for female mice, and 1.45- and 2.00-fold of control for group 1 and 2 males, respectively. These groups represent the most accurate data for TCE-induced changes in liver weight not affected by initial differences in body weight or systemic effects of TCE, which resulted in decreased body weight gain. These results suggest that there is more variability in TCE-induced liver weight gain between groups of male than female mice.

The C57BL, B6CBA, NZB, and NMRI groups all had at least one group of male mice with changes in body weight due to TCE exposure. The A/sn group had not only decreased body weight in both male groups after TCE exposure (along with differences between exposed and control groups at the initiation of exposure), but also decreased body weight in one of the female groups. Thus, the results for TCE-induced liver weight change in these male groups also reflected changes in body weight. These results suggest a strain-related increased sensitivity to TCE toxicity as reflected by decreased body weight.

For C57BL mice, there was 1.65- and 1.60-fold of control for liver weight after TCE exposure was reported in groups 1 and 2 for female mice, and for males, 1.28-fold (the group with decreased body weight) and 1.82-fold of control values for groups 1 and 2, respectively. For B6CBA mice there was 1.70- and 1.69-fold of controls values for liver weight after TCE exposure in groups 1 and 2 for female mice, and for males, 1.21-fold (the group with decreased body weight) and 1.47-fold of control values reported for groups 1 and 2, respectively. For the NZB mice, there was 2.09-fold (n = 3) and 2.08-fold of control values for liver weight after TCE exposure in groups 1 and 2 for female mice, and for males, 2.34- and 3.57-fold (the group with increased body weight) of control values reported for groups 1 and 2, respectively. For the NMRI mice, whose results were reported for one group with 10 mice, there was 1.66-fold of control value for liver weight after TCE exposure for female mice, and for males, 1.68-fold of control value reported (a group with decreased body weight). Finally, for the A/sn strain that had decreased body weight in all groups but one after TCE exposure and significantly smaller body weights in the control groups before TCE exposure in both male groups, the results still show

TCE-related liver weight increases. For the As/n mice, there was 1.56- and 1.72-fold (a group with decreased body weight) of control value for liver weight in groups 1 and 2 for female mice, and for males, 1.62-fold (a group with decreased body weight) and 1.58-fold (a group with decreased body weight) of control values reported for groups 1 and 2, respectively.

The consistency between groups of female mice of the same strain for TCE-induced liver weight gain, regardless of strain examined, is striking. The largest difference within female strain groups occurred in the only strain in which there was a decrease in TCE-induced body weight. For males, even in strains that did not show TCE-related changes in body weight, there was greater variation between groups than in females. For strains in which one group had TCE-related changes in body weight and another did not, the group with the body weight decrease always had a lower liver weight as well. Groups that had increased body weight after TCE exposure also had an increased liver weight in comparison to the groups without a body weight change. These results demonstrate the importance of carefully matching control animals to treated animals and the importance of the effect of systemic toxicity, as measured by body weight decreases, on the determination of the magnitude of liver weight gain induced by TCE exposure. These results also show the increased variation in TCE-induced liver weight gain between groups of male mice and an increase incidence of body weight changes due to TCE exposure in comparison to females, regardless of strain.

In terms of strain sensitivity, it is important not only to take into account differing effects on body weight changes due to TCE exposure but also to compare animals of the same age or beginning weight as these, parameters may also affect liver weight gain or toxicity induced by TCE exposure. The authors do not state the age of the animals at the beginning of exposure and report, as stated above, a range of initial body weights between the groups as much as 44% for males and 38% for females. These differences can be due to strain and age. The differences in final body weight between the groups of controls, when all animals would have been 30 days older and more mature, was still as much as 48% for males and 44% for females.

The data for female mice, in which body weight was decreased by TCE exposure only in one group in one strain, suggest that the magnitude of TCE-induced liver weight increase was correlated with body weight of the animals at the beginning of the experiment. For the C57BL and As/n strains, female mice starting weights were averaged 17.5 and 15.5 g, respectively, while the average liver weights were 1.63- and 1.64-fold of control after TCE exposure, respectively. For the B6CBA, wild-type, DBA, and NZB female groups, the starting body weights averaged 22.5, 21.0, 23.0, and 21.0 g, respectively, while the average liver weight increases were 1.70-, 1.78-, 1.88-, and 2.09-fold of control after TCE exposure. Thus, groups of female mice with higher body weights, regardless of strain, generally had higher increases in TCE-induced liver weight increases.

The NMRI group of female mice, did not follow this general pattern and had the highest initial body weight for the single group of 10 mice reported (i.e., 27 g) associated with a

1.66-fold of control value for liver weight. It is probable that the data for these mice had been collected from another study. In fact, the starting weights reported for these groups of 10 mice are identical to the starting weights reported for 26 mice examined in Kjellstrand et al. (1981b). However, while this study reports a 1.66-fold of control value for liver weight after 30 days of TCE exposure, the extrapolated percent liver/body weight given in the 1981 study for 30 days of TCE exposure was 1.74-fold of control in female NMRI mice. In the Kjellstrand et al. (1983a) study, discussed below, 10 female mice were reported to have a 1.66-fold of control value for liver weight after 30 days exposure to 150 ppm TCE with an initial starting weight of 26.7 g. Thus, these data appear to be from that study. Thus, differences in study design, variation between experiments, and strain differences may account for the differences results reported in Kjellstrand et al. (1983b) for NMRI mice and the other strains in regard to the relationship to initial body weight and TCE response of liver weight gain.

These data suggest that initial body weight is a factor in the magnitude of TCE-induced liver weight induction rather than just strain. For male mice, there appeared to be a difference between strains in TCE-induced body weight reduction, which in turn affects liver weight. The DBA and wild-type mice appeared to be the most resistant to this effect (with no groups affected), while the C57BL, B6CBA, and NZB strains appearing to have at least one group affected, and the A/sn strain having both groups of males affected. Only one group of NMRI mice were reported in this study and that group had TCE-induced decreases in body weight.

As stated above, there appeared to be much greater differences between groups of males within the same strain in regard to liver weight increases than for females and that the increases appeared to be affected by concurrent body weight changes. In general, the strains and groups within strains, that had TCE-induced body weight decreases had the smallest increases in liver weight, while those with no TCE-induced changes in body weight in comparison to untreated animals (i.e., wild-type and DBA) or had an actual increase in body weight (one group of NZB mice) had the greatest TCE-induced increase in liver weight. Therefore, only examining liver weight in males rather than percent liver/body weight ratios would not be an accurate predictor of strain sensitivity at this dose due to differences in initial body weight and TCE-induced body weight changes.

E.2.2.6. Kjellstrand et al. (1983a)

This study was conducted in male and female NMRI mice with a similar design as Kjellstrand et al. (1983b). The ages of the mice were not given by the authors. Animals were housed 10 animals per cage and exposed from 30 to 120 days at concentrations ranging from 37 to 3,600 ppm TCE. TCE was stabilized with 0.01% thymol and 0.03% diisopropylene. Animals were exposed continuously with exposure chambers being opened twice a week for change of bedding food and water resulting in a drop in TCE concentration of ~1 hour. A group of mice was exposed intermittently with TCE at night for 16 hours. This paradigm results not

only in inhalation exposure, but also oral exposure from TCE adsorption to food and grooming behavior. The authors state that "the different methodological aspects linked to statistical treatment of body and organ weights have been discussed earlier (Kjellstrand et al., 1981b). The same air-exposed control was used in three cases." The design of the experiment, in terms of measurement of individual organ and body weights and the inability to assign a percent liver/body weight for each animal, and limitations are similar to that of Kjellstrand et al. (1983a).

The exposure design was for groups of male and female mice to be exposed to 37, 75, 150, and 300 ppm TCE continuously for 30 days (n = 10 per gender and group except for the 37 ppm exposure groups) and then for liver weight and body weight to be determined. Additional groups of animals were exposed for 150 ppm continuously for 120 days (n = 10). Intermittent exposure of 4 hours/day for 7 days/week were conducted for 120 days at 900 ppm and examined immediately or 30 days after cessation of exposure (n = 10). Intermittent exposures of 16 hours/day at 255-ppm group (n = 10), 8 hours/day at 450 ppm, 4 hours/day at 900 ppm, 2 hours/day at 1,800 ppm, and 1 hour/day at 3,600 ppm 7 days/week for 30 days were also conducted (n = 10 per group).

As in Kjellstrand et al. (1983b), body weights for individual animals were not recorded in a way that the initial and final body weights could be compared. The approach taken by the authors was to match the control group at the initiation of exposure and compare control and treated average values. At the beginning of the experiment, only one group began the experiment with a statistically significant change in body weight between treated and control animals (female mice exposed 16 hours a day for 30 days). In regard to final body weight, which would indicate systemic TCE toxicity, five groups had significantly decreased body weight (i.e., males exposed to 150 ppm continuously for 30 or 120 days, males and females exposed continuously to 300 ppm for 30 days) and two groups significantly increased body weight (i.e., males exposed to 1,800 ppm for 2 hours/day and 3,600 ppm for 1 hour/day for 30 days) after TCE exposure.

Thus, the accuracy of determining the effect of TCE on liver weight changes, reported by the authors in this study for groups in which body weight were also affected by TCE exposure, would be affected by similar issues as for data presented by Kjellstand et al. (1983b). In addition, comparison in results between the 37 ppm exposure groups and those of the other groups would be affected by difference in number of animals examined (10 vs. 20). As with Kjellstrand et al. (1983b), the ages of the animals in this study are not given by the author. Difference in initial body weight (which can be affected by age and strain) reported by Kjellstrand et al. (1983b) appeared to be correlated with the degree of TCE-induced change in liver weight. Although each exposed group was matched to a control group with a similar average weight, the average initial body weights in this study varied between groups (i.e., as much as 14% in female control, 16% in TCE-exposed female mice, 12% in male control, and 16% in male exposed mice).

For female mice exposed to 37–300 ppm TCE continuously for 30 days, only the 300 pm group experienced a 16% decrease in body weight between control and exposed animals. Thus, liver weight increased reported by this study after TCE exposure were not affected by changes in body weight for exposures <300 ppm in female mice. Initial body weights in the TCE-exposed female mice were similar in each of these groups (i.e., range of 29.2–31.6 g, or 8%), with the exception of the females exposed to 150 ppm TCE for 30 days (i.e., initial body weight of 27.3 g), reducing the effects of differences in initial body weight on TCE-induced liver weight induction. Exposure to TCE continuously for 30 days resulted in a dose-dependent change in liver weight in female mice with 1.06-, 1.27-, 1.66-, and 2.14-fold of control values reported for liver weight at 37, 75, 150, and 300 ppm TCE, respectively. In females, the increase at 300 ppm was accompanied by statistically significant decreased body weight in the TCE exposed groups compared to control (~16%). Thus, the response in liver weight gain at that exposure is in the presence of toxicity. However, the TCE-induced increases in liver weight consistently increased with dose of TCE in a linear fashion.

For male mice exposed to 37–300 ppm TCE continuously for 30 days, both the 150 and 300 ppm groups experienced a 10 and 18% decrease in body weight after TCE exposure, respectively. The 37 and 75 ppm groups did not have decreased body weight due to TCE exposure, but varied by 12% in initial body weight. Thus, there are more factors affecting reported liver weight increases from TCE exposure in the male than female mice, most importantly toxicity. Exposure to TCE continuously for 30 days resulted in liver weights of 1.15-, 1.50-, 1.69-, and 1.90-fold of control for 37, 75, 150, and 300 ppm, respectively. The flattening of the dose-response curve for liver weight in the male mice is consistent with the effects of toxicity at the two highest doses, and thus, the magnitude of response at these doses should be viewed with caution. Consistent with Kjellstrand et al. (1983b) results, male mice in this study appeared to have a higher incidence of TCE-induced body weight changes than female mice.

The effects of extended exposure, lower durations of exposure but at higher concentrations, and of cessation of exposure were examined for ≥150 ppm TCE. Mice exposed to TCE at 150 ppm continuously for 120 days were reported to have increased liver weight (i.e., 1.57-fold of control for females and 1.49-fold of control for males), but in the case of male mice, also to have a significant decrease in body weight of 17% in comparison to control groups. Increasing the exposure concentration to 900-ppm TCE and reducing exposure time to 4 hours/day for 120 days also resulted in increased liver weight (i.e., 1.35-fold of control for females and 1.49-fold of controls for males) but with a significant decrease in body weight in females of 7% in comparison to control groups. For mice that were exposed to 150 ppm TCE for 30 days and then examined 120 days after the cessation of exposure, liver weights were 1.09-fold of control for female mice and the same as controls for male mice.

With the exception of 1,800 and 3,600 ppm TCE groups exposed at 2 and 1 hour, respectively, exposure from 225, 450, and 900 ppm at 16, 8, and 4 hours, respectively, for 30 days did not result in decreased body weight in males or female mice. These exposures did result in increased liver weights in relation to control groups and for female mice the magnitude of increase was similar (i.e., 1.50-, 1.54-, and 1.51-fold of control for liver weight after exposure to 225 ppm TCE 16 hours/day, 450 ppm TCE 8 hours/day, and 900 ppm TCE 4 hours/day, respectively). For these groups, initial body weights varied by 13% in females and 14% in males. Thus, under circumstances without body weight changes due to TCE toxicity, liver weight appeared to have a consistent relationship with the product of duration and concentration of exposure in female mice.

For male mice, the increases in TCE-induced liver weight were more variable (i.e., 1.94-, 1.74-, and 1.61-fold of control for liver weight after exposure to 225 ppm TCE 16 hours/day, 450 ppm TCE 8 hours/day, and 900 ppm TCE 4 hours/day, respectively) with the product of exposure duration and concentration did not result in a consistent response in males (e.g., a lower dose for a longer duration of exposure resulted in a greater response than a larger dose at a shorter duration of exposure).

Kjellstrand et al. (1983a) reported light microscopic findings from this study and report that:

> after 150 ppm exposure for 30 days, the normal trabecular arrangement of the liver cells remained. However, the liver cells were generally larger and often displayed a fine vacuolization of the cytoplasm. The nucleoli varied slightly to moderately in size and shape and had a finer, granular chromatin with a varying basophilic staining intensity. The Kupffer cells of the sinusoid were increased in cellular and nuclear size. The intralobular connective tissue was infiltrated by inflammatory cells. There was no sign of bile stasis. Exposure to TCE in higher or lower concentrations during the 30 days produced a similar morphologic picture. After intermittent exposure for 30 days to a time weighted average concentration of 150 ppm or continuous exposure for 120 days, the trabecular cellular arrangement was less well preserved. The cells had increased in size and the variations in size and shape of the cells were much greater. The nuclei also displayed a greater variation in basophilic staining intensity, and often had one or two enlarged nucleoli. Mitosis was also more frequent in the groups exposed for longer intervals. The vacuolization of the cytoplasm was also much more pronounced. Inflammatory cell infiltration in the interlobular connective tissue was more prominent. After exposure to 150 ppm for 30 days, followed by 120 days of rehabilitation, the morphological picture was similar to that of the air-exposure controls except for changes in cellular and nuclear sizes.

Although not reporting comparisons between changes in male and female mice in the results section of the paper, the authors stated in the discussion section that “However, liver mass

increase and the changes in liver cell morphology were similar in TCE-exposed male and female mice."

The authors do not present any quantitative data on the lesions they describe, especially in terms of dose-response. Most of the qualitative description is for the 150 ppm exposure level, in which there are consistent reports of TCE induced body weight decreases in male mice. The authors suggest that lower concentrations of TCE give a similar pathology as those at the 150 ppm, but did not present data to support that conclusion. Although stating that Kupffer cells were increased in cellular and nuclear size, no differential staining was applied light microscopy sections distinguish Kupffer from endothelial cells lining the hepatic sinusoid in this study. Without differential staining, such a determination is difficult at the light microscopic level. Indeed, Goel et al. (1992) describe proliferation of sinusoidal endothelial cells after 1,000 and 2,000 mg/kg-day TCE exposure for 28 days in male Swiss mice. However, the described inflammatory cell infiltrates in the Kjellstrand et al. (1983a) study are consistent with invasion of macrophages and well as polymorphonuclear cells into the liver, which could activate resident Kupffer cells.

Although not specifically describing the changes as consistent with increased polyploidization of hepatocytes, the changes in cell size and especially the continued change in cell size and nuclear staining characteristics after 120 days of cessation of exposure are consistent with changes in polyploidization induced by TCE. Of note is that in the histological description provided by the authors, although vacuolization is reported and consistent with hepatotoxicity or lipid accumulation, which is lost during routine histological slide preparation, there is no mention of focal necrosis or apoptosis resulting from these exposures to TCE.

E.2.2.7. Buben and O'Flaherty (1985)

This study was conducted with older mice than those generally used in chronic exposure assays (male Swiss-Cox outbred mice between 3 and 5 months of age) with a weight range reported between 34 and 45 g. The mice were administered distilled TCE in corn oil by gavage 5 times/week for 6 weeks at exposure concentrations of either 0, 100, 200, 400, 800, 1,600, 2,400, or 3,200 mg TCE/kg-day. While 12–15 mice were used in most exposure groups, the 100 and 3,200 mg/kg groups contained 4–6 mice and the two control groups consisted of 24 and 26 mice. Liver toxicity was determined by "liver weight increases, decreases in liver glucose-6-phosphate (G6P) activity, increases in liver triglycerides, and increases in serum glutamate-pyruvate transaminase (SGPT) activity." Livers were perfused with cold saline prior to testing for weight and enzyme activity and hepatic DNA was measured.

The authors reported the mice to tolerate the 6-week exposed with TCE with few deaths occurring except at the highest dose and that such deaths were related to CNS depression. Mice in all dose groups were reported to continue to gain weight throughout the 6-week dosing period. However, TCE exposure caused "dose-related increases in liver weight to body weight ratio and

since body weight of mice were generally unaffected by treatment, the increases represent true liver weight increases." Exposure concentrations, as low as 100 mg/kg-day, were reported to be "sufficient to cause statistically significant increase in the liver weight/body weight ratio," and the increases in liver size to be "attributable to hypertrophy of the liver cells, as revealed by histological examination and by a decrease in the DNA concentration in the livers."

Mice in the highest dose group were reported to display liver weight/body weight ratios that were about ~75% greater than those of controls and even at the lowest dose there was a statistically significant increase (i.e., control liver/body weight percent was reported to be 5.22 ± 0.09 vs. 5.85 ± 0.20% in 100 mg/kg-day exposed mice). The percent liver/body ratios were 5.22 ± 0.09, 5.84 ± 0.20, 5.99 ± 0.13, 6.51 ± 0.12, 7.12 ± 0.12, 8.51 ± 0.20, 8.82 ± 0.15, and 9.12 ± 0.15% for control (n = 24), 100 (n = 5), 200 (n = 12), 400 (n = 12), 800 (n = 12), 1,600 (n = 12), 2,400 (n = 12), and 3,200 (n = 4) mg/kg-day TCE. This represents 1.12-, 1.15-, 1.25-, 1.36-, 1.63-, 1.69-, and 1.75-fold of control for these doses. All dose groups of TCE induced a statistically significant increase in liver/body weight ratios. For the 200–1,600 mg/kg-day exposure levels, the magnitudes of the increases in TCE exposure concentrations were similar to the magnitudes of TCE-induced increases in percent liver/body weight ratios (i.e., an approximately twofold increase in TCE dose resulted in ~1.7-fold increase change in percent liver/body weight).

TCE exposure was reported to induce a dose-related trend towards increased triglycerides (i.e., control values of 3.08 ± 0.29 vs. 6.89 ± 1.40 at 2,400 mg/kg TCE) with variation of response increased with TCE exposure. For liver triglycerides, the reported values in mg/g liver were 3.08 ± 0.29 (n = 24), 3.12 ± 0.49 (n = 5), 4.41 ± 0.76 (n = 12), 4.53 ± 1.05 (n = 12), 5.76 ± 0.85 (n = 12), 5.82 ± 0.93 (n = 12), 6.89 ± 1.40 (n = 12), and 7.02 ± 0.69 (n = 4) for control, 100, 200, 400, 800, 1,600, 2,400, and 3,200 mg/kg-day dose groups, respectively.

For G6P, the values in μg phosphate/mg protein/20 minutes were 125.5 ± 3.2 (n = 12), 117.8 ± 6.0 (n = 5), 116.4 ± 2.8 (n = 9), 117.3 ± 4.6 (n = 9), 111.7 ± 3.3 (n = 9), 89.9 ± 1.7 (n = 9), 83.8 ± 2.1 (n = 8), and 83.0 ± 7.0 (n = 3) for the same dose groups. Only the 2,400 mg/kg-day group was reported to be statistically significantly increased for triglycerides after TCE exposure although there appeared to be a dose-response. For decreases in G6P, doses ≥800 mg/kg-day were statistically significant.

The numbers of animals varied between groups in this study but, in particular, only a subset of the animals were tested for G6P with the authors providing no rationale for the selection of animals for this assay. The differences in the number of animals per group and small number of animals per group affected the ability to determine a statistically significant change in these parameters but the changes in liver weights were robust enough and the variation was small enough between groups that all TCE-induced changes were described as statistically significant. The livers of TCE treated mice, although enlarged, were reported to appear normal.

A dose-related decrease in G6P activity was reported with similar small decreases (~10%) observed in the TCE exposed groups that did not reach statistical significance until the dose reached 800 mg/kg TCE exposure. SGPT activity was not observed to be increased in TCE-treated mice except at the two highest doses and even at the 2,400 mg/kg-day dose half of the mice had normal values. The large variability in SGPT activity was indicative of heterogeneity of this response between mice at the higher exposure levels for this indicator of liver toxicity. However, the results of this study also demonstrate that hepatomegaly was a robust response that was observed at the lowest dose tested, was dose-related, and was not accompanied by toxicity.

Liver histopathology and DNA content were determined only in control, 400, and 1,600 mg/kg-day TCE exposure groups. DNA content was reported to be significantly decreased from 2.83 ± 0.17 mg/g liver in controls to 2.57 ± 0.14 in 400 mg/kg-day TCE treated group, and to 2.15 ± 0.08 mg/kg-day liver in the 1,600 mg/kg-day exposed group. This result was consistent with a decreased number of nuclei/g of liver and hepatocellular hypertrophy.

Liver degeneration was reported as swollen hepatocytes and to be common with treatment. "Cells had indistinct borders; their cytoplasm was clumped and a vesicular pattern was apparent. The swelling was not simply due to edema, as wet weight/dry weight ratios did not increase." Karyorrhexis (the disintegration of the nucleus) was reported to be present in nearly all specimens and suggestive of impending cell death. A qualitative scale of negative, 1, 2, 3, or 4 was given by the authors to rate their findings without further definition or criterion given for the ratings. "No karyorrhexis, necrosis, or polyploidy was reported in controls, but a score of 1 for karyorrhexis was given for 400 mg/kg TCE and 2 for 1,600 mg/kg TCE." Central lobular necrosis reported to be present only at the 1,600 mg/kg-day TCE exposure level and as a score of 1. "Polyploidy was also characteristic in the central lobular region" with a score of 1 for both 400 and 1,600 mg/kg TCE. The authors reported that "hepatic cells had two or more nuclei or had enlarged nuclei containing increased amounts of chromatin, suggesting that a regenerative process was ongoing" and that there were no fine lipid droplets in TCE-exposed animals.

The finding of "no polyploidy" in control mouse liver is unexpected given that binucleate and polyploid hepatocytes are a common finding in the mature mouse liver. It is possible that the authors were referring to unusually high instances of "polyploidy" in comparison to what would be expected for the mature mouse. The score given by the authors for polyploidy did not indicate a difference between the two TCE exposure treatments and that it was of the lowest level of severity or occurrence.

No score was given for centrolobular hypertrophy although the DNA content and liver weight changes suggested a dose response. The "karyorrhexis" described in this study could have been a sign of cell death associated with increased liver cell number or dying of maturing hepatocytes associated with the increased ploidy, and suggests that TCE treatment was inducing polyploidization. Consistent with enzyme analyses, centrilobular necrosis was only seen at the

highest dose and with the lowest qualitative score, indicating that even at the highest dose there was little toxicity.

Thus, the results of this study of TCE exposure for 6 weeks are consistent with acute studies and show that the region of the liver affected by TCE is the centrilobular region, that hepatocellular hypertrophy is observed in that region, and that increased liver weight is induced at the lowest exposure level tested and much lower than those inducing overt toxicity. These authors suggest that polyploidization is occurring as a result of TCE exposure, although a quantitative dose-response cannot be determined from these data.

E.2.2.8. Channel et al. (1998)

This study was performed in male hybrid B6C3F_1/CrlBR mice (13 weeks old, 25–30 g) and focused on indicators of oxidative stress. TCE was administered by gavage 5 days/week in corn oil for up to 55 days for some groups. Although the study design indicated that water controls, corn oil controls, and exposure levels of 400, 800, and 1,200 mg/kg-day TCE in corn oil, results were not presented for water controls for some parameters measured. Initial body weights and those recorded during the course of the study were not reported for individual treatment groups. Liver samples were collected on study days 2, 3, 6, 10, 14, 21, 28, 35, 42, 49, and 56. Histopathology was studied from a single section taken from the median lobe. Thiobarbituric acid-reactive substances (TBARS) were determined from whole-liver homogenates. Nuclei were isolated from whole-liver homogenates and DNA assayed for 8-hydroxy-2' deoxyguanosine (8-OHdG). There was no indication that parenchymal cell and nonparenchymal cells were distinguished in the assay. Free radical electron paramagnetic resonance (EPR) for total radicals was analyzed in whole-liver homogenates. For peroxisome detection and analysis, livers from three mice from the 1,200 mg/kg-day TCE and control (oil and water) groups were analyzed via electron microscopy. Only centrilobular regions, the area stated by the authors to be the primary site of peroxisome proliferation, were examined. For each animal, 7 micrographs of randomly chosen hepatocytes immediately adjacent to the central vein were examined with peroxisomal area to cytoplasmic area, the number of peroxisomes per unit area of cytoplasm, and average peroxisomal size quantified. Proliferation cell nuclear antigen (PCNA), described as a marker of cell cycle except G0, was examined in histological sections for a minimum of 18 fields per liver section. The authors did not indicate what areas of the liver lobule were examined for PCNA. Apoptosis was detected on liver sections using a apoptosis kit using a single liver section from the median lobe and based on the number of positively labeled cells per 10 mm^2 in combination with the morphological criteria for apoptosis of Columbano et al. (1985). However, the authors did not indicate what areas of the liver lobule were specifically examined.

The authors reported that body weight gain was not adversely affected by TCE dosing of the time course of the study but did not show the data. No gross lesions were reported to be

observed in any group. For TBARS, no water control data were reported by the authors. Data were presented for six animals per group for the corn oil control group and the 1,200 mg/kg-day group (error bars representing the SE). No data were presented without corn oil so that the effects of corn oil on the first day of the study (day 2 of dosing) could not be determined.

After 2 and 3 days of dosing, the corn oil and 1,200 mg/kg-day TCE groups appeared to have similar levels of TBAR detected in whole liver as nmol TBARS/mg protein. However, by day 6, the corn oil treated control had a decrease in TBAR that continued until day 15 where the level was ~50% of that reported on days 2 and 3. The variation between animals as measured by SE was reported to be large on day 10. By day 20, there was a slight increase in variation that declined by day 35 and stayed the same through day 55. For the TCE-exposed group, the TBARS remained relatively consistent and began to decline by about day 20 to a level that similar to the corn oil declines by day 35. Therefore, corn oil alone had a significant effect on TBAR detection inducing a decline by 6 days of administration that persisted thought 55 days. TCE administration at the 1,200 mg/kg-day dose in corn oil appeared to have a delayed decline in TBARS. The authors interpreted this pattern to show that lipid peroxidation was elevated in the 1,200 mg/kg-day TCE group at day 6 over corn oil. However, corn oil alone induced a decrease in TBARs. At no time was TBARS in the TCE treatment groups reported to be greater than the initial levels at days 2 and 3, a time in which TCE and corn oil treatment groups had similar levels. Rather than inducing increasing TBARS over the time course of the study, TCE, at the 1,200 mg/kg-day dose, appeared to delay the corn oil induced suppression of TBARS detection. Because the authors did not present data for aqueous control animals, the time course of TBARS detection in the absence of corn oil cannot be established.

For the 800 and 400 mg/kg-day TCE data, the authors presented a figure, without SE information, for up to 35 days that shows little difference between 400 mg/kg TCE treatment and corn oil suppression of TBAR induction. There was little difference between the patterns of TBAR detection for 800 and 400 mg/kg-day TCE, indicating that both delayed TBARS suppression by corn oil to a similar extent and did not induce greater TBARS than corn oil alone.

For 8-OHdG levels, the authors reported that elevations were modest with the greatest increase noted in the 1,200 mg/kg-day TCE treatment group of 196% of oil controls on day 56. Levels fluctuated throughout the study with most of the time points that were elevated showing 129% of control for the 1,200 mg/kg-day group. Statistically significant elevations were noted on days 2, 10, 28, 49, and 56 with depression on day 3. On all other days (i.e., days 6, 14, 21, 35, and 42), the 8-OHdG values were similar to those of corn oil controls. No statistically significant effects were reported to be observed at lower doses.

The figure presented by the authors shows the percent of controls by TCE treatment at 1,200 mg/kg-day but not the control values themselves. The pattern by corn oil is not shown and neither is the SE of the data. As a percent of control values, the variations were very large for many of the data points and largest for the data given at day 55 in which the authors report the

largest difference between control and TCE treatment. There was no apparent pattern of elevation in 8-OHdG when the data were presented in this manner. Because the data for the corn oil control was not given, as well as no data given for aqueous controls, the effects of corn oil alone cannot be discerned.

Given that for TBARS corn oil had a significant effect and showed a pattern of decline after 6 days, with TCE showing a delayed decline, it is especially important to discern the effects of corn oil and to see the pattern of the data. At time points when TBARS levels were reported to be the same between corn oil and TCE (days 42, 49 and 56), the pattern of 8-OHdG was quite different with a lower level at day 42, a slightly increased level at day 49, and the highest difference reported at day 56 between corn oil control and TCE treated animals. The authors reported that the pattern of "lipid peroxidation" was similar between the 1,200 and 800 mg/kg-day doses of TCE, but that there was no significant difference between 800 mg/kg-day TCE and corn oil controls. Thus, the pattern of TBARS as a measure of lipid peroxidation and 8-OHdG level in nuclear DNA did not match.

In regard to total free radical levels as measured by EPR, results were reported for the 1,200 mg/kg TCE as a signal that was subtracted from control values with the authors stating that only this dose level induced an elevation significantly different from controls. Again, aqueous control values were not presented to discern the effects of corn oil or the pattern that may have arisen with time of corn oil administration.

The pattern of total free radical level appeared to differ from that of lipid peroxidation and for that of 8-OHdG DNA levels, with no changes at days 2, 3, a peak level at day 6, a rapid drop at day 10, mild elevation at day 20, and a significant decrease at day 49. The percentage differences between control and treated values reported at days 6 and 20 by the authors was not proportional to the fold-difference in signal indicating that there was not a consistent level for control values over the time course of the experiment. While differences in lipid peroxidation detection between 1,200 mg/kg-day TCE and corn oil control were greatest at day 14, total free radicals showed their biggest change between corn oil controls and TCE exposure on day 6, time points in which 8-OHdG levels were similar between TCE treatment and corn oil controls. Again, there was no reported difference between corn oil control and the 800 mg/kg-day TCE exposed group in total free radical formation, but for lipid peroxidation, the 800 mg/kg-day TCE exposed group had a similar pattern as that of 1,200 mg/kg-day TCE.

Only the 1,200 mg/kg-day group was evaluated for peroxisomal proliferation at days 6, 10, and 14. Thus, correlations with peroxisome proliferation and other parameters in the report at differing times and TCE exposure concentrations could not be made. The authors reported that there was a treatment and time effect for percent peroxisomal area, a "treatment only" effect for number of peroxisome and no effect for peroxisomal size. They also reported that hepatocytes examined from corn oil control rats were no different than those from water control rats for all peroxisomal parameter, thus discounting a vehicle effect.

However, there was an effect on peroxisomal size between corn oil control and water with corn oil decreasing the peroxisomal size in comparison to water on all days tested. The highest TCE-induced percent peroxisomal area and number occurred on day 10 of the three time points measured for this dose and the fold increase was ~4.5- and ~3.1-fold increase, respectively. The day-10 peak in peroxisomal area and number did not correlate with the reported pattern of free radical or 8-OHdG generation.

For cell proliferation and apoptosis, data were given for days 2, 6, 10, 14, and 21 in a figure. PCNA cells, a measure of cells that have undergone DNA synthesis, was elevated only on day 10 and only in the 1,200 mg/kg-day TCE exposed group with a mean of ~60 positive nuclei per 1,000 nuclei for six mice (~6%). Given that there was little difference in PCNA positive cells at the other TCE doses or time points studied, the small number of affected cells in the liver could not account for the increase in liver size reported in other experimental paradigms at these doses.

The PCNA positive cells as well as "mitotic figures" were reported to be present in centrilobular, midzonal, and periportal regions with no observed predilection for a particular lobular distribution. No data were shown regarding any quantitative estimates of mitotic figures and whether they correlated with PCNA results. Thus, whether the DNA synthesis phases of the cell cycle indicated by PCNA staining were indentifying polyploidization or increased cell number cannot be determined. The authors reported that there was no cytotoxicity manifested as hepatocellular necrosis in any dose group and that there was no significant difference in apoptosis between treatment and control groups with data not shown. The extent of apoptosis in any of the treatment groups, or which groups and timepoints were studied for this effect cannot be determined. No liver weight or body weight data were provided in this study.

These results confirm that as a vehicle corn oil is not neutral in its affects in the liver. The TBARS results indicate a reduction in detection of TBARS in the liver with increasing time of exposure to corn oil alone. Although control animals "treated with water" gavage were studied, only the results for peroxisome proliferation were presented by the study, so that the effects of corn oil gavage were not easy to discern. In addition, the data were presented in such a way for 8-OHdG and total free radical changes that the pattern of corn oil administration was obscured. It is not apparent from this study that TCE exposure induces oxidative damage.

E.2.2.9. Dorfmueller et al. (1979)

The focus of this study was the evaluation of "teratogenicity and behavioral toxicity with inhalation exposure of maternal rats" to TCE. Female Long-Evans hooded rats (n = 12) of ~210 g weight were treated with 1,800 ± 200 ppm TCE for 6 hours/day, 5 days/week, for 22 ± 6 days (until pregnancy confirmation) continuing through GD 20. Control animals were exposed 22 ± 3 days before pregnancy confirmation. The TCE used in this study contained 0.2% epichlorohydrin. Body weights were monitored as well as maternal liver weight at the end of

exposure. Other than organ weight, no other observations regarding the liver were reported in this study. The initial weights of the dams were 212 ± 39 g (mean ± SD) and 204 ± 35 g for treated and control groups, respectively. The final weights were 362 ± 32 g and 337 ± 48 g for treated and control groups, respectively. There was no indication of maternal toxicity by body weight determinations as a result of TCE exposure in this experiment and there was also no significant difference in absolute or relative percent liver/body weight between control and treated female rats in this study.

E.2.2.10. Kumar et al. (2001a)

In this study, adult male Wistar rats (130 ± 10 g body weight) were exposed to 376 ± 1.76 ppm TCE ("AnalaR grade") for 8, 12, and 24 weeks for 4 hours/day 5 days/week. The ages of the rats were not given by the authors. Each group contained six rats. The animals were exposed in whole-body chambers and thus, additional oral exposure was probable. Along with histopathology of light microscopic sections, enzymatic activities of ALP and acid phosphatase, glutamic oxoacetate transaminase, glutamic pyruvate transaminase, reduced GSH, and "total sulphydryl" were assayed in whole-liver homogenates as well as total protein. The authors stated that "the size and weight of the liver were significantly increased after 8, 12, and 24 weeks of TCE exposure." However, the authors did not report the final body weight of the rats after treatment nor did they give quantitative data of liver weight changes. In regard to histopathology, the authors stated:

> After 8 weeks of exposure enlarged hepatocytes, with uniform presence of fat vacuoles were found in all of the hepatocytes affecting the periportal, midzonal, and centrilobular areas, and fat vacuoles pushing the pyknosed nuclei to one side of hepatocytes. Moreover congestion was not significant. After exposure of 12 and 24 weeks, the fatty changes became more progressive with marked necrosis, uniformly distributed in the entire organ.

No other description of pathology was provided in this report. In regard to the description of fatty change, the authors only did conventional H&E staining of sections with no precautions to preserve or stain lipids in their sections. The authors provided a table with histological scoring of simply + or – for minimal, mild, or moderate effects and do not define the criteria for that scoring. There was also no quantitative information given as to the extent, nature, or location of hepatocellular necrosis. The authors reported "no change was observed in GOT and GPT levels of liver in all the three groups. The GSH level was significantly decreased while TSH level was significantly increased during 8, 12, and 24 weeks of TCE exposure. The acid and ALPs were significantly increased during 8, 12, and 24 weeks of TCE exposure." The authors presented a series of figures that are poor in quality to demonstrate histopathological

TCE-induced changes. No mortality was observed from TCE exposure in any group despite the presence of liver necrosis.

E.2.2.11. Kawamoto et al. (1988b)

The focus of this study was the long-term effects of TCE treatment on induction of metabolic enzymes in male adult Wistar rats. The authors reported that eight rats weighing 200 g were treated with 2 g/kg TCE in olive oil administered subcutaneously twice a week for 15 weeks with seven rats serving as olive oil controls. In a separate experiment, five rats were injected with 1 g/kg TCE in olive oil i.p. once a day for 5 continuous days. For comparative purposes, groups of five rats each were administered 3-methylcholanthrene (20 mg/kg in olive oil i.p.), phenobarbital (80 mg/kg in saline i.p.) for 4 days as well as ethanol administered in drinking water containing 10% ethanol for 14 days. Microsomes were prepared 1 week after the last exposure from rats administered TCE for 15 weeks and 24 hours after the last exposure for the other treatments.

Body weights were reported to be slightly less for the TCE treated group than for controls with the initial weights, shown in a figure, to be similar for the first weeks of exposure. At 15 weeks, there appeared to be ~7.5% difference in mean body weights between control and TCE treated rats, which the authors reported to not be significantly different. Organ weights at the termination of the experiment were reported to only be different for the liver with a 1.21-fold of control value reported as a percentage of body weight with TCE treatment. The authors reported their increase in liver weights in male rats from subcutaneous exposure to TCE in olive oil (2.0 g/kg) to be consistent with the range of liver weight gain in rats reported by Kjellstrand et al. (1981b) for 150 ppm TCE inhalation exposure (see comments on that study above). The 5-day i.p. treatment with TCE was also reported to only produce increased liver weight but the data were not shown and the magnitude of the percentage increase was not given by the authors. No liver pathology results were studied or reported.

Along with an increase in liver weight, 15-week treatment with TCE was reported to cause a significant increase of microsomal protein/g liver of ~20% (10.64 ± 0.88 vs. 12.58 ± 0.71 mg/g liver for olive oil controls and TCE treatment, respectively). Microsomal CYP content was reported to show a mild increase that was not statistically significant of 1.08-fold (1.342 ± 0.205 vs. 1.456 ± 0.159 nmol/mg protein for olive oil controls and TCE treatment, respectively) of control. However, CYP content showed 1.28-fold of control value (14.28 ± 2.41 vs. 18.34 ± 2.31 nmol/g liver for olive oil controls and TCE treatment, respectively) in terms of g/liver. Chronic treatment of TCE was also reported to cause a significant increase in cytochrome b-5 level (~1.35-fold of control) and NADPH-cytochrome c reductase activity (~1.50-fold of control) in g/liver.

The 5-day TCE treatment via the i.p. route of administration was reported to cause a significant increase in microsomal protein (~20%) and induce CYP (~50% increase g/liver and

22% increase in microsomal protein), but to also increase cytochrome b-5 and NADPH-cytochrome c reductase activity by 50 and 70% in g/liver, respectively. Although weaker, 5-day i.p. treatment with TCE induced an enzyme pattern more similar to that of phenobarbital and ethanol rather methylcholanthrene (i.e., increased CYP but not microsomal protein and NADPH-cytochrome c reductase). Direct quantitative comparisons of vehicle effects and potential impact on response to TCE treatments for 15 weeks subcutaneous exposure and 5-day i.p. exposure could not be made as baseline levels of all enzyme and protein levels changed as a function of age.

Of note is that, in the discussion section of the paper, the authors disclosed that injection of TCE 2 or 3 g/kg i.p. for 5 days resulted in paralytic ileus from TCE exposure as unpublished observations. They noted that the rationale for injecting TCE subcutaneously was that it not only did not require an inhalation chamber, but also guarded against peritonitis that sometimes occurs following repeated i.p. injection. In terms of comparison with inhalation or oral results, the authors noted that the subcutaneous treatment paradigm will result in TCE not immediately being metabolized but retained in the fatty tissue and that after cessation of exposure, TCE metabolites continued to be excreted into the urine for >2 weeks.

E.2.2.12. NTP (1990)

E.2.2.12.1. 13-Week studies

The NTP conducted a 13-week study of 7-week-old F344/N rats (10 rats per group) that received doses of 125–2,000 mg/kg (males [0, 125, 250, 500, 1,000, or 2,000 mg/kg]) and 62.5 to 1,000 mg/kg (females [0, 62.5, 125, 250, 500, or 1,000 mg/kg] TCE via corn oil gavage 5 days/week (see Table E-1). For 7-week-old B6C3F$_1$mice (n = 10 per group), the dose levels were reported to be 375–6,000 mg/kg TCE (0, 375, 750, 1,500, 3,000, or 6,000 mg/kg). Animals were exposed via corn oil gavage to TCE that was epichlorhydrin-free.

Table E-1. Mice data for 13 weeks: mean body and liver weights

Dose (mg/kg TCE)	Survival	Body weight (mean in g)		Liver weight (mean final in g)	% liver weight/body weight (fold change vs. control)
		Initial	Final		
Male					
0	10/10	21	36	2.1	5.8
375	10/10	20	35	1.74	5.0 (0.86)
750	10/10	21	32	2.14	6.8 (1.17)
1,500	8/10	19	29	2.27	7.6 (1.31)
3,000	3/10	20	30	2.78	8.5 (1.46)
6,000	0/10	22	–	–	–
Female					
0	10/10	18	26	1.4	5.5
375	10/10	17	26	1.31	5.0 (0.91)
750	9/10	17	26	1.55	5.8 (1.05)

1,500	9/10	17	26	1.8	6.5 (1.18)
3,000	9/10	15	26	2.06	7.8 (1.42)
6,000	1/10	15	27	2.67	9.5 (1.73)

All rats were reported to survive the 13-week study, but males receiving 2,000 mg/kg exhibited a 24% difference in final body weight. However, there was great variation in initial weights between the dose groups with mean initial weights at the beginning of the study reported to be 87, 88, 92, 95, 101, and 83 g for the control, 125, 250, 500, 1,000, and 2,000 mg/kg dose groups in male rats, respectively. This represents a 22% difference between the highest and lowest initial weights between groups. Thus, changes in final body weight after TCE treatment also reflect differences in starting weights between the groups that, in the case of the 500 and 1,000 mg/kg groups, would result in a lower-than-expected change in weight due to TCE exposure.

For female rats, the mean initial starting weights were reported to be 81, 72, 74, 75, 73, and 76 g, respectively for the control, 62.5, 125, 250, 500, and 1,000 mg/kg dose groups. This represents a ~13% difference between initial weights. In the case of female rats, the larger mean initial weight in the control group would tend to exaggerate the effects of TCE exposure on final body weight. The authors did not report the variation in initial or final body weights within the dose groups. At the lowest doses for male and female rats, body mean weights were reported to be decreased by 6 and 7% in male and female rats, respectively. Organ weight changes were not reported for rats.

For male mice, mean initial body weights ranged from 19 to 22 g (~16% difference) and for female mice ranged between 18 and 15 g (20% difference), and thus, similar to rats, the final body weights in the groups dose with TCE reflect not only the effects of the compound but also differences in initial weights. For male mice, the mean final body weights were reported to be 3–17% less than controls for the 375–3,000 mg/kg doses. For female mice, the percent difference in final body weight was reported to be the same except for the 6,000 mg/kg dose group, but this lack of difference between controls and treated female mice reflected no change in mice that started at differing weights.

Male mice started to exhibit mortality at 1,500 mg/kg with 8/10 surviving the 1,500 mg/kg dose, 3/10 surviving the 3,000 mg/kg dose, and none surviving the 6,000 mg/kg dose of TCE until the end of the study. For females, 1 animal out of 10 died in the 750, 1,500, and 3,000 mg/kg dose groups and 1/10 survived the 6,000 mg/kg group.

In general, the magnitude of increase in TCE exposure concentration was similar to the magnitude of increase in percent liver/body weight for the 750 and 1,500 mg/kg TCE exposure groups in male B6C3F_1 mice and for the 750–3,000 mg/kg TCE exposure groups in female mice (i.e., a twofold increase in TCE exposure resulted in an approximate twofold increase in percent liver/body weight).

The descriptions of pathology in rats and mice given by this study were not very detailed. For rats, only control and high-dose rats were examined histologically. For mice, only controls and the two highest dose groups were examined histologically. Only mean liver weights were reported with no statistical analyses provided to ascertain quantitative differences between study groups.

Pathological results were reported to reveal that 6/10 males and 6/10 female rats had pulmonary vasculitis at the highest concentration of TCE. This change was also reported to have occurred in 1/10 control male and female rats. Most of those animals were also reported to have had mild interstitial pneumonitis. The authors report that viral titers were positive during this study for Sendai virus.

In mice, liver weights (both absolute and as a percent of body weight) were reported to increase with TCE-exposure level. Liver weights were reported to have increased by >10% relative to controls for males receiving ≥750 mg/kg and for females receiving ≥1,500 mg/kg. The most prominent hepatic lesions detected in the mice were reported to be centrilobular necrosis, observed in 6/10 males and 1/10 females administered 6,000 mg/kg.

> Although centrilobular necrosis was not seen in either males or females administered 3000 mg/kg, 2/10 males had multifocal areas of calcifications scattered throughout their livers. These areas of calcification were considered to be evidence of earlier hepatocellular necrosis. Multifocal calcification was also seen in the liver of a single female mouse that survived the 6000 mg/kg dosage regime. One female mouse administered 3000 mg/kg also had a hepatocellular adenoma, an extremely rare lesion in female mice of this age (20 weeks).

There appeared to be consistent decrease in liver weight at the lowest dose in both female and male mice after 13 weeks of TCE exposure. Liver weight was increased at exposure concentrations in which there was not increased mortality due to TCE exposure at 13 weeks of TCE exposure.

E.2.2.12.2. **2-Year Studies**

In the 2-year phase of the NTP study, TCE was administered by corn oil gavage to groups of 50 male and 50 female F344/N rats, and B6C3F$_1$ mice. Dosage levels were 500 and 1,000 mg/kg for rats and 1,000 mg/kg for mice. TCE was administered 5 times/week for 103 weeks and surviving animals were killed between weeks 103 and 107. The same number of animals receiving corn oil gavage served as controls. The animals were 8 weeks old at the beginning of exposure. The focus of this study was to determine if there was a carcinogenic response due to TCE exposure so there was little reporting of non-neoplastic pathology or toxicity. There was no report of liver weight at termination of the study, only body weight.

The authors reported that there was no increase in necrosis in the liver from TCE exposure in comparison to control mice. In control male mice, the incidence of HCC (tumors with markedly abnormal cytology and architecture) was reported to be 8/48 in controls, and 31/50 in TCE-exposed male mice. For female control mice, HCCs were reported in 2/48 of controls and 13/49 of TCE-exposed female mice. Specifically, the authors described liver pathology in mice as follows:

> Microscopically the hepatocellular adenomas were circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent but the tumors lacked typical lobular organization. The hepatocellular carcinomas had markedly abnormal cytology and architecture. Abnormalities in cytology included increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies, and variations in nuclear appearance. In many instance, several or all of the abnormalities were present in different areas of the tumor. There were also variations in architecture with some of the hepatocellular carcinomas having areas of trabecular organization. Mitosis was variable in amount and location.

The authors reported that the non-neoplastic lesion in male mice differing from controls was focal necrosis in four vs. one animal in the dosed group (8 vs. 2%). There was no fatty metamorphosis in treated male mice vs. two animals in control. In female mice, there was focal inflammation in 29 vs. 19% of animals (dosed vs. control) and no other changes. Therefore, the reported pathological results of this study did not show that the liver was showing signs of toxicity after 2 years of TCE exposure except for neoplasia.

For hepatocellular adenomas, the incidence was reported to be "7/48 control vs. 14/50 dosed in males and 4/48 in control vs. 16/49 dosed female mice." The administration of TCE to mice was reported to cause increased incidences of HCCs in males (control, 8/48; dosed, 31/50: $p = 0.001$) and in females (control 2/48; dosed 13/49; $p < 0.005$). HCCs were reported to metastasize to the lungs in five dosed male mice and one control male mouse, while none were observed in females. The incidences of hepatocellular adenomas were reported to be increased in male mice (control 7/48; dosed 14/50) and in female mice (control 4/48; dosed 16/49; $p < 0.05$).

The survival of both low- and high-dose male rats and dosed male mice was reported to be less than that of vehicle controls with body weight decreases dose dependent. Female mice body weights were comparable to controls. The authors report adjusted rates of 20.6% for control vs. 53.1% for dosed males for adenoma, 22.1% control, and 92.9% for carcinoma in males, and liver carcinoma or adenoma adjusted rates of 100%. For female mice, the adjusted rates were reported to be 12.5% adenoma for control vs. 55.6% for dosed, and 6.2% control carcinoma vs. 43.9% dosed, with liver carcinoma or adenoma adjusted rates of 18.7% for control

vs. 69.7% for dosed. All of the liver results for male and female mice were reported to be statistically significant. The administration of TCE was reported to cause earlier expression of tumors as the first animals with carcinomas were 57 weeks for TCE-exposed animals and 75 weeks for control male mice.

In male rats, there was no reported treatment-related non-neoplastic liver lesions. In female rats, a decrease in basophilic cytological change was reported to be of note in TCE treated rats (~50% in controls but ~5% in TCE treatment groups). However, the authors reported that "the results in male F344/N rats were considered equivocal for detecting a carcinogenic response because both groups receiving TCE showed significantly reduced survival compared to vehicle controls (35/70, 70%; 20/50, 40%; 16/50, 32%) and because 20% of the animals in the high-dose group were killed accidently by gavage error." Specifically 1 male control, 3 low-dose males, 10 high-dose males, 2 female controls, 5 low-dose females, and 5 high-dose female rats were killed by gavage error.

E.2.2.13. NTP (1988)

The studies described in the NTP (1988) TCE report were conducted "to compare the sensitivities of four strains of rats to diisopropylamine-stabilized TCE." However, the authors concluded:

> that because of chemically induced toxicity, reduced survival, and incomplete documentation of experimental data, the studies are considered inadequate for either comparing or assessing TCE-induced carcinogenesis in these strains of rats. TCE (more than 99% pure, stabilized with 8ppm diisopropylamine) was administered via corn oil gavage at exposure concentrations of 0, 500 or 1000 mg/kg per day, 5 days per week, for 103 weeks to 50 male and female rats of each strain. The survival of "high-dose male Marshal rats was reduced by a large number of accidental deaths (25 animals were accidentally killed).

However, the report stated that survival was decreased at both exposure levels of TCE because of mortality that occurred during the administration of the chemical. The number of animals accidently killed were reported to be: 11 male ACI rats at 500 mg/kg, 18 male ACI rats at 1,000 mg/kg, 2 vehicle control female ACI rats, 14 female ACI rats at 500 mg/kg, 12 male ACI rats at 1,000 mg/kg, 6 vehicle control male August rats, 12 male August rats at 500 mg/kg, 11 male August rats at 1,000 mg/kg, 1 vehicle control female August rats, 6 female August rats at 500 mg/kg, 13 male August rats at 1,000 mg/kg, 2 vehicle control male Marshal rats, 12 male Marshal rats at 500 mg/kg, 25 male Marshal rats at 1,000 mg/kg, 3 vehicle control female Marshal rats, 14 female Marshal rats at 500 mg/kg, 18 female Marshal rats at 1,000 mg/kg, 1 vehicle control male Osborne-Mendel rat, 6 male Osborne-Mendel rats at 500 mg/kg, 7 male Osborne-Mendel rats at 1,000 mg/kg, 8 vehicle control female Osborne-Mendel rats, 6 female Osborne-Mendel rats at 500 mg/kg, and 6 female Osborne-Mendel rats at 1,000 mg/kg. The ages

of the rats "when placed on the study" were reported to differ and were for ACI rats (6.5 weeks), August rats (8 weeks), Marshal rats (7 weeks), and Osborne-Mendel rats (8 weeks). The ages of sacrifice also varied and were 17–18 weeks for the ACI and August rats and 110–111 weeks for the Marshal rats.

Results from a 13-week study were briefly mentioned in the report. For the 13-week duration of exposure, groups of 10 male ACI and August rats were administered 0,125, 250, 500, 1,000, or 2,000 mg/kg TCE in corn oil gavage. Groups of 10 female ACI and August rats were administered 0, 62.5, 125, 250, 500, or 1,000 mg/kg TCE. Groups of 10 male Marshal rats received 0, 268, 308, 495, 932, or 1,834 mg/kg and groups of female Marshal rats were given 0, 134, 153, 248, 466, or 918 mg/kg TCE. With the exception of three male August rats receiving 2,000 mg/kg TCE, all animals survived to the end of the 13-week experimental period. "The administration of the chemical for 13 weeks was not associated with histopathological changes."

In the 2-year study the report noted that there:

> was no evidence of liver toxicity described as non-neoplastic changes in male ACI rats due to TCE exposure with 4% or less incidence of any lesion in control or treated animals. For female ACI rats, the incidence of fatty metamorphosis was 6% in control vehicle, 9% in low dose TCE, and 13% in high dose TCE groups. There was also a 2%, 11%, and 8% incidence of clear cell change, respectively. A 6% incidence of hepatocytomegaly was reported in vehicle control and 15% incidence in the high dose group.

All other descriptors had reported incidences of <4%.

For August rats, there was also little evidence of liver toxicity. In male August rats, there was a reported incidence of 8, 4, and 10% focal necrosis in vehicle control, low dose, and high dose, respectively. Fatty metamorphosis was reported to be 8% in control, and 2 and 4% in low and high dose. All other descriptors were reported to be <4%. In female August rats, all descriptors of pathology were reported to have a <4% incidence except for hepatomegaly, which was 10% for vehicle control, 6% for the low dose, and 2% for high dose TCE.

For male Marshal rats, there was a reported 63% incidence of inflammation, NOS in vehicle control, 12% in low dose, and values not recorded at the high dose. There was a reported 6 and 14% incidence of fatty metamorphosis in control and low-dose male rats. Clear cell change was 8% in vehicle with all other values ≤4%. For female Marshal rats, all values were ≤4% except for fatty metamorphosis in 6% of vehicle controls.

For male Osborne-Mendel rats, there was a reported 4, 10, and 4% incidence of focal necrosis in vehicle control, low, and high dose, respectively. For "cytoplasmic change/NOS," there were reported incidences of 26, 32, and 27% in vehicle, low-dose, and high-dose animals, respectively. All other descriptors were reported to be ≤4%. In female Osborne-Mendel rats,

there was a reported incidence of 10% of focal necrosis at the low dose with all other descriptors reported at ≤4%.

Obviously, the negative results in this bioassay are confounded by the killing of a large portion of the animals accidently by experimental error. Still, these large exposure concentrations of TCE did not seem to be causing overt liver toxicity in the rat. Organ weights were not reported in this study, which would have been hard to interpret if they had been reported because of the mortality.

E.2.2.14. Fukuda et al. (1983)

In this 104-week bioassay designed primarily to determine a carcinogenic response, female noninbred Crj:CD-1 (ICR) mice and female Crj:CD (Sprague-Dawley) rats 7 weeks of age were exposed to "reagent grade" TCE at 0, 50, 150, and 450 ppm for 7 hours/day, 5 days/week. During the 2-year duration of the experiment, inhalation concentrations were reported to be within 2% of target values. The numbers of animals per group were reported to be 49–50 mice and 49–51 rats at the beginning of the experiment. The impurities in the TCE were reported to be 0.128% carbon tetrachloride benzene, 0.019% epichlorohydrin, and 0.019% 1,1,2-trichloroethane. After 107 weeks from commencement of the exposure, surviving animals were reported to be killed and completely necropsied. "Tumors and abnormal organs as well as other major organs were excised and prepared for examination in H&E sections." No other details of the methodologies used for pathological examination of tissues were given including what areas of the liver and number of sections examined by light microscopy.

Body weights were not given, but the authors reported that "body weight changes of the mice and rats were normal with a normal range of standard deviation." It was also reported that there were no significant differences in average body weight of animals at specified times during the experiments and no significant difference in mortality between the groups of mice. The report included a figure showing, that for the first 60 weeks of the experiment, there was a difference in cumulative mortality at the 450 ppm dose in ICR mice and the other groups. The authors reported that significantly increased mortalities in the control group of rats compared to the other dosed groups were observed at 85 weeks and after 100 weeks, reflecting many deaths during the 81–85-week and 96–100-week periods for control rats. No significant comparable clinical observations were reported to be noted in each group but that major symptoms such as bloody nasal discharge (in rats), local alopecia (in mice and rats), hunching appearance (in mice), and respiratory disorders (in mice and rats) were observed in some animals mostly after 1 year.

The authors reported that "the numbers of different types of tumors were counted and only malignant tumors were counted when both malignant and benign tumors were observed within one organ." They also reported that "all animals were included in the effective numbers except for a few that were killed accidently, severely autolyzed or cannibalized, and died before the first appearance of tumors among the groups."

In mice, the first tumors were observed at 286 days as thymic lymphoma and most of the malignant tumors appearing later were described as lymphomas or lymphatic leukemias. The incidences of mice with tumors were 37, 36, 54, and 52% in the control, 50, 150 and 450 ppm groups, respectively, by the end of the experiment. "Tumors of the ovary, uterus, subcutaneous tissue, stomach, and liver were observed in the dose groups at low incidences (2–7%) but not in the controls." For the liver, the control, 50 and 150 ppm groups were all reported to have no liver tumors with one animal (2%) having an adenoma at the 450 ppm dose.

For rats, the first tumor was reported to be observed at 410 days and the incidences of animals with tumors were 64, 78, 66, and 63% for control, 50, 150, and 450 ppm TCE, respectively, by the end of the experiment. Most tumors were distributed in the pituitary gland and mammary gland with other tumors reported at a low incidence of 2–4% with none in the controls. For the liver, there were no liver tumors in the control or 150 ppm groups, but one animal (2%) had a cystic cholangioma in 50 ppm group and one animal (2%) had a HCC in the 450 ppm group of rats. No details concerning the pathology of the liver or organ weight changes were given by the authors, including any incidences of hepatomegaly or preneoplastic foci. Of note is that there were no background liver tumors in either strain, indicative of the relative insensitivity of these strains to hepatocarcinogenicity. However, the carcinogenic potential of TCE was reflected by a number of other tumor sites in this paradigm.

E.2.2.15. Henschler et al. (1980)

This report focused on the potential carcinogenic response of TCE in mice (NMRI random bred), rats (WIST random bred), and hamsters (Syrian random bred) exposed to 0, 100, and 500 ppm TCE for 6 hours/day 5 days/week for 18 months. The TCE used in the experiment was reported to be pure with the exception of trace amounts of chlorinated hydrocarbons, epoxides, and triethanolamines (<0.000025% w/w) and stabilized with 0.0015% triethanolamine. The number of animals in each group was 30 and the ages and initial and final body weights of the animals were not provided in the report. For the period of exposure (8 am–2 pm), animals were deprived of food and water. The exposure period was for 18 months with mice and hamsters sacrificed after 30 months and rats after 36 months. "Deceased animals" were reported to be autopsied; spleen, liver, kidneys, lungs, and heart were weighed; and these organs, as well as stomach, CNS, and tumorous tissues, were examined in H&E sections.

Body weight gain was reported to be normal in all species with no noticeable differences between control and exposed groups but data were not shown. However, a "clearly dose-dependent decrease in the survival rate for both male and female mice" was reported to be statistically significant in both sexes and concentrations of TCE with no other significant differences reported in other species. The increase in mortality was more pronounced in male mice, especially after 50 weeks of exposure. Hence, the opportunity for tumor development was diminished due to decreased survival in TCE treated groups.

No organ weights were provided for the study due to the design, in which a considerable period of time occurred between the cessation of exposure and the sacrifice of the animals. Liver weights changes due to TCE may have been diminished with time.

For the 30 autopsied male mice in the control group, one hepatocellular adenoma and one HCC was reported. Whether they occurred in the same animal cannot be determined from the data presentation. In the 29 animals in the 100 ppm TCE exposure group, two hepatocellular adenomas and one mesenchymal liver tumor were reported but no HCCs also without a determination as whether they occurred in the same animal or not. In the 30 animals autopsied in the 500 ppm exposure group, no liver tumors were reported. In female mice, of the 29 animals autopsied in the control group, 30 animals autopsied in the 100 ppm group, and the 28 animals autopsied in the 500 ppm group, there were also no liver tumors reported.

In both the 100 and 500 ppm exposure groups, of male mice especially, low numbers of animals studied, abbreviated TCE exposure duration, and lower numbers of animals surviving to the end of the experiment limit the power of this study to determine a treatment-related difference in liver carcinogenicity. As discussed in Section E.2.3.2 below, the use of an abbreviated exposure regime or study duration and low numbers of animals examined limits the power of a study to detect a treatment-related response. The lack of any observed background liver tumors in the female mice and a very low background level of two tumors in the male mice are indicative of a low sensitivity to detect liver tumors in this paradigm, which may have occurred either through its design, or a low sensitivity of mouse strain used for this endpoint. However, the carcinogenic potential of TCE in mice was reflected by a number of other tumor sites in this paradigm.

For rats and hamsters the authors reported “no dose-related accumulation of any kind of tumor in either sex of these species.” For male rats, there was only one hepatocellular adenoma reported at 100 ppm in the 30 animals autopsied and no carcinomas. For female rats, there were no liver tumors reported in control animals but, more significantly, at 100 ppm, there was one adenoma and one cholangiocarcinoma reported at 100 ppm, and at 500 ppm, there were two cholangioadenomas. Although not statistically significant, the occurrence of this relatively rare biliary tumor was observed in both TCE dose groups in female rats. The difference in survival, as reported in mice, did not affect the power to detect a response in rats, but the low numbers of animals studied, abbreviated exposure duration, and apparent low sensitivity to detect a hepatocarcinogenic response suggest a study of low power. Nevertheless, the occurrence of cholangioadenomas and one cholangiocarcinoma in female rats after TCE treatments is of concern, especially given the relationship in origin and proximity of the bile and liver cells and the low incidence of this tumor. For hamsters, the low background rate of tumors of any kind suggests that in this paradigm, the sensitivity for detection of this tumor is relatively low.

E.2.2.16. Maltoni et al. (1986)

The report by Maltoni et al. (1986) included a series of "systematic and integrated experiments (BT 301, 302, 303, 304, 304bis, 305, 306 bis) started in sequence, testing TCE by inhalation and by ingestion." The first experiment (BT 301) was begun in 1976 and the last in 1983, with this report representing the completed summary of the findings and results of project. The focus of the study was detection of a neoplastic response with only a generalized description of tumor pathology phenotype given and no reporting of liver weight changes induced by TCE exposure.

In experiment BT 301, TCE was administered in male and female Sprague-Dawley rats (13 weeks at start of experiment) via olive oil gavage at control, 50, or 250 mg/kg exposure levels for 52 weeks (4–5 days weekly). The animals (30 male, 30 female for each dose group) were examined during their lifetime. In experiment BT 302, male and female Sprague-Dawley rats (13 weeks old at start of the experiment) were exposed to TCE via inhalation at 0, 100, and 600 ppm, 7 hours/day, 5 days/week, for 8 weeks. The animals (90 animals in each control group, 60 animals in each 100 ppm group, and 72 animals in each 600 ppm group) were examined during their lifetime. In experiment BT 304, male and female Sprague-Dawley rats (12 weeks old at start of the experiment) were exposed TCE via inhalation at 0, 100, 300, and 600 ppm 7 hours/day, 5 days/week, for 104 weeks. The animals (95 male, 100 female rats control groups, 90 animals in each 100 ppm group, 90 animals in each 300 ppm group, and 90 animals in each 600 ppm group) were examined during their lifetime. In experiment BT304bis, male and female Sprague-Dawley rats (12 weeks old at start of the experiment) were exposed to TCE via inhalation at 0, 100, 300, and 600 ppm for 7 hours/day, 5 days/week, for 104 weeks. The animals (40 male, 40 female rats control groups, 40 animals in each 100 ppm group, 40 animals in each 300 ppm group, and 40 animals in each 600 ppm group) were examined during their lifetime.

In experiment BT 303, Swiss mice (11 weeks old at the start of the experiment) were exposed to TCE via inhalation in for 8 weeks using the same exposure concentrations as for experiment BT 302. The animals (100 animals in each control group, 60 animals in the 100 ppm exposed group, and 72 animals in each 600 ppm group) were examined during their lifetime. In experiment BT 305, Swiss mice (11 weeks old at the start of the experiment) were exposed to TCE via inhalation in for 78 weeks, 7 hours/day, 5 days/week. The animals (90 animals in each control group, 90 animals in the 100 ppm exposed group, 90 animals in the 300 ppm group, and 90 animals in each 600 ppm group) were examined during their lifetime. In experiment BT 306, $B6C3F_1$ mice (from NCI source) (12 weeks old at the start of the experiment) were exposed to TCE via inhalation in for 78 weeks, 7 hours a day, 5 days a week. The animals (90 animals in each control group, 90 animals in the 100-ppm-exposed group, 90 animals in the 300-ppm group, and 90 animals in each 600-ppm group) were examined during their lifetime. In experiment BT 306bis, $B6C3F_1$ mice (from Charles River Laboratory as source) (12 weeks old at the start of

the experiment) were exposed to TCE via inhalation for 78 weeks, 7 hours/day, 5 days/week. The animals (90 animals in each control group, 90 animals in the 100 ppm exposed group, 90 animals in the 300 ppm group, and 90 animals in each 600 ppm group) were examined during their lifetime.

In all experiments, TCE was supplied, tested, and reported by the authors of the study to be was highly purified and epoxide free with butyl-hydroxy-toluene at 20 ppm used as a stabilizer. Extra virgin olive oil was used as the carrier for ingestion experiments and was reported to be free of pesticides. The authors described the treatment of the animals and running of the facility in detail and reported that:

> Animal rooms were cleaned every day and room temperature varied from 19 degrees to 22 degrees and was checked 3 times daily. Bedding was changed every two days and cages changes and washed once weekly. The animals were handled very gently and, therefore, were neither aggressive nor nervous. Concentrations of TCE were checked by continuous gas-chromatographic monitoring. Treatment was performed by the same team. In particular, the same person carried out the gavage of the same animals. This is important, since animals become accustomed to the same operators. The inhalation chambers were maintained at 23 ± 2 degrees C and 50 ± 10% relative humidity. Ingestion from Monday to Friday was usually performed early in the morning. The status and behavior of the animals were examined at least three times daily and recorded. Every two weeks the animals were submitted to an examination for the detection of the gross changes, which were registered in the experimental records. The animals which were found moribund at the periodical daily inspection were isolated in order to avoid cannibalism. The animals were weight every two weeks during treatment and then every eight weeks. Animals were kept under observation until spontaneous death. A complete necropsy was performed. Histological specimens were fixed in 70% ethyl alcohol. A higher number of samples was taken when particular pathological lesions were seen. All slides were screened by a junior pathologist and then reviewed by a senior pathologist. The senior pathologist was the same throughout the entire project. Analysis of variance was used for statistical evaluation of body weights. Results are expressed as means and standard deviations. Survival time is evaluated using the Kruskal-Wallis test. For different survival rates between groups, the incidence of lesions is evaluated by using the Log rank test. Non-neoplastic, preneoplastic, and neoplastic lesions were evaluated using the Chi-square of Fisher's exact test. The effect of different doses was evaluated using the Cochran-Armitage test for linear trends in proportions and frequencies.

The authors stated that: "Although the BT project on TCE was started in 1976 and most of the experiments were performed from the beginning of 1979, the methodological protocol adopted substantially met the requirements of the Good Laboratory Practices Act." Finally, it was reported that "the experiments ran smoothly with no accidents in relation to the conduct of the experiment and the health of the animals, apart from an excess in mortality in the male

$B6C3F_1$ mice of the experiment BT 306, due to aggressiveness and fighting among the animals." This is in contrast to the description of the gavage studies conducted by NTP (1990, 1988) in which gavage error resulted in significant loss of experimental animals.

Questions have been raised about the findings, experimental conditions, and experimental paradigm of the European Ramazzini Foundation (ERF) from which the Maltoni et al. (1986) experiments were conducted (EFSA, 2006). However, these concerns were addressed by Caldwell et al. (2008a), who concluded that the ERF bioassay program produced credible results that were generally consistent with those of NTP

In regards to effects of TCE exposure on survival:

> a nonsignificant excess in mortality correlated to TCE treatment was observed only in female rats (treated by ingestion with the compound) and in male $B6C3F_1$ mice. In $B6C3F_1$ mice of the experiment BT 306 bis, the excess in mortality in treated animals was higher ($p < 0.05$ after 40 weeks) but was not dose correlated. No excess in mortality was observed in the other experiments.

The authors reported that "no definite effect of TCE on body weight was observed in any of the experiments, apart from experiment BT 306 bis, in which a slight nondose correlated decrease was found in exposed animals."

In mice, "hepatoma" was the term used by the authors of these studies to describe all malignant tumors of hepatic cells, of different subhistotypes, and of various degrees of malignancy. The authors reported that the hepatomas induced by exposure to TCE:

> may be unique or multiple, and have different sizes (usually detected grossly at necropsy). Under microscopic examination these tumors proved to be of the usual type observed in Swiss and $B6C3F_1$ mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens. They frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. The hepatomas may produce distant metastases, more frequently in the lungs.

In regard to the induction of "hepatomas" by TCE exposure, the authors report that in Swiss mice exposed to TCE by inhalation for 8 weeks (BT303), the percentage of animals with hepatomas was 1.0% in male mice and 1.0% in female mice in the control group ($n = 100$ for each gender). For animals exposed to 100 ppm TCE, the percentage in female mice was 1.7% and male mice 5.0% ($n = 60$ for each gender). For animals exposed to 600 ppm TCE, the percentage in female mice was 0% and in male mice 5.5% ($n = 72$ for each gender).

The relatively larger number of animals used in this bioassay, in comparison to NTP standard assays, allows for a greater power to detect a response. It is also apparent from these results that Swiss mice in this experimental paradigm are a "less sensitive" strain in regard to spontaneous liver cancer induction over the lifetime of the animals. These results suggest that

8 weeks of TCE exposure via inhalation at 100 or 600 ppm may have been associated with a small increase in liver tumors in male mice in comparison to concurrent controls.

In Swiss mice exposed to TCE via inhalation for 78 weeks (BT 305), the percentage of animals with hepatomas was reported to be 4.4% in male mice and 0% in female mice in the control group (n = 90 for each gender). For animals exposed to 100 ppm TCE, the percentage in female mice was reported to be 0% and male mice 2.2% (n = 90 for each gender). For animals exposed to 300 ppm TCE, the percentage in female mice was reported to be 0% and in male mice 8.9% (n = 90 for each gender). For animals exposed to 600 ppm TCE, the percentage in female mice was reported to be 1.1% and in male mice 14.4%. As with experiment BT303, there is a consistency in the relatively low background level of hepatomas reported for Swiss mice in this paradigm. After 78 weeks of exposure, there appears to be a dose-related increase in hepatomas in male but not female Swiss mice via inhalation exposure.

In $B6C3F_1$ mice exposed to TCE by inhalation for 78 weeks (BT306), the percentage of animals with hepatomas was reported to be 1.1% in male mice and 3.3% in female mice in the control group (n = 90 for each gender). For animals exposed to 100 ppm TCE, the percentage in female mice was reported to be 4.4% and in male mice 1.1% (n = 90 for each gender). For animals exposed to 300 ppm TCE, the percentage in female mice was reported to be 3.3% and in male mice 4.4% (n = 90 for each gender). For animals exposed to 600 ppm TCE, the percentage in female mice was reported to be 10.0% and in male mice 6.7%. This was the experimental group with excess mortality in the male group due to fighting. The excess mortality could have affected the results. The authors reported that there was a difference in the percentage of males bearing benign and malignant tumors that was due to early mortality among males in experiment BT306. It is unexpected for the liver cancer incidence to be less in male mice than female mice and not consistent with the results reported for the Swiss mice.

In $B6C3F_1$ male mice exposed to TCE via inhalation (BT 306 bis), the percentage of animals with hepatomas was reported to be 18.9% in male mice in the control group (n = 90). For animals exposed to 100 ppm TCE, the percentage in male mice was reported to be 21.1% (n = 90). For animals exposed to 300 ppm TCE, the percentage in male mice was reported to be 30.0% (n = 90). For animals exposed to 600 ppm TCE, the percentage in male mice was reported to be 23.3%. This experiment did not examine female mice. The authors reported a decrease in survival in mice from this experiment that could have affected results. It is apparent from the BT 306 and BT 306 bis experiments that the background level of liver cancer was significantly different in male mice, although they were supposed to be of the same strain. The finding of differences in response in animals of the same strain but from differing sources has also been reported in other studies for other endpoints (see Section E.3.1.2).

The authors reported four liver angiosarcomas: one in an untreated male rat (BT 304); one in a male and one in a female rat exposed to 600 ppm TCE for 8 weeks (experiment BT302);

and one in a female rat exposed to 600 ppm TCE for 104 weeks (BT 304). The authors concluded that:

> the tumors observed in the treated animals cannot be considered to be correlated to TCE treatment, but are spontaneously arising. These findings are underlined because of the extreme rarity of this tumor in control Sprague-Dawley rats, untreated or treated with vehicle materials. The morphology of these tumors is of the liver angiosarcoma type produced by vinyl chloride in this strain of rats.

In rats treated for 104 weeks, TCE was reported to not affect the percentages of animals bearing benign and malignant tumor and of animals bearing malignant tumors. Moreover, it did not affect the number of total malignant tumors per 100 animals. This study did not report a treatment-related increase in liver cancer in rats. The report only explicitly described positive findings so it is assumed that there were no increases in "hepatomas" in rat liver associated with TCE treatment. The authors concluded that "under the tested experimental conditions, the evidence of TCE (without epoxide stabilizer) carcinogenicity, gives the result of TCE treatment-related hepatomas in male Swiss and $B6C3F_1$ mice. A borderline increased frequency of hepatomas was also seen after 8 weeks of exposure in male Swiss mice." Thus, the increase in liver tumors in both strains of mice exposed to TCE via inhalation reported in this study is consistent with the gavage results from the NTP (1990) study in $B6C3F_1$ mice, where male mice had a higher background level and greater response from TCE exposure than females.

E.2.2.17. Maltoni et al. (1988)

This report was an abbreviated description of an earlier study (Maltoni et al., 1986) focusing on the identification of a carcinogenic response in rats and mice by chronic TCE exposure.

E.2.2.18. Van Duuren et al. (1979)

This study exposed male and female noninbred HA:ICR Swiss mice at 6–8 weeks of age to distilled TCE with no further descriptions of purity. Gavage feeding of TCE was once weekly in 0.1 mL trioctanoin. Neither initial nor final body weights were reported by the authors. The authors reported that, at the termination of the experiments or at death, animals were completely autopsied with specimens of all abnormal-appearing tissues and organs excised for histopathologic diagnosis. Tissues from the stomachs, livers, and kidneys were reported to be taken routinely for the intragastric feeding experiments. Tissues were reported to be stained for H&E for pathologic examination, but no further description of the lobe(s) of the liver examined or the sections examined was provided by the authors.

Results were only reported for the no of mice with forestomach tumors exposed to 0.5 mg/mouse of TCE treatment given once a week in 0.1 mL trioctanoin. Mouse body weights

were not given, so the dose in mg/kg for the mice cannot be ascertained. The protocol used in this experiment kept the mg/mouse constant with a 1-week dosing schedule so that as the mice increased weight with age, the dose as a function of body weight was decreased. The days on test were reported to be 622 for 30 male and female mice.

Two male and one female mice were reported as having forestomach tumors. For 30 mice treated with trioctanoin alone, the number of forestomach tumors was reported to be zero. For mice with no TCE treatment, 5 of 100 male mice were reported to have forestomach tumors and of 8 of 60 female mice were reported to have forestomach tumors for 636 and 649 days on test. No results for liver were presented by the authors by the intragastric route of administration including background rates of the incidences of liver tumors or treatment results. The authors noted that except for repeated skin applications of certain chemicals, no significant difference between the incidence of distant tumors in treated animals compared with no treatment and vehicle control groups was noted. Given the uncertainties in regard to dose, the once-a-week dosing regime, the low number of animals tested with resulting low power, and the lack of reporting of experimental results, the ability to use the results from this experiment in regard to TCE carcinogenicity is very limited.

E.2.2.19. NCI (1976)

This bioassay was "initiated in 1972 according to the methods used and widely accepted at that time" with the design of carcinogenesis bioassays having "evolved since then in some respects and several improvements" having been developed. The most notable changes reported in the foreword of the report are changes "pertaining to preliminary toxicity studies, numbers of controls used, and extent of pathological examination." Industrial-grade TCE was tested (99% TCE, 0.19% 1,2,-epoxybutane, 0.04% ethyl acetate, 0.09% epichlorhydrin, 0.02% *N*-methyl pyrrole, and 0.03% diisobutylene) with rats and mice exposed via gavage in corn oil 5 times/week for 78 weeks using 50 animals per group at two doses with both sexes of Osborne-Mendel rats and B6C3F_1 mice. However, for control groups, only 20 of each sex and species were used. Rats were killed after 110 weeks and mice after 90 weeks. Rats and mice were initially 48 and 35 days of age, respectively, at the start of the experiment with control and treated animals born within 6 days of each other. Initial weight ranges were reported for treated and control animals to be 168–229 g for male rats, 130–170 g for female rats, 11–22 g for male mice, and 11–18 g for female mice. Animals were reported to be "randomly assigned to treatment groups so that initially the average weight in each group was approximately the same." Mice treated with TCE were reported to be:

> maintained in a room housing other mice being treated with one of the following 17 compounds: 1,1,2-2-tetrachloroethane, chloroform, 3-chloropropene, chloropicrin, 1,2-dibromochloropropane, 1,2, dibromoethane, ethylene dichloride, 1,1-diochloroethane, 3-sulfolene, idoform, methyl chloroform, 1,1,2-trichloro-

ethane, tetrachloroethylene, hexachloroethane, carbon disulfide, trichlorofluoromethane, and carbon tetrachloride. Nine groups of vehicle controls and 9 groups of untreated controls were also housed in this same room.

The authors noted that:

TCE-treated rats and their controls were maintained in a room housing other rats being treated with one of the following compounds: dibromochloropropane, ethylene dichloride, 1,1-dichloroethane, and carbon disulfide. Four groups of vehicle-treated controls were in the same room." Thus, there was the potential of co-exposure to a number of other chemicals, especially for the mice, resulting from exhalation in treated animals housed in the same room, including the control groups, as noted by the authors. The authors also noted that "samples of ambient air were not tested for presence of volatile materials" but state that "although the room arrangement is not desirable as is stated in the Guidelines for Carcinogen Bioassay in Small Rodents, there is no evidence the results would have been different with a single compound in a room.

The initial doses of TCE for rats were reported to be 1,300 and 650 mg/kg. However, these levels were changed based on survival and body weight data "so that the TWA doses were 549 and 1,097 mg/kg for both male and female rats." For mice, the initial doses were reported to be 1,000 and 2,000 mg/kg for males and 700 and 1,400 mg/kg for females. The "doses were increased so that the time weighted average doses were 1,169 and 2,339 mg/kg for male mice and 869 and 1,739 mg/kg for female mice."

The authors reported that signs of toxicity, including reduction in weight, were evident in treated rats, which, along with increased mortality, "necessitated a reduction in doses during the test." In contrast "very little evidence of toxicity was seen in mice, so doses were increased slightly during the study." Doses were "changed for the rats after 7 and 16 weeks of treatment, and for the mice after 12 weeks." At 7 weeks of age, male and female rats were dosed with 650 mg/kg TCE, at 14 weeks they were dosed with 750 mg/kg TCE, and at 23 weeks of age 500 mg/kg TCE. For the high exposure level, the exposure concentrations were 1,300, 1,500, and 1,000 mg/kg TCE, respectively, for the same changes in dosing concentration. For rats the percentage of TCE in corn oil remained constant at 60%. For female mice, the TCE exposure at the beginning of dosing was 700 mg/kg TCE (10% in corn oil) at 5 weeks of age for the "lower dose" level. The dose was increased to 900 mg/kg-day (18% in corn oil) at 17 weeks of age and maintained until 83 weeks of age. For male mice, the TCE exposure at the beginning of dosing was 1,000 mg/kg TCE (15% in corn oil) at 5 weeks of age for the "lower dose" level. At 11 weeks, the level of TCE remained the same but the percentage of TCE in corn oil was reduced to 10%. The dose was increased to 1,200 mg/kg-day at 17 weeks of age (24% in corn oil) and maintained until 83 weeks of age. For the "higher dose," the TCE exposure at the beginning of dosing was 1,400 mg/kg TCE (10% in corn oil) at 5 weeks of age in female mice.

At 11 weeks of age, the exposure level of TCE was kept the same but the percentage of TCE in corn oil increased to 20%. By 17 weeks of age, the exposure concentration of TCE in corn oil was increased to 1,800 mg/kg (18% in corn oil) in female mice. For the "higher dose" in male mice, the TCE exposure at the beginning of dosing was 2,000 mg/kg (15% in corn oil) which was maintained at 11 weeks in regard to TCE administered but the percent of TCE corn oil was increased to 20%. For male mice, the exposure concentration was increased to 2,400 mg/kg (24% in corn oil). For all of the mice, treatment continued on a 5 days/week schedule of gavage dosing throughout the timecourse of treatment (78 weeks of treatment). Thus, not only did the total dose administered to the animals change, but the volumes of vehicle in which TCE was administered changed throughout the experiment.

The authors stated that at 37 weeks of age, "To help assure survival until planned termination the dosing schedule was changed for rats to a cycle of 1 week of no treatment followed by 4 weeks of treatment." for male and female rats. Thus, the duration of exposure in rats was also changed. All lobes of the liver were reported to be taken including the free margin of each lobe with any nodule or mass represented in a block 10 × 5 × 3 mm cut from the liver and fixed in a marked capsule.

Body weights (mean ± SD) were reported to be 193 ± 15.0 g (n = 20), 193 ± 15.8 g (n = 50), and 195 ± 16.7 g (n = 50) for control, low-, and high-dose male rats at initiation of the experiment. By 1 year of exposure (50 weeks), 20/20 control male rats were still alive to be weighed, 42/50 of the low dose rats were alive and 34/50 of high dose rats were still alive. The body weights of those remaining were decreased by 6.2 and 17% in the low- and high-dose animals in comparison with the controls. For female rats, the mean body weights were reported to be 146 ± 11.4 g (n = 20), 144 ± 11.0 g (n = 50), and 144 ± 9.5 g (n = 50) for control, low-, and high-dose female rats at initiation of the experiment. By 1 year of exposure (50 weeks), 17/20 control female rats were still alive; 28/50 low-dose and 39/50 high-dose rats were alive. The body weights of those remaining were decreased by 25 and 30% in the low- and high-dose animals in comparison with the controls.

For male mice, the initial body weights were 17 ± 0.5 g (n = 20), 17 ± 2.0 g (n = 50), and 17 ± 1.1 g (n = 50) for control, low, and high doses. By 1 year of exposure (50 weeks), 18/20 control male mice were still alive; 47/50 or the low-dose and 34/50 high-dose mice were still alive. The body weights of those remaining were unchanged in comparison to controls. For female mice, the initial body weights were 14 ± 0.0 g (n = 20), 14 ± 0.6 g (n = 50), and 14 ± 0.7 g (n = 50) for control, low, and high doses. By 1 year of exposure (50 weeks), 18/20 control male mice were still alive; 45/50 of the low dose and 41/50 of the high-dose groups were still alive. The body weights of those remaining were unchanged in comparison to controls.

A high proportion of rats were reported to die during the experiment with 17/20 control, 42/50 low-dose, and 47/50 high-dose animals dying prior to scheduled termination. For female rats, 12/20 control, 35/48 low-dose, and 37/50 high-dose animals were reported to die before

scheduled termination with two low-dose females reported to be missing and not counted in the denominator for that group. The authors reported that earlier death was associated with higher TCE dose. A decrease in the percentage of tumor-bearing animals was reported to be lower in treated animals and attributed by the authors to be likely related to the decrease in their survival.

A high percentage of respiratory disease was reported to be observed among the rats without any apparent difference in the type, severity, or morbidity as to sex or group. The authors reported that "no significant toxic hepatic changes were observed" but no other details regarding results in the liver of rats were provided.

Carbon tetrachloride was administered to rats as a positive control. A low incidence of both HCC and neoplastic nodule was reported to be found in both colony controls (1/99 HCC and 0/99 neoplastic nodule in male rats and 0/98 HCC and 2/98 neoplastic nodules in female rats) and carbon-tetrachloride-treated rats. Hepatic adenomas were included in the description of neoplastic nodules in this study with the diagnosis of HCC to be "based on the presence of less organized architecture and more variability in the cells comprising the neoplasms."

The authors reported that "increased mortality in treated male mice appears to be related to the presence of liver tumors." For both male and female mice, the incidences of HCC were reported to be high from TCE treatment with 1/20 in age matched controls, 26/50 in low-dose, and 31/48 in high-dose males. Colony controls for male mice were reported to be 5/77 for vehicle and 5/70 for untreated mice. For female mice HCCs were reported to be observed in 0/20 age-matched controls, 4/50 low-dose, and 11/47 high-dose mice. Colony controls for female mice were reported to be 1/80 for vehicle and 2/75 for untreated mice. In male mice, HCCs were reported to be observed early in the study with the first seen at 27 weeks. HCCs were not observed so early in low-dose male or female mice.

The diagnosis of HCC was reported to be based on histologic appearance and the presence of metastasis especially to the lung with no other lesions significantly elevated in treated mice. The tumors were reported to be:

> varied from those composed of well differentiated hepatocytes in a relatively uniform trabecular arrangement to rather anaplastic lesions in which mitotic figures occurred in cells which varied greatly in size and tinctorial characteristics. Many of the tumors were characterized by the formation of relatively discrete areas of highly anaplastic cells within the tumor proper which were, in turn, surrounded by relatively well differentiated neoplastic cells. In general, various arrangements of the hepatocellular carcinoma occurred, as described in the literature, including those with an orderly cord-like arrangement of neoplastic cells, those with a pseudoglandular pattern resembling adenocarcinoma, and those composed of sheets of highly anaplastic cells with minimal cord or gland-like arrangement. Multiple metaplastic lesions were observed in the lung, including several neoplasms which were differentiated and relative benign in appearance." The authors noted that almost all mice treated with carbon tetrachloride exhibited

liver tumors and that the "neoplasms occurring in treated [sic carbon tetrachloride treated] mice were similar in appearance to those noted in the TCE-treated mice.

Thus, phenotypically this study reported that the liver tumors induced in mice by TCE were heterogeneous and typical of those arising after carbon tetrachloride administration. The descriptions of liver tumors in this study and the tendency of metastasis to the lung are similar to the descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation.

In terms of noncancer pathology of the liver, one control male rat was reported to display fatty metamorphosis of the liver at 102 weeks. However, for the low dose, three male rats were reported to display fatty metamorphosis (90, 110, and 110 weeks), two rats to display cystic inflammation (76, 110 weeks), and one rat to display general inflammation (110 weeks). At the high dose, six rats were reported to display fatty metamorphosis (12, 35, 49, 52, 52, and 58 weeks), one rat to display cytomegaly (42 weeks), two rats to display centrilobular degeneration (53 and 58 weeks), one rat to display diffuse inflammation (62 weeks), 1 rat to display congestion (Week 12), and five rats to display angiectasis or abnormally enlarged blood vessels, which can be manifested by hyperproliferation of endothelial cells and dilatation of sinusoidal spaces (35, 42, 52, 54, and 65 weeks). One control female rat was reported o display fatty metamorphosis of the liver at 110 weeks, and one control female rats to display "inflammation" of the liver at 110 weeks. Of the TCE dosed female rats, only one high-dose female rat displayed fatty metamorphosis at week 96.

Thus, for male rats, there was liver pathology present in some rats due to TCE exposure examined from 12 weeks to a year at their time of their premature death. For mice, the liver pathology was dominated by the presence of HCC with additional hyperplasia noted in two mice of the high-dose male and female groups and one or less mouse exhibiting hyperplasia in the control or low-dose groups.

The authors noted that "while the absence of a similar effect in rats appears most likely attributable to a difference in sensitivity between the Osborne-Mendel rat and $B6C3F_1$ mouse, the early mortality of rats due to toxicity must also be considered." They concluded that "the test in rats is inconclusive: large numbers of rats died prior to planned termination; in addition, the response of this rat strain to the hepatocarcinogenicity of the positive control compound, carbon tetrachloride, appeared relatively low." Finally, the authors noted that "while the results obtained in the present bioassay could possibly have been influenced by an impurity in the TCE used, the extremely low amounts of impurities found make this improbable."

E.2.2.20. Herren-Freund et al. (1987)

This study gave results primarily in initiated male $B6C3F_1$ mice that were also exposed to TCE metabolites in drinking water for 61 weeks. However, in Table 1 of the report, results were

given for mice that received no initiator but were given 40 mg/L TCE or 2 g/L sodium chloride as control. The mice were reported to be 28 days of age when placed on drinking water containing TCE. The authors reported that concentrations of TCE fell by about half at the 40 mg/L dose of TCE during the twice a week change in drinking water solution. For control animals (n = 22), body weight at termination was reported to be 32.93 ± 0.54 g, liver weight 1.80 ± 0.05 g, and percent liver/body weight 5.47% ± 0.16%. For TCE treated animals (n = 32), body weight at termination was reported to be 35.23 ± 0.66 g, liver weight was 1.97 ± 0.10 g, and percent liver/body weight was 5.57% ± 0.24%. Thus, hepatomegaly was not reported for this paradigm at this time of exposure. The study reported that for 22 control animals the prevalence of adenomas was 2/22 animals (or 9%), with the mean number of adenomas per animal to be 0.09 ± 0.06 (SEM). The prevalence of carcinomas in the control group was reported to be 0/22. For 32 animals exposed to 40 mg/L TCE, the prevalence of adenomas was 3/32 animals (or 9%), with the mean number of adenomas per animal to be 0.19 ± 0.12 (SEM). The prevalence of animals with HCCs was 3/32 animals (or 9%) with the mean number of HCCs to be 0.10 ± 0.05 (SEM).

Thus, similar to the acute study of Tucker et al. (1982), significant loss of TCE is a limitation for trying to evaluate TCE hazard in drinking water. However, despite difficulties in establishing accurately the dose received, an increase in adenomas per animal and an increase in the number of animals with HCCs were reported to be associated with TCE exposure after 61 weeks of exposure. Also of note is that the increase in tumors was reported without significant increases in hepatomegaly at the end of exposure. The authors did not report these increases in tumors as being significant but did not do a statistical test between TCE exposed animals without initiation and control animals without initiation. The low numbers of animal tested limits the statistical power to make such a determination. However, for carcinomas, there was none reported in controls but 9% of TCE-treated mice had HCCs.

E.2.2.21. Anna et al. (1994)

This report focused on presenting incidence of cancer induction after exposure to TCE or its metabolites and included a description of results for male $B6C3F_1$ mice (8 weeks old at the beginning of treatment) receiving 800 mg/kg-day TCE via gavage in corn oil, 5 days/week for 76 weeks. There was very limited reporting of results other than tumor incidence. There was no reporting of liver weights at termination of the experiment. Although the methods section of the report gives 800 mg/kg-day as the exposure level, Table 1 in the results section reports that TCE was administered at 1,700 mg/kg-day. This could be a typographical error in the table as a transposition with the dose of "perc" administered to other animals in the same study. The methods section of the report states that the authors based their dose in mice that used in the 1990 (NTP) study. The NTP study only used a 1,000 mg/kg-day in mice, suggesting that the table is mislabeled and that the actual dose is 800 mg/kg-day in the Anna et al. (1994) study.

All treated mice were reported to be alive after 76 weeks of treatment. For control animals, 10 animals exposed to corn oil and 10 untreated controls were killed in a 9-day period. The remaining controls were killed at 96, 103, and 134 weeks of treatment. Therefore, the control group (all) contains a heterogeneous group of animals that were sacrificed from 76 to 134 weeks and were not comparable to the animals sacrificed at 76 weeks.

At 76 weeks, 3 of 10 the untreated and two of the 10 corn oil treated controls were reported to have one small hepatocellular adenoma. None of the controls examined at 76 weeks were reported to have any observed HCCs. The authors reported no cytotoxicity for TCE, corn oil, and untreated control group. At 76 weeks, 75 mice treated with 800 mg/kg-day TCE were reported to have a prevalence of 50/75 animals having adenomas with the mean number of adenomas per animal to be 1.27 ± 0.14 (SEM). The prevalence of carcinomas in these same animals was reported to be 30/70 with the mean number of HCCs per animal to be 0.57 ± 0.10 (SEM).

Although not comparable in terms of time until tumor observation, corn oil control animals examined at much later time points did not have as great a tumor response as did those exposed to TCE. At 76–134 weeks, 32 mice treated with corn oil were reported to have a prevalence of 4/32 animals having adenomas with the mean number of adenomas per animal to be 0.13 ± 0.06 (SEM). The prevalence of carcinomas in these same animals was reported to be 4/32 with the mean number of HCCs per animal to be 0.12 ± 0.06 (SEM). Despite only examining one exposure level of TCE and the limited reporting of findings other than incidence data, this study also reported that TCE exposure in male $B6C3F_1$ mice to be associated with increased induction of adenomas and HCC, without concurrent cytotoxicity.

In terms of liver tumor phenotype, Anna et al. (1994) reported the percent of H-ras codon 61 mutations in tumors from concurrent control animals (water and corn oil treatment groups combined) examined in their study, historical controls in $B6C3F_1$ mice, and in tumors from TCE or DCA (0.5% in drinking water) treated animals. From their concurrent controls, they reported H-ras codon 61 mutations in 17% (n = 6) of adenomas and 100% (n = 5) of carcinomas. For historical controls (published and unpublished), they reported mutations in 73% (n = 33) of adenomas and in 70% (n = 30) of carcinomas. For tumors from TCE-treated animals, they reported mutations in 35% (n = 40) of adenomas and 69% (n = 36) of carcinomas, while for DCA-treated animals, they reported mutations in 54% (n = 24) of adenomas and in 68% (n = 40) of carcinomas. The authors reported that "in this study, the H-ras codon 61 mutation frequency was not statistically different in liver tumors from DCA and TCE-treated mice and combined controls (62, 51 and 69%, respectively)." In regard to mutation spectra in H-ras oncogenes detected $B6C3F_1$ mouse liver "tumors," the authors reported combined results for concurrent and historical controls of 58% AAA, 27% CGA, and 14% CTA substitutions for CAA at codon 61 out of 58 mutations. For TCE "tumors" the substitution pattern was reported to be 29% AAA,

24% CGA, and 40% CTA substitutions for CAA at codon 61 out of 39 mutations and for DCA 28% AAA, 35% CGA, and 38% CTA substitutions for CAA at codon 61 out of 40 mutations.

E.2.2.22. Bull et al. (2002)

This study primarily presented results from exposures to TCE, DCA, TCA, and combinations of DCA and TCA after 52 weeks of exposure with some animals examined at 87 weeks. It only examined and described results for liver. In a third experiment, 1,000 mg/kg TCE was administered once daily 7 days/week for 79 weeks in 5% alkamuls in distilled water to 40 $B6C3F_1$ male mice (6 weeks old at the beginning of the experiment). At the time of euthanasia, the livers were removed, tumors were identified, and the tissues section was examined by a pathologist and immunostaining. Liver weights were not reported. For the TCE gavage experiment, there were 6 gavage-associated deaths during the course of this experiment among a total of 10 animals that died with TCE treatment. No animals were lost in the control group.

The limitations of this experiment were discussed in Caldwell et al. (2008b). Specifically, for the DCA- and TCA-exposed animals, the experiment was limited by low statistical power, a relatively short duration of exposure, and uncertainty in reports of lesion prevalence and multiplicity due to inappropriate lesions grouping (i.e., grouping of hyperplastic nodules, adenomas, and carcinomas together as "tumors"), and incomplete histopathology determinations (i.e., random selection of gross lesions for histopathology examination).

For the TCE results, Bull et al. (2002) reported a high prevalence (23/36 $B6C3F_1$ male mice) of adenomas and HCC (7/36) and gave results of an examination of approximately half of the lesions induced by TCE exposure. Tumor incidence data were provided for only 15 control mice and reported as 2/15 (13%) having adenomas and 1/15 (7%) carcinomas. Thus, this study presents results that are consistent with other studies of chronic exposure that show TCE induction of HCC in male $B6C3F_1$ mice.

For determinations of immunoreactivity to c-Jun as a marker of differences in "tumor" phenotype, Bull et al. (2002) did include all lesions in most of their treatment groups, decreasing the uncertainty of his findings. The exceptions were the absence of control lesions and inclusion of only 16/27 and 38/72 lesions for 0.5 g/L DCA + 0.05 g/L TCA and 1 g/kg-day TCE exposure groups, respectively. Immunoreactivity results were reported for the group of hyperplastic nodules, adenomas, and carcinomas. Thus, changes in c-Jun expression between the differing types of lesions were not determined.

Bull et al. (2002) reported lesion reactivity to c-Jun antibody to be dependent on the proportion of the DCA and TCA administered after 52 weeks of exposure. Given alone, DCA produced lesions in mouse liver for which approximately half displayed a diffuse immunoreactivity to a c-Jun antibody, half did not, and none exhibited a mixture of the two. After TCA exposure alone, no lesions were reported to be stained with this antibody. When

given in various combinations, DCA and TCA co-exposure induced a few lesions that were only c-Jun+, many that were only c-Jun–, and a number with a mixed phenotype whose frequency increased with the dose of DCA. For TCE exposure of 79 weeks, TCE-induced lesions also had a mixture of phenotypes (42% c-Jun+, 34% c-Jun–, and 24% mixed) and were most consistent with those resulting from DCA and TCA co-exposure but not either metabolite alone.

Mutation frequency spectra for the H-ras codon 61 in mouse liver "tumors" induced by TCE (n = 37 tumors examined) were reported to be significantly different than that for TCA (n = 41 tumors examined), with DCA-treated mice tumors giving an intermediate result (n = 64 tumors examined). In this experiment, TCA-induced "tumors" were reported to have more mutations in codon 61(44%) than those from TCE (21%) and DCA (33%). This frequency of mutation in the H-ras codon 61 for TCA is the opposite pattern as that observed for a number of peroxisome proliferators in which the mutation spectra in tumors has been reported to be much lower than spontaneously arising tumors (see Section E.3.4.1.5).

Bull et al. (2002) noted that the mutation frequency for all TCE-, TCA-, or DCA-induced tumors was lower in this experiment than for spontaneous tumors reported in other studies (they had too few spontaneous tumors to analyze in this study), but that this study utilized lower doses and was of shorter duration than that of Ferreira-Gonzalez et al. (1995). These are additional concerns along with the effects of inappropriate lesion grouping, in which a lower stage of progression is grouped with more advanced stages. In a limited subset of tumor that were both sequenced and characterized histologically, only 8 of 34 (24%) TCE-induced adenomas but 9/15 (60%) of TCE-induced carcinomas had mutated H-ras at codon 61, which the authors suggest is evidence that this mutation is a late event.

The issues involving identification of mode of action through tumor phenotype analysis are discussed in detail below for the more general case of liver cancer as well as for specific hypothesized modes of action (see Sections E.3.1.4, E.3.1.8, E.3.2.1, and E.3.4.1.5). In an earlier paper, Bull (2000) suggested that "the report by Anna et al. (1994) indicated that TCE-induced tumors possessed a different mutation spectra in codon 61 of the H-ras oncogene than those observed in spontaneous tumors of control mice." Bull (2000) stated that "results of this type have been interpreted as suggesting that a chemical is acting by a mutagenic mechanism" but went on to suggest that it is not possible to a priori rule out a role for selection in this process and that differences in mutation frequency and spectra in this gene provide some insight into the relative contribution of different metabolites to TCE-induced liver tumors. Bull (2000) noted that data from Anna et al. (1994), Ferreira-Gonzalez et al. (1995), and Maronpot et al. (1995a) indicated that mutation frequency in DCA-induced tumors did not differ significantly from that observed in spontaneous tumors, that the mutation spectra found in DCA-induced tumors has a striking similarity to that observed in TCE-induced tumors, and that DCA-induced tumors were significantly different than that of TCA-induced liver tumors.

What is clear from these observations is that the phenotype of TCE-induced tumors appears to be more like DCA-induced tumors (which are consistent with spontaneous tumors), or those resulting from a co-exposure to both DCA and TCA, than from those induced by TCA. More importantly, these data suggest that using measures other than dysplasticity and tincture indicate that mouse liver tumors induced by TCE are heterogeneous in phenotype. The descriptions of tumors in mice reported by the NTP (1990) and Maltoni et al. (1986) studies are also consistent with phenotypic heterogeneity as well as consistency with spontaneous tumor morphology.

E.2.3. Mode of Action: Relative Contribution of TCE Metabolites

Several metabolites of TCE have also been shown to induce liver cancer in rodents with DCA and TCA having been the focus of study as potential active agent(s) of TCE liver toxicity and/or carcinogenesis and both able to induce peroxisome proliferation (Caldwell and Keshava, 2006). A variety of DCA effects from exposure have been noted that are consistent with conditions that increase risk of liver cancer (e.g., effects on the cytosolic enzyme GST-zeta, diabetes, and glycogen storage disease), with the pathological changes induced by DCA on whole liver consistent with changes observed in preneoplastic foci from a variety of agents (Caldwell and Keshava, 2006). CH is one of the first metabolites from oxidative metabolism of TCE with a large fraction of TCE metabolism appearing to go through CH and then subsequent metabolism to TCA and TCOH (Chiu et al., 2006b). Similarities in toxicity may indicate that common downstream metabolites may be toxicologically important, and differences may indicate the importance of other metabolic pathways.

Although both induce liver tumors, DCA and TCA have distinctly different actions (Caldwell and Keshava, 2006) and apparently differ in induced tumor phenotype (see discussions above in Section E.2.2. and many studies have been conducted to try to elucidate the nature of those differences (Caldwell et al., 2008b). Limitations of all of the available chronic studies of TCA and most of the studies of DCA include less-than-lifetime exposures, varying and small numbers of animals examined, and few exposure concentrations that were relatively high.

E.2.3.1. Acute studies of DCA/TCA

The studies in this section focus on studies of DCA and TCA that examine, to the extent possible, similar endpoints using similar experimental designs as those of TCE examined above and that give insight into proposed modes of action for all three. Of note for any experiment involving TCA is whether exposure solutions were neutralized. Unbuffered TCA is commonly used as a reagent to precipitate proteins so that any result from studies using unbuffered TCA could potentially be confounded by the effects on pH.

E.2.3.1.1. **Sanchez and Bull (1990)**

In this report TCA and DCA were administered to male B6C3F$_1$ mice (9 weeks of age) and male and female Swiss-Webster mice (9 weeks of age) for up to 14 days. At 2, 4, or 14 days, mice were injected with tritiated thymidine. Experiments were replicated at least once but results were pooled so that variation between experiments could not be determined. B6C3F$_1$ male mice were given DCA or TCA at 0, 0.3, 1.0, or 2.0 g/L in drinking water (n = 4 for each group for 2 and 5 days, but n = 15 for control and n = 12 for treatment groups at day 14). Swiss-Webster mice (n = 4) at were exposed to DCA only on day 14 at 0, 1.0, or 2.0 g/L. Mice were injected with tritiated thymidine 2 hours prior to sacrifice. The pH of the drinking water was adjusted to 6.8–7.2 with sodium hydroxide. Concentrations of TCA and DCA were reported to be stable for a minimum of 3 weeks.

Hepatocyte diameters were reported to be determined by randomly selecting five different high power fields (400×) in five different sections per animals (total of 25 fields/animal with "cells in and around areas of necrosis, close to the edges of the section, or displaying mitotic figures were not included in the cell diameter measurements." PAS staining was reported to be done for glycogen and lipofuscin determined by autofluorescence. Tritiated thymidine was reported to be given to the animals 2 hours prior to sacrifice. In two of three replications of the 14-day experiment, a portion of the liver was reported to be set aside for DNA extraction with the remaining group examined autoradiographically for tritiated thymidine incorporation into individual hepatocytes. Autoradiographs were also reported to be examined in the highest dose of either DCA or TCA for the 2- and 5-day treatment groups. Autoradiographs were reported to be analyzed in randomly selected fields (5 sections per animal in 10 different fields) for a total of 50 fields/animal and reported as percentage of cells in the fields that were labeled. There was no indication by the authors that they characterized differing zones of the liver for preferential labeling. DNA thymidine incorporation results were not examined in the same animals as those for individual hepatocyte incorporation and also not examined at 2- or 5-day time periods. The only analyses reported for the Swiss-Webster mice were of hepatic weight change and histopathology. Variations in results were reported as SE of the mean.

Liver weights were reported but not body weights, so the relationship of liver/body weight ratio could not be determined for the B6C3F$_1$ mice. For liver weight, the numbers of animals examined varied greatly between and within treatment groups. The number of control animals examined were reported to be n = 4 on day 2, n = 8 on day 5, and n = 15 on day 14. There was also a large variation between control groups in regard to liver weight. Control liver weights for day 2 were reported to be 1.3 ± 0.1, day 5 to be 1.5 ± 0.05, and for day 14 to be 1.3 ± 0.04 g. Liver weights in Day 5 control animals were much greater than those for day 2 and day 14 animals and thus, the means varied by as much as 15%.

For DCA, there was no reported change in liver weights compared to controls values at any exposure level of DCA after 2 days of exposure. After 5 days of exposure, there was no

difference in liver weight between controls and 0.3 g/L exposed animals. However, the animals exposed at 1.0 or 2.0 g/L DCA had identical increases in liver weight of 1.7 ± 0.13 and 1.7 ± 0.8 g, respectively. Due to the low power of the experiment, only the 2.0 g/L DCA result was identified by the authors as significantly different from the control value. For TCA, there was a slight decrease reported between control values and the 0.3 g/L treatment group (1.2 ± 0.1 g vs. 1.3 ± 0.1 g), but the 1.0 and 2.0 g/L treatment groups had similar slight increases over control (for 1.0 g/L liver weight was 1.5 ± 0.1 and for 2.0 g/L liver weight was 1.4 ± 0.1 g). The same pattern was apparent for the 5-day treatment groups for TCA as for the 2-day treatment groups.

For 14-day exposure periods, the number of animals studied was increased to12 for the TCA and DCA treatment groups. After 14 days of DCA treatment, there was a reported dose-related increase in liver weight that was statistically significant at the two highest doses (i.e., at 0.3 g/L DCA liver weight was 1.4 ± 0.04, at 1.0 g/L DCA liver weight was 1.7 ± 0.07 g, and at 2.0 g/L DCA liver weight was 2.1 ± 0.08 g). This was 1.08-, 1.31-, and 1.62-fold of controls, respectively. After 14 days of TCA exposure, there was a dose-related increase in liver weight that the authors reported to be statistically significant at all exposure levels (i.e., at 0.3 g/L liver weight was 1.5 ± 0.06, at 1.0 g/L liver weight was 1.6 ± 0.07 g, and at 2.0 g/L liver weight was 1.8 ± 0.10 g). This represents 1.15-, 1.23-, and 1.38-fold of control.

The authors note that at 14 days, that DCA-associated increases in hepatic liver weight were greater than that of TCA. What is apparent from these data are that while the magnitude of difference between the exposures was ~6.7-fold between the lowest and highest dose, the differences between TCA exposure groups for change in liver weight was ~2.5. For DCA, the slope of the dose-response curve for liver weight increases appeared to be closer to the magnitude of difference in exposure concentrations between the groups (i.e., a difference of 7.7-fold between the highest and lowest dose for liver weight induction). Given that the control animal weights varied as much as 15%, the small number of animals examined, and that body weights were also not reported, there are limitations for making quantitative comparisons between TCA and DCA treatments. However, after 14 days of treatment, it is apparent that there was a dose-related increase in liver weight after either DCA or TCA exposure at these exposure levels. For male and female Swiss-Webster mice, 1 and 2 g/L DCA treatment (n = 4) was reported to also induce an increase in percent liver/body weight that was similar to the magnitude of exposure difference (see below).

Grossly, livers of $B6C3F_1$ mice treated with DCA for 1 or 2 g/L were reported to have "pale streaks running on the surface" and occasionally, discrete, white, round areas were also observed on the surface of these livers. Such areas were not observed in TCA-treated or control $B6C3F_1$ mice. Pale streaks on the surface of the liver were not observed in Swiss-Webster mice. Again there was no significant effect on total body or renal weights (data not shown).

Swiss-Webster mice were reported to have dose-related increases in hepatic weight and hepatic/body weight ratios were observed. DCA-associated increases in relative hepatic weights

in both sexes were comparable to those in $B6C3F_1$ mice. The authors report liver weights for the Swiss-Webster male mice (n = 4 for each group) to be 2.1 ± 0.1 g for controls, 2.1 ± 0.1 g for 1.0 g/L DCA, and 2.4 ± 0.2 g for 2.0 g/L DCA 14-day treatment groups. The percent liver/body weights for these same groups were reported to be 6.4 ± 0.4, 6.9 ± 0.2, and 8.1 ± 0.3%, respectively. For female Swiss-Webster mice (n = 4 for each group), the liver weights were reported to be 1.1 ± 0.1 g for controls, 1.5 ± 0.1 g for 1.0 g/L DCA, and 1.7 ± 0.2 g for 2.0 g/L DCA 14-day treatment groups. The percent liver/body weights for these same groups of Swiss mice were reported to be 4.8 ± 0.2, 6.0 ± 0.2, and 6.8 ± 0.4%, respectively.

Thus, while there was no significant difference in "liver weight" between the control and the 1.0 g/L DCA treatment group for male or female Swiss-Webster mice, there was a statistically significant difference in liver/body weight ratio reported by the authors. These data illustrate the importance of reporting both measures and the limitations of using small numbers of animals (n = 4 for the Swiss Webster vs. n = 12–14 for $B6C3F_1$ 14-day experiments).

Relative liver weights were reported by the authors for male $B6C3F_1$ mice only for the 14-day groups, as a function of calculated mean water consumption, as pooled data from the three experiments, and as a figure that was not comparable to the data reported for Swiss-Webster mice. The liver weight data indicate that male mice of the same age appeared to differ in liver weight between the two strains without treatment (i.e., male $B6C3F_1$ mice had control liver weights at 14 days of 1.3 ± 0.04 g for 15 mice, while Swiss-Webster mice had control values of 2.1 ± 0.1 for 4 mice). While the authors report that results were "comparable" between the $B6C3F_1$ mice in regard to DCA-induced changes in liver weight, the increase in percent liver/body weight ratios were 1.27-fold of control for Swiss-Webster male mice (n = 4) and 1.42-fold of control for females while the increase in liver weight for $B6C3F_1$ male mice (n = 12–14) was 1.62-fold of controls after 14 days of exposure to 2 g/L DCA.

The concentration of DNA in the liver was reported as mg hepatic DNA/g of liver. This measurement can be associated with hepatocellular hypertrophy when decreased, or increased cellularity (of any cell type), increased DNA synthesis, and/or increased hepatocellular ploidy in the liver when increased. The number of animals examined for this parameter varied. For control animals, there were four animals reported to be examined at 2 days, eight animals examined at 5 days, and at 14 days eight animals were examined.

The mean DNA content in control livers were not reported to vary greatly, however, and the variation between animals was relatively low in the 5- and 14-day control groups (i.e., 1.67 ± 0.27, 1.70 ± 0.05, and 1.69 mg DNA/g, for 2-, 5-, or 14-day control animals, respectively). For treatment groups, the number of animals reported to be examined appeared to be the same as the control animals.

For DCA treatment, there did not appear to be a dose-response in hepatic DNA content with the 1 g/L exposure level having the same reported value as control but the 0.3 and 2.0 g/L values reported to be lower (mean values of 1.49 and 1.32 mg DNA/g, respectively). After

5 days of exposure, all treatment groups were reported to have a lower DNA content that the control value (i.e., 1.44 ± 0.06, 1.47 ± , and 1.30 ± 0.14 mg DNA/g, for 0.3, 1.0, and 2.0 g/L exposure levels of DCA, respectively). After 14 days of exposure, there was a reported increase in hepatic DNA at the 0.3 g/L exposure level, but significant decreases at the 1.0 and 2.0 g/L exposure levels (i.e., 1.94 ± 0.20, 1.44 ± 0.14, and 1.19 ± 0.16 mg DNA/g for the 0.3, 1.0, and 2.0 g/L exposure levels of DCA, respectively).

Changes in DNA concentration in the liver were not correlated with the pattern of liver weight increases after DCA treatment. For example, while there was a clear dose-related increase in liver weight after 14 days of DCA treatment, the 0.3 g/L DCA exposed group was reported to have a higher rather than lower level of hepatic DNA than controls. After 2 or 5 days of DCA treatment, liver weights were reported to be the same between the 1.0 and 2.0 g/L treatment groups but hepatic DNA was reported to be decreased.

For TCA, there appeared to be a dose-related decrease in reported hepatic DNA after 2 days of treatment (i.e., 1.63 ± 0.07, 1.53 ± 0.08, and 1.43 ± 0.04 mg DNA/g for the 0.3, 1.0, and 2.0 g/L exposure levels of TCA, respectively). After 5 days of TCA exposure, there was a reported decrease in hepatic DNA for all treatment groups that was similar at the 1.0 and 2.0 g/L exposure groups (i.e., 1.45 ± 0.17, 1.29 ± 0.18, and 1.26 ± 0.22 mg DNA/g for the 0.3, 1.0, and 2.0 g/L exposure levels of TCA, respectively). After 14 days of TCA treatment, there was a reported decrease in all treatment groups in hepatic DNA content that did not appear to be dose-related (i.e., 1.31 ± 0.17, 1.21 ± 0.17, and 1.33 ± 0.18 mg DNA/g for the 0.3, 1.0, and 2.0 g/L exposure levels of TCA, respectively).

Thus, similar to the results reported for DCA, the patterns of liver weight gain did not match those of hepatic DNA decrease for TCA-treated animals. For example, although there appeared to be a dose-related increase in liver weight gain after 14 days of TCA exposure, there was a treatment- but not dose-related decrease in hepatic DNA content.

In regard to the ability to detect changes, the low number of animals examined after 2 days of exposure (n = 4) limited the ability to detect a significant change in liver weight and hepatic DNA concentration. For hepatic DNA determinations, the larger number of animals examined at 5- and 14-day time points and the similarity of values with relatively smaller SE of the mean reported in the control animals made quantitative differences in this parameter easier to determine. However, animals varied in their response to treatment and this variability exceeded that of the control groups. For DCA, results reported at 14 days and those for TCA reported at 5 and 14 days, the SEs for treated animals showed a much greater variability than those of the control animals (range of 0.04–0.05 mg DNA/g for control groups, but ranges of 0.17–0.22 mg DNA/g for TCA at 5 days and 0.14–0.20 mg DNA/g for DCA or TCA at 14 days). The authors stated that:

> the increases in hepatic weights were generally accompanied by decreases in the concentration of DNA. However, the only clear changes were in animals treated with DCA for 5 or 14 days where the ANOVAs were clearly significant (P<0.020 and 0.005, respectively). While changes of similar magnitude were observed in other groups, the much greater variation observed in the treated groups resulted in not significant differences by ANOVA (p = 0.41, 0.66. 0.26, 0.15 for DCA – 2 days, and TCA for 2,5, and 14 days, respectively).

The size of hepatocytes is heterogeneous and correlated with its ploidy, zone, and age of the animal (see Section E.1.1). The authors did not indicate if there was predominance in zone or ploidy for hepatocytes included in their analysis of average hepatocyte diameter in the random selection of 25 fields per animal (n = 3–7 animals). There appeared to be a dose-related increase in cell diameter associated with DCA exposure and a treatment but not dose-related increase with TCA treatment after 14 days of treatment. For control B6C3F_1 male mice (n = 7), the hepatocyte diameter was reported to be 20.6 ± 0.4 microns. For mice exposed to DCA, hepatocyte diameter was reported to be 22.2 ± 0.2, 25.2 ± 0.6, and 26.0 ± 1.0 microns for 0.3, 1.0, and 2.0 g/L treated mice (n = 4 for each group), respectively. For mice exposed to TCA hepatocyte diameter was reported to be 22.2 ± 0.2, 22.4 ± 0.6, and 23.2 ± 0.4 microns for 0.3, 1.0, and 2.0 g/L treated mice (n = 4 for the 0.3 and 1.0 g/L groups and n = 3 for the 2.0 g/L group), respectively.

The small number of animals examined limited the power of the experiment to determine statistically significant differences with the authors reporting that only the 1.0 g/L DCA and 2.0 g/L DCA- and TCA-treated groups statistically significant from control values. The dose-related increases in reported cell diameter were consistent with the dose-related increases in liver weight reported for DCA after 14 days of exposure. However, the pattern for hepatic DNA content did not. For TCA, the dose-related increases in cell diameter were also consistent with the dose-related increases in liver weight after 14 days of exposure. Similar to DCA results, the changes in hepatic DNA content did not correlate with changes in cell size. In regard to the magnitude of increases over control values, the 68 vs. 38% increase in liver weight for DCA vs. TCA at 2.0 g/L, was less than the 26 and 13% increases in cell diameter for the same groups, respectively. Therefore, for both DCA and TCA exposure, there appeared to be dose-related hepatomegaly and increased cell size after 14-days of exposure.

The authors reported PAS staining for glycogen content as an attempt to examine the nature of increased cell size by DCA and TCA. However, they did not present any quantitative data and only provided a brief discussion. The authors reported that:

> hepatic sections of DCA-treated B6C3F_1 mice (1 and 2 g/L) contained very large amounts of perilobular PAS-positive material within hepatocytes. PAS stained hepatic sections from animals receiving the highest concentration of TCA displayed a much less intense staining that was confined to periportal areas. Amylase digesting confirmed the majority of the PAS-positive material to by glycogen. Thus, increased hepatocellular size in groups receiving DCA appears

to be related to increased glycogen deposition. Similar increases in glycogen deposition were observed in Swiss-Webster mice.

There is no way to discern whether DCA-induced glycogen deposition was dose-related and therefore correlated with increased liver weight and cell diameter. While the authors suggest that Swiss-Webster mice displayed "similar increased in glycogen deposition," the authors did not report a similar increase in liver weight gain after DCA exposure at 14 days (1.27-fold of control percent liver/body weight ratio in Swiss male mice and 1.42-fold in female Swiss-Webster mice vs. 1.62-fold of control in $B6C3F_1$ mice after 14 days of exposure to 2 g/L DCA). Thus, the contribution of glycogen deposition to DCA-induced hepatomegaly and the nature of increased cell size induced by acute TCA exposure cannot be determined by this study. However, this study does show that DCA and TCA differ in respect to their effects on glycogen deposition after short-term exposure.

The authors report that:

> localized areas of coagulative necrosis were observed histologically in both $B6C3F_1$ and Swiss-Webster mice treated with DCA at concentrations of 1 and 2 g/L for 14 days. The necrotic areas corresponded to the pale streaked areas seen grossly. These areas varied in size, shape and location within sections and occupied up to several mm^2. An acute inflammatory response characterized by thin rims of neutrophils was associated with the necrosis, along with multiple mitotic figures. No such areas of necrosis were observed in animals treated at lower concentrations of DCA, or in animals receiving the chemical for 2 or 5 days. Mice treated with 2 g/L TCA for 14 days have some necrotic areas, but at such low frequency that it was not possible to determine if it was treatment-related (2 lesions in a total of 20 sections examined). No necrosis was observed in animals treated at the lower concentrations of TCA or at earlier time points.

Again there were no quantitative estimates given of the size of necrotic areas, variation between animals, variation between strain, or dose-response of necrosis reported for DCA exposure by the authors. The lack of necrosis after 2 and 5 days of exposure at all treatment levels and at the lower exposure level at 14 days of exposure is not correlated with the increases in liver weight reported for these treatment groups.

Autoradiographs of randomly chosen high powered fields (400×) (50 fields/animal) were reported as the percentage of cells in the fields that were labeled. There was significant variation in the number of animals examined and in the reported mean percent of labeled cells between control groups. The number of control animals was not given for the 2-day group but for the 5- and 14-day groups were reported to be n = 4 and n = 11, respectively. The mean percent of labeling in control animals was reported at 0.11 ± 0.03, 0.12 ± 0.04, and 0.46 ± 0.07% of hepatocytes for 2-, 5-, and 14-day control groups, respectively. Only the 2.0 g/L exposures of

DCA and TCA were examined at all three times of exposure, while all groups were examined at 14 days. However, the number of animals examined in all treatment groups appeared to be only four animals in each group.

There was not an increase over controls reported in the 2.0 g/L DCA or TCA 2- and 5-day exposure groups in hepatocyte labeling with tritiated thymidine. After 14 days of exposure, there was a statistically significant but very small dose-related increase over the control value after DCA exposure (i.e., 0.46 ± 0.07, 0.64 ± 0.15, 0.75 ± 0.22, and 0.94 ± 0.05% labeling of hepatocytes in control, 0.3, 1.0, and 2.0 g/L DCA treatment groups, respectively). For TCA, there was no change in hepatocyte labeling except for a 50% decrease from control values at after 14 days of exposure to 2.0 g/L TCA (i.e., 0.46 ± 0.07, 0.50 ± 0.14, 0.52 ± 0.26, and 0.26 ± 0.14% labeling of hepatocytes in control, 0.3, 1.0, and 2.0 g/L TCA treatment groups, respectively). The authors report that:

> labeled cells were localized around necrotic areas in these [sic DCA treated] groups. Since counts were made randomly, the local increased in DCA-treated animals at concentrations of 1 and 2 g/L are in fact much higher than indicated by the data. Labeling indices in these areas of proliferation were as high as 30%. Labeled hepatocytes in TCA-treated and the control animals were distributed uniformly throughout the sections. There was an apparent decrease in the percentage of labeled cells in the group of animals treated with the highest dose of TCA. This is because no labeled cells were found in any of the fields examined for one animal.

The data for control mice in this experiment are consistent with others showing that the liver is quiescent in regard to hepatocellular proliferation with few cells undergoing mitosis (see Section E.1.1). For up to 14 days of exposure with either DCA or TCA, there was little increase in hepatocellular proliferation except in instances and in close proximity to areas of proliferation. The increases in liver weight reported for this study were not correlated with and cannot be a result of hepatocellular proliferation as only a very small population of hepatocytes is undergoing DNA synthesis. For TCA, there was no increase in DNA synthesis in hepatocytes, even at the highest dose, as shown by autoradiographic data of tritiated thymidine incorporation in random fields.

Whole-liver sections were examined for tritiated thymidine incorporation from DNA extracts. The number of animals examined varied (i.e., n = 4 for the 2-day exposure groups and n = 8 for 5- and 14-day exposure groups), but the number of control animals examined was the same as the treated groups for this analysis. The levels of tritiated thymidine incorporation in hepatic DNA (dpm/mg DNA expressed as mean × 10^3 ± SE of the number of animals) were reported to be similar across control groups (i.e., 56 ± 11, 56 ± 6, and 56 ± 7 dpm/mg DNA, for 2-, 5-, and 14-day treatment groups, respectively).

After 2 days of DCA exposure, there appeared to be a slight treatment-related, but not dose-related, increase in reported tritiated thymidine incorporation into hepatic DNA (i.e., 72 ± 23, 80 ± 6, and 68 ± 7 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L DCA, respectively). After 5 days of DCA exposure, there appeared to be a dose-related increase in reported tritiated thymidine incorporation into hepatic DNA (i.e., 68 ± 18, 110 ± 20, and 130 ± 7 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L DCA, respectively). However, after 14 days of DCA exposure, levels of tritiated thymidine incorporation were less than those reported at 5 days and the level for the 0.3 g/L exposure group was less than the control value (i.e., 33 ± 11, 77 ± 9, and 81 ± 12 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L DCA, respectively).

After 2 days of TCA exposure, there did not appear to be a treatment-related increase in tritiated thymidine incorporation into hepatic DNA (i.e., 82 ± 16, 52 ± 7, and 54 ± 7 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L TCA, respectively). Similar to the reported results for DCA, after 5 days of TCA exposure, there appeared to be a dose-related increase in reported tritiated thymidine incorporation into hepatic DNA (i.e., 79 ± 23, 86 ± 17, and 158 ± 33 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L TCA, respectively). After 14 days of TCA exposure, there were treatment-related increases, but not a dose-related increase, in reported tritiated thymidine incorporation into hepatic DNA (i.e., 71 ± 10, 73 ± 14, and 103 ± 14 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L TCA, respectively).

It would appear that for both TCA and DCA, the increase in tritiated thymidine incorporation into hepatic DNA was dose related and peaked after 5 days of exposure. The authors report that the decrease in incorporation into hepatic DNA observed after 14 days of DCA treatment at 0.3 g/L to be statistically significant as well as the increases after 5 and 14 days of TCA exposure at the 2.0 g/L level. The small numbers of animals examined, the varying number of animals examined, and the degree of variation in treatment-related effects limit the statistical power of this experiment to detect quantitative changes.

Given the limitations of this experiment, determination of an accurate measure of the quantitative differences in tritiated thymidine incorporation into whole-liver DNA or that observed in hepatocytes are hard to determine. In general, the results for tritiated thymidine incorporation into hepatic DNA were consistent with those for tritiated thymidine incorporation into hepatocytes in that they show that there were, at most, a small population of hepatocytes undergoing DNA synthesis after up to 14 days of exposure at relative high levels of exposure to DCA and TCA (i.e., the largest percentage of hepatocytes undergoing DNA synthesis for any treatment group was <1% of hepatocytes). The highest increases over control levels for hepatic DNA incorporation for the whole liver were reported at the highest exposure level of TCA treatment after 5 days of treatment (threefold of control) and after 14 days of TCA treatment (twofold of control).

Although the authors report small areas of focal necrosis with concurrent localized increases in hepatocyte proliferation in DCA-treated animals exposed to1.0 g/L and 2.0 g/L

DCA, the levels of whole-liver tritiated thymidine incorporation were only slightly elevated over controls at these concentrations, and were decreased at the 0.3 g/L exposure concentration for which no focal necrosis was reported. The whole-liver DNA incorporation of tritiated thymidine was not consistent with the pattern of tritiated thymidine incorporation observed in individual hepatocytes. The authors state that "at present, the mechanisms for increased tritiated thymidine uptake in the absence of increased rates of cell replication with increasing doses of TCA cannot be determined." The authors do not discuss the possibility that the difference in hepatocyte labeling and whole-liver DNA tritiated thymidine incorporation could have been due to the labeling representing increased polyploidization rather than cell proliferation, as well as increased numbers of proliferating nonparenchymal and inflammatory cells. The increased cell size due from TCA exposure without concurrent increased glycogen deposition could have been indicative of increased polyploidization. Finally, although both TCA- and DCA-induced increases in liver weight were generally consistent with cell size increases, they were not correlated with patterns of change in hepatic DNA content, incorporation of tritiated thymidine in DNA extracts from whole liver, or incorporation of tritiated thymidine in hepatocytes. In regard to cell size, although increased glycogen deposition with DCA exposure was noted by the authors of this study, lack of quantitative analyses of that accumulation precludes comparison with DCA-induced liver weight gain.

E.2.3.1.2. Nelson et al. (1989) and Nelson and Bull (1988)

Nelson and Bull (1988) administered TCE (0, 3.9, 11.4, 22.9, and 30.4 mmol/kg) in Tween 80® via gavage to male Sprague-Dawley rats and male B6C3F$_1$ mice, sacrificed them 4 hours after treatment (n = 4–7), and measured the rate of DNA unwinding under alkaline conditions. They assumed that this assay represented increases in SSBs. For rats, there was little change from controls up to 11.4 mmol/kg (1.5 g/kg TCE) but a significantly increased rate of unwinding at 22.9 and 30.4 mmol/kg TCE (approximately twofold greater at 30.4 mmol). For mice, there was a significantly increased level of DNA unwinding at 11.4 and 22.9 mmol. Concentrations >22.9 mmol/kg were reported to be lethal to the mice. In this same study, TCE metabolites were administered in unbuffered solution using the same assay. DCA was reported to be most potent in this assay with TCA being the lowest, while CH closely approximated the dose-response curve of TCE in the rat. In the mouse, the most potent metabolite in the assay was reported to be TCA followed by DCA with CH considerably less potent.

The focus of the Nelson et al. (1989) study was to examine whether reported SSBs in hepatic DNA induced by DCA and TCA (Nelson and Bull, 1988) were secondary to peroxisome proliferation also reported to be induced by both. Male B6C3F$_1$ mice (25–30 g but no age reported) were given DCA (10 mg/kg or 500 mg/kg) or TCA (500 mg/kg) via gavage in 1% aqueous Tween 80® with no pH adjustment. The animals were reported to be sacrificed 1, 2, 4, or 8 hours after administration, and livers were examined for SSBs as a whole-liver homogenate.

In a separate experiment (experiment #2), treatment was parallel to the first (500 mg/kg treatment of DCA or TCA), but levels of PCO activity were measured as an indication of peroxisome proliferation and expressed as μmol/minute/g liver. In a separate experiment (experiment #3), mice were administered 500 mg/kg DCA or TCA for 10 days with Clofibrate administered at a dose of 250 mg/kg as a positive control. Twenty-four hours after the last dose, animals were killed, and liver was examined by light microscopy and PCO activity. Finally, in an experiment parallel in design to experiment #3, SSBs were measured in total hepatic DNA after 500 mg/kg exposure to TCA (experiment #4). Electron microscopy was performed on two animals/group for vehicle, DCA, or TCA treatment, with six randomly chosen micrographic fields utilized for peroxisome profiles. These micrographs were analyzed without identification as to what area of the liver lobules they were being taken from. Hence, there is a question as to whether the areas that are known to be peroxisome rich were assayed or not.

The data from all control groups were reported as pooled data in figures, but statistical comparisons were made between concurrent control and treated groups. The results for DNA SSBs were reported for "13 control animals" and each experimental time point "as at least 6 animals."

DNA strand breaks were reported to be significantly increased over concurrent control by a single exposure to 10 or 500 mg/kg DCA or 500 mg/kg TCA for 1, 2, or 4 hours after administration but not at 8 or 24 hours. There did not appear to be a difference in the magnitude of response between the three treatments (the fraction of unwound DNA was ~2.5 times that of control). PCO activity was reported to be not increased over control within 24 hours of either DCA or TCA treatment (n = 6 animals per group). The fraction of alkaline unwinding rates as an indicator of SSBs were reported to not be significantly different from controls and TCA-treated animals after 10 days of exposure (n = 5).

Relative to controls, body weights were reported to not be affected by exposures to DCA or TCA for 10 days at 500 mg/kg (data were not shown.) (n = 6 per group). However, both DCA and TCA were reported to significantly increase liver weight and liver/body weight ratios (i.e., liver weights were 1.3 ± 0.05, 2.1 ± 0.10, and 1.7 ± 0.09 g for control, 500 mg/kg DCA, and 500 mg/kg TCA treatment groups, respectively while percent liver/body weights were 4.9 ± 0.14, 7.5 ± 0.18, and 5.7 ± 0.14% for control, 500 mg/kg DCA, and 500 mg/kg TCA treatment groups, respectively).

PCO activity (μmol/minute/g liver) was reported to be significantly increased by DCA (500 mg/kg), TCA (500 mg/kg), and Clofibrate (250 mg/kg) treatment (i.e., levels of oxidation were 0.63 ± 0.07, 1.03 ± 0.09, 1.70 ± 0.08, and 3.26 ± 0.05 for control, 500 mg/kg DCA, 500 mg/kg TCA, and 250 mg/kg Clofibrate treatment groups, respectively). Thus, the increases were ~1.63-, 2.7-, and 5-fold of control for DCA, TCA, and Clofibrate treatments.

Results from randomly selected electron photomicrographs from two animals (six per animal) were reported for DCA and TCA treatment and to show an increase in peroxisomes per

unit area that was reported to be statistically significant (i.e., 9.8 ± 1.2, 25.4 ± 2.9, and 23.6 ± 1.8 for control, 500 mg/kg DCA, and 500 mg/kg TCA, respectively). The 2.5- and 2.4-fold of control values for DCA and TCA gave a different pattern than that of PCO activity. The small number of animals examined limited the power of the experiment to quantitatively determine the magnitude of peroxisome proliferation via electron microscopy. The enzyme analyses suggested that both DCA and TCA were weaker inducers of peroxisome proliferation than Clofibrate.

The authors reported that there was no evidence of gross hepatotoxicity in vehicle or TCA-treated mice. Light microscopic sections from mice exposed to TCA or DCA for 10 days were stained with H&E and PAS for glycogen. For TCA treatment, PAS staining "produced approximately the same intensity of staining and amylase digesting revealed that the vast majority of PAS-positive staining was glycogen." Hepatocytes were reported to be "slightly larger in TCA-treated mice than hepatocytes from control animals throughout the liver section with the architecture and tissue pattern of the liver intact." The histopathology after DCA treatment was reported to be "markedly different than that observed with either vehicle or TCA treatments" with the "most pronounced change in the size of hepatocytes." DCA was reported to:

> produce marked cellular hypertrophy uniformly throughout the liver. The hepatocytes were approximately 1.4 times larger in diameter than control liver cells. This hypertrophy was accompanied by an increase in PAS staining; indicating greater glycogen deposition than in TCA-treated and control liver tissue. Multiple white streaks were grossly visible on the surface of the liver of DCA-treated mice. The white areas corresponded with subcapsular foci of coagulative necrosis. These localized necrotic areas were not encapsulated and varied in size. The largest necrotic foci occupied the area of a single lobule. These necrotic areas showed a change in staining characteristics. Often this change consisted of increased eosinophilia. A slight inflammatory response, characterized by neutrophil infiltration, was present. These changed were evident in all DCA-treated mice.

The results from this experiment cannot inform as to dose-response relationships for the parameters tested with the exception of DNA SSBs where two concentrations of DCA were examined (10 and 500 mg/kg). For this parameter, the 10 mg/kg exposure of DCA was as effective as the 500 mg/kg dose where toxicity was observed. This effect on DNA was observed before evidence of induction of peroxisome proliferation. The authors did not examine Clofibrate for effects on DNA so whether it too, would have produced this effect is unclear. The results from this study are consistent with those of Sanchez and Bull (1990) for induction of hepatomegaly by DCA and TCA, the lack of hepatotoxicity at this dose by TCA, and the difference in glycogen deposition between DCA and TCA.

E.2.3.1.3. **Styles et al. (1991)**

In this report, a similar paradigm is used as Nelson et al. (1989) for the intention of repeating that work on SSBs and to study DNA synthesis and peroxisome proliferation. In regard to the findings of SSBs, Styles et al. (1991) reported for a similar paradigm of 500 mg/kg neutralized TCA administered to male $B6C3F_1$ mice (7–8 weeks of age) and examined at 1, 4, 8, and 24 hours after dosing. They reported no increased unwinding of DNA 1 or 24 hours after TCA administration. In a separate experiment, tritiated thymidine was administered to mice 1 hour before sacrifice at 24, 36, 48, 72, and 96 hours after the first dose of 500 mg/kg TCA for 3 days via gavage (n = 5 animals per group).

The hepatic DNA uptake of tritiated thymidine was reported to be similar to control levels up to 36 hours after the first dose and then to increase to a level approximately sixfold greater than controls by 72 hours after the first dose of TCA. By 96 hours, the level of tritiated thymidine incorporation had fallen to approximately fourfold greater than controls. The variation, reported by SD, was very large in treated animals (e.g., SD was equal to approximately ±1.3-fold of control for 48 hour time point). Individual hepatocytes were examined with the number of labeled hepatocytes/1,000 cells reported for each animal.

The control level was reported to be ~1 with a SD of similar magnitude. The number of labeled hepatocytes was reported to decrease between 24 and 36 hours and then to rise slowly back to control levels at 48 hours and then to be significantly increased 72 hours after the first dose of TCA (~9 cells/1,000 with a SD of 3.5) and then to decrease to a level of ~5 cells/1,000. Thus, it appears that increases in hepatic DNA tritiated thymidine uptake preceded those of increased labeled hepatocytes and did not capture the decrease in hepatocyte labeling at 36 hours. By either measure, the population of cells undergoing DNA synthesis was small, with the peak level being <1% of the hepatocyte population.

The authors go on to report the zonal distribution of mean number of hepatocytes incorporating tritiated thymidine but no variations between animals were reported. The decrease in hepatocyte labeling at 36 hours was apparent at all zones. By 48 hours, there appeared to be slightly more periportal than midzonal cells undergoing DNA synthesis with centrilobular cells still below control levels. By 72 hours, all zones of the liver were reported to have a similar number of labeled cells. By 96 hours, the midzonal and centrilobular regions have returned almost to control levels while the periportal areas were still elevated. These results are consistent with all hepatocytes showing a decrease in DNA synthesis by 36 hours and then a wave of DNA synthesis occurring starting at the periportal zone and progressing through to the pericentral zone until 72 hours and then the midzonal and pericentral hepatocytes completing their DNA synthesis activity.

Peroxisome proliferation was assessed via electron photomicrographs taken in mice (four controls and four treated animals) given 10 daily doses of 500 mg/kg TCA and killed 14 hours after the last dose. No details were given by the authors as to methodology for peroxisome

volume estimate (e.g., how many photos per animals were examined and whether they were randomly chosen). The mean percent cell volume occupied by peroxisome was reported to be 2.1 ± 0.386 and 3.9 ± 0.551% for control and 500 mg/kg TCA, respectively. Given that there were no time points examined before 10 days for peroxisome proliferation, correlations with DNA synthesis activity induced by TCA cannot be made from this experiment. However, it is clear from this study that a wave of DNA synthesis occurs throughout the liver after treatment of TCA at this exposure concentration and that it has peaked by 72 hours even with continuous exposure to 96 hours. Whether the DNA synthesis represents polyploidization or cell proliferation cannot be determined from these data; neither can a dose-response be determined.

E.2.3.1.4. **Carter et al. (1995)**

The aim of this study was to "use correlative biochemical, pathologic and morphometric techniques to characterize and quantify the acute, short-term responses of hepatocytes in the male B6C3F_1 mouse to drinking water containing DCA." This report used tritiated thymidine incorporation, DNA concentration, hepatocyte number per field (cellularity), nuclear size, and binuclearity (polyploidy) parameters to study 0, 0.5, and 5 g/L neutralized DCA exposures up to 30 days. Male B6C3F_1 mice were started on treatment at 28 days of age. Tritiated thymidine was administered by miniosmotic pump 5 days prior to sacrifice.

The experiment was conducted in two phases, which consisted of 5–15 days of treatment (Phase I) and 20–30 days of treatment (Phase II) with five animals per group in groups sacrificed at 5-day intervals. Liver sections were stained for H&E, PAS (for glycogen) or methyl green pyronin stain (for RNA). DNA was extracted from liver homogenates and the amount of tritiated thymidine determined as dpm/μg DNA. Autoradiography was performed with the number of hepatocyte nuclei scored in 1,000 hepatocytes selected randomly to provide a labeling index of "number of labeled cells/1000 X 100%." Changes in cellularity, nuclear size and number of multinucleate cells were quantified in H&E sections at 40 × power. Hepatocyte cellularity was determined by counting the number of nuclei in 50 microscopic fields with multinucleate cells being counted as one cell and nonparenchymal cells not counted. Nuclear size was also measured in 200 nuclei with the mean area plus 2 SD was considered to be the largest possible single nucleus. Therefore, polyploid diploid cells were identified by the authors but not cells that had undergone polyploidy with increased DNA content in a single nucleus.

Mean body weights at the beginning of the experiment varied between 18.7 and 19.6 g in the first three exposure groups of Phase I of the study. Through 15 days of exposure, there did not appear to be a change in body weight in the 0.5 g/L exposure groups but in the 5 g/L exposure group body weight was reduced at 5, 10, and 15 days with that reduction statistically significant at 5 and 15 days. Liver weights did not appear to be increased at day 5 but were increased at days 10 and 15 in both treatment groups (i.e., means ± SEM. for day 10, 1.36 ± 0.03, 1.46 ± 0.03, and 1.59 ± 0.08 g for control, 0.5, and 5 g/L DCA, respectively; and for day 15,

1.51 ± 0.06, 1.72 ± 0.05, and 2.08 ± 0.11 g for control, 0.5, and 5 g/L DCA, respectively). The percent liver/body weight followed a similar pattern with the exception that at day 5, the 5 g/L exposure group had a statistically significant increase over control (i.e., for day 10, 6.00 ± 0.10, 6.72 ± 0.17, and 8.21 ± 0.10% for control, 0.5, and 5 g/L DCA, respectively; and for day 15, 6.22 ± 0.08, 6.99 ± 0.15, and 10.37 ± 0.27% g for control, 0.5, and 5 g/L DCA, respectively).

In Phase II of the study, control body weights were smaller than Phase I and varied between 16.6 and 16.9 g in the first three exposure groups. Liver weights of controls were also smaller making it difficult to quantitatively compare the two groups in terms of absolute liver weights. However, the pattern of DCA-induced increases in liver weight and percent liver/body weight remained. The patterns of body weight reduction only in the 5 g/L treatment groups and increased liver weight with DCA treatment at both concentrations continued from 20 to 30 days of exposure.

For liver weight, there was a slight but statistically significant increase in liver weight for the 0.5 g/L treatment groups over controls (i.e., for day 20, 1.02 ± 0.02, 1.18 ± 0.05, and 1.98 ± 0.05 g for control, 0.5, and 5 g/L DCA, respectively; for day 25, 1.15 ± 0.03, 1.34 ± 0.04, and 2.06 ± 0.12 g for control, 0.5, and 5 g/L DCA, respectively, for day 30, 1.15 ± 0.03, 1.39 ± 0.08, and 1.90 ± 0.12 g for control, 0.5, and 5 g/L DCA, respectively). For percent liver/body weight, there was a small increase at 0.5 g/L that was not statistically significant but all other treatments induced increases in percent liver/body weight that were statistically significant (i.e., for day 20, 4.82 ± 0.07, 5.05 ± 0.09, and 9.71 ± 0.11% for control, 0.5, and 5 g/L DCA, respectively; for day 25, 5.08 ± 0.04, 5.91% ± 0.09, and 10.38 ± 0.58% for control, 0.5, and 5 g/L DCA, respectively; for day 30, 5.17 ± 0.09, 6.01 ± 0.08, and 10.28 ± 0.28% for control, 0.5, and 5 g/L DCA, respectively).

Of note is the dramatic decrease in water consumption in the 5 g/L treatment groups that were consistently reduced by 64% in Phase I and 46% in Phase II. The 0.5 g/L treatment groups had no difference from controls in water consumption at any time in the study. The effects of such water consumption decreases would affect body weight as well as dose received. Given the differences in the size of the animals at the beginning of the study and the concurrent differences in liver weights and percent liver/body weight in control animals between the two phases, the changes in these parameters through time from DCA treatments cannot be accurately determined (e.g., control liver/body weights averaged 6.32% in Phase I but 5.02% in Phase II). However, percent liver/body weight increase were reported to be consistently increased within and between both phases of the study for the 0.5 g/L DCA treatment from 5 to 30 days of treatment (i.e., for Phase I, the average increase was 9.5% and for Phase II, the average increased was 12.5% for 0.5 g/L DCA treated groups). Although increased at 5 days, the nonsignificance of the change may be resultant from the small number of animals examined. The difference in magnitude of dose and percent liver/body weight increase is difficult to determine given that the 5 g/L dose of DCA reduced body weight and significantly reduced water consumption by ~50% in both phases

of the study. Of note is that the differences in DCA-induced percent liver/body weight were ~6-fold for the 15, 25, and 30-day data between the 0.5 and 5 g/L DCA exposures rather than the 10-fold difference in exposure concentration in the drinking water.

The incorporation of tritiated thymidine into total hepatic DNA control treatment groups was reported to be 73.34 ± 11.74 dpm/μg DNA at 5 days, 34 ± 4.12 dpm/μg DNA at 15 days, and 28.48 ± 3.24 dpm/μg DNA at 20 days but was not reported for other treatments. The results for 0.5 g/L treatments were not reported quantitatively but the authors stated that the results "showed similar trends of initial inhibition followed by enhancement of labeling, the changes relative to controls were not statistically significant." For 5 g/L treatment groups, the 5-day treated groups DNA tritiated thymidine incorporation was reported to be 42.8% of controls and followed by a transient increase at 15 and 20 days (i.e., 2.65- and 2.45-fold of controls, respectively) but after 25 and 30 days, was not significantly different from controls (data not shown).

Labeling indices of hepatocytes were reported as means, but variations as either SEM or SD were not reported. Control means were reported as 5.5, 4, 2, 2, 3.2, and 3.5% of randomly selected hepatocytes for 5, 10, 15, 20, 25, and 30 days, respectively, for four to five animals per group. In contrast to the DNA incorporation results, no increase in labeling of hepatocytes was reported to be observed in comparison to controls for any DCA treatment group from 5 to 30 days of DCA exposure. The 5 g/L treatment group showed an immediate decrease in hepatocyte labeling from day 5 onwards that gradually increased approximately half of control levels by day 30 of exposure (i.e., <0.5% labeling index at day 5, ~1% labeling index at day 10, ~0.6% labeling index at day 20, 1% labeling index at day 25, and 2% labeling index at day 30). For the 0.5 g/L treatment, the labeling index was reported to not differ from controls from days 5 though 15, but to be significantly decreased between days 20 and 30 to levels similar to those observed for the 5 g/L exposures. The relatively higher number of hepatocytes incorporating label reported in this study than others can be a reflection of the longer times of exposure to tritiated thymidine. Here, incorporation was shown for 1 weeks worth of exposure and reflects the percent of cell undergoing synthesis during that time period. Also, the higher labeling index in control animals at the 5- and 10-day exposure periods is probably a reflection of the age of the animals at the time of study.

From the data reported by the authors, there was a correlation between the patterns of total DNA incorporation of label and hepatocyte labeling indices in control groups (i.e., higher level of labeling at 5 days than at 15 and 20 days). However, the patterns of decreased thymidine labeling reported for hepatocytes were not correlated with a transient increase in total DNA thymidine incorporation reported with DCA treatment, especially at the 5 g/L exposure level with a large decrease reported for the number of labeled hepatocytes at the same time an increase in total DNA thymidine incorporation was reported.

Although reported to be transiently increased, the total hepatic DNA labeling still represented at most a 2.5-fold increase over control liver, which represents a small population of cells. Given that the study examined hepatocyte labeling in random fields and did not report quantitative zonal differences in proliferation, a more accurate determination of what hepatocytes were undergoing proliferation cannot be made from the labeling index results. Also, although the authors report signs of inflammatory cells for 5-day treatment there is no reference to any inflammatory changes that may have been observed at later time periods when cellular degeneration and loss of nuclei were apparent. Such an increase inflammatory infiltrates can increase the DNA synthesis measurements in the liver. The difference in labeling index and total DNA synthesis could reflect differences in nonparenchymal cell proliferation or ploidy changes vs. mitoses in hepatocytes. Clearly, the increases in liver weight that were reported as early as 5 days of exposure could not have resulted from increased hepatocyte proliferation.

The H&E sections were reported to have been fixed in an aqueous solution that reduced glycogen content. However, residual PAS positive material (assumed to be glycogen) was reported to be present indicating that not all of the glycogen had been dissolved. The authors report changes in pathology between 5 and 30 days in control animals that included straightening of hepatocyte cording, decreased mitoses, less clarity and more fine granularity of pericentral hepatocellular cytoplasm, increased numbers of larger nuclei that were not labeled, and reported differences between animals in the amount of glycogen present (i.e., two or three animals out of the five had less glycogen than other members of the group with less glycogen in the central and midzonal areas). These changes are consistent with increased polyploidization expected for maturing mice (see Sections E.1.1 and E.1.2).

After 5 days of treatment, 0.5 g/L exposed animals were reported to have livers with fewer mitoses and tritiated thymidine hepatocyte labeling, but by 10 days, there was an increase in nuclear size. Labeling was reported to be predominantly in small nuclei. Animals given 0.5 g/L DCA for 15, 20, and 25 days were reported to have “focal cells in the middle zone with less detectable or no cell membranes and loss of the coarse granularity of the cytoplasm” with some cells not having nuclei or cells having a loss of nuclear membrane and apparent karyolysis. “Cells without nuclei because the plane of the section did not pass through the nuclei had the same type of nuclei. Cells without nuclei not related to plane of section had a condensed cytoplasm.” Livers from 20-day and later sacrifice groups treated with 0.5 g/L DCA were reported to have normal architecture. After 25 days of treatment, apoptotic bodies were reported to be observed with fewer nuclei around the central veins nuclei that were larger in central and midzonal areas.

In animals treated with 5 g/L DCA, the authors report similar features as for 0.5 g/L but in a zonal pattern. Inflammatory cells were reported to not be observed, and after 5 and 10 days, a marked decrease in labeled nuclei. After 5 days of 5 g/L DCA, nuclear depletion in the central and mid-zonal areas was reported. In methyl green pyronin-stained slides a marked loss of

cellular membranes was reported at 5 days with a loss of nuclei and formation of "lakes of liver cell debris." After 15 days of treatment, there was a reported increase in labeling in comparison to animals sacrificed after 5 or 10 days. The cells nearest to the triads were reported to have clearing of their cytoplasms and an increase in PAS positivity. Hepatocytes of both 0.5 and 5 g/L DCA treatment groups were reported to have "enlarged, presumably polyploidy nuclei." Some of the nuclei were reported to be "labeled, usually in hepatocytes in the mid-zonal area."

The morphometric analyses of liver sections were reported to reveal statistically significant changes in cellularity, nuclear size (as measured by either nuclear area or mean diameter of the nuclear area equivalent circle), and multinucleated cells during 30 days of exposure to DCA. The authors reported that the concentration of total DNA in the liver, reported as total μg nuclear DNA/g liver, ranged between 278.17 ± 16.88 and 707.00 ± 25.03 in the control groups (i.e., two- to fivefold range). No 0.5 g/L DCA treatment groups differed from their control group in terms of liver DNA concentration. However, for 10–30 days of exposure, hepatic DNA concentrations were reported to be decreased in the 5 g/L treatment groups (at 5 days, there appeared to be ~30% increase over control). The number of cells per field was reported to range between 24.28 ± 1.94 and 43.81 ± 1.93 in control livers (i.e., 1.8-fold range). From 5 to 15 days, the number of cells/field decreased with 0.5 g/L DCA treatment, although only at day 15 was the change statistically significant. From 20 to 30 days of treatment, only the 30-day treatment showed a slight decrease in cells/field and that change was statistically significant. After 5 days of treatment, the number of cells/field was 1.6-fold of control, by 15 days, it was reduced by ~20%, and for 20–30 days, it continued to be reduced by as much as 40%.

Although the authors reported that the changes in cellularity and DNA concentration to be closely correlated, the patterns in the number of cells/field varied in their consistency with those of DNA concentration (i.e., for days 5, 20, and 25 the direction of change with dose was similar between the two parameters but not for days 10, 15, and 30). If changes in liver weight were due to hepatocellular hypertrophy, the increased liver size would be matched by a decrease in liver DNA concentration and by the number of cells/field. The large increases in liver/body weight induced by 5 g/L DCA were matched by decreases in liver DNA concentration except for the 5-day exposure group. In general, the small increases in liver/body weight consistently induced by 0.5 g/L treatment from days 5 through 30 were not correlated with DNA concentrations or cells/field.

The small number of animal examined for these parameters (i.e., n = 4–5) and the highly variable control values limit the power to accurately detect changes. The apparent dehydration in the animals treated at 5 g/L DCA was cited by the authors for the transient increase in cellularity and DNA concentration in the 5-day exposure group. However, drinking water consumption was reported to be similarly reduced at all treatment periods for 5 g/L DCA-treated animals so that all groups would experience the same degree of dehydration.

The percentage of mononucleated cells was reported as percent of mononucleated hepatocytes with results given as means, but with no reports of variation within groups. The mean control values were reported to range between 60 and 75% for Phase I and between 58 and 71% for Phase II of the experiment (n = 4–5 animals per group). The percent of mononucleated hepatocytes was reported to be similar between control and DCA treatment groups at 5- and 10-day exposures. At 15 days, both DCA treatments were reported to give a similar increase in mononucleated hepatocytes (~80 vs. 60% in control) with only the 5 g/L DCA group statistically significant. The increase in mononucleated cells reported for DCA treatment is similar in size to the variation between control values. For Phase II of the study, DCA treatment was reported to increase the number of mononucleated cells in at all concentrations and exposure time periods in comparison to control values. However, only the increases for the 5 g/L treatments at days 20 and 25, and the 0.5 g/L treatment at day 30 were reported to be statistically significant. Again, small numbers of animals limit the ability to accurately determine a change. However, the consistent reporting of an increasing number of mononucleated cells between 15 and 30 days could be associated with clearance of mature hepatocytes as suggested by the report of DCA-induced loss of cell nuclei.

Mean nuclear area was reported to range between 45 and 54 μ^2 in Phase I and between 41 and 48 μ^2 in Phase II of the experiment with no variation in measurements given by the authors. The only statistically significant differences reported between control and treated groups in Phase I was a decrease from 54 to ~42 μ^2 in the 0.5 g/L DCA 10-day treatment group and a small increase from 50 to ~52 μ^2 in the 15-day treatment group. Clearly, the changes reported by the authors as statistically significant did not show a dose-related pattern and were within the range of variation reported between control groups. For Phase II of the experiment, both DCA treatment concentrations were reported to induce a statistically significant increase the nuclear area that was dose-related, with the exception of day 30 in which the nuclear area was similar between the 0.5 and 5 g/L treatment groups. The largest increase in nuclear area was reported at 20 days for the 5 g/L treatment group (~72 vs. 41 μ^2 for control).

The patterns of increases in nuclear area were correlated with those of increased percentage of mononucleated cells in Phase II of the study (20–30 days of treatment) as well as the small changes seen in Phase I of the experiment. An increase in nuclear cell area is consistent with increase polyploidization without mitosis, as cells are induced towards polyploidization. A decrease in the numbers of binucleated cells in favor of mononucleated cells is consistent with clearance of mature binucleated hepatocyte as well induction of further polyploidization of diploid or tetraploid binucleated cell to tetraploid or octoploid mononucleated cells. The authors suggested that the “large hyperchromatic mononucleated hepatocytes are tetraploid” and suggest that such increases in tetraploid cells have also been observed with nongenotoxic carcinogens and with di(2-ethylhexyl) phthalate (DEHP).

In terms of increased cellular granularity observed by the authors with DCA treatment, this result is also consistent with a more differentiated phenotype (Sigal et al., 1999). Thus, these results for DCA are consistent with a DCA-induced change in polyploidization of the cells without cell proliferation.

The pattern of consistent increase in percent liver/body weight induced by 0.5 g/L DCA treatment from days 5 though 30 was not consistent with the increased numbers of mononucleated cells and increase nuclear area reported from day 20 onward. The large differences in liver weight induction between the 0.5 and 5 g/L treatment groups at all times studied also did not correlate with changes in nuclear size and percent of mononucleated cells. Thus, increased liver weight was not a function of cellular proliferation, but probably included both aspects of hypertrophy associated with polyploidization and increased glycogen deposition induced by DCA. The similar changes reported after short-term exposure for both the 0.5 and 5 g/L exposure concentration were suggested by the authors to indicate that the carcinogenic mechanism at both concentrations would be similar. Furthermore, they suggest that although there is evidence of cytotoxicity (e.g., loss of cell membranes and apparent apoptosis), DeAngelo et al. (1999) suggested that the present study does not support that the mechanism of DCA-induced hepatocellular carcinogenesis is one of regenerative hyperplasia following massive cell death nor peroxisome proliferation as the 0.5 g/L exposure concentration has been shown to increase hepatocellular lesions after 100 weeks of treatment without concurrent peroxisome proliferation or cytotoxicity.

E.2.3.1.5. **DeAngelo et al. (1989)**

Various strains of rats and mice were exposed to TCA (12 and 31 mM) or DCA (16 and 39 mM) for 14 days with Sprague-Dawley rats and $B6C3F_1$ mice exposed to an additional concentration of 6 mM TCA and 8 mM DCA. Although noting that in a previous study, with high concentrations of chloracids, there was decreased water consumption, the authors did not measure drinking water consumption in this study.

This study exposed several strains of male rats and mice to TCA at two concentrations in drinking water (12 and 31 mM neutralized TCA) for 14 days. The conversion of mmol/L or mM TCA is 5, 2, and 1 g/L TCA for 31, 12, and 6 mM TCA, respectively. The conversion of mmol/L of mM DCA is 5, 2, and 1 g/L DCA for 39, 16, and 8 mM DCA, respectively. The strains of mice tested were Swiss-Webster, $B6C3F_1$, C57BL/6, and C3H and for rats were Sprague-Dawley, Osborne-Mendel, and F344. For the F344 rat and $B6C3F_1$ mice, data from two separate experiments were reported for each. The number of animals in each group was reported to be six for most experiments with the exception of the Sprague-Dawley rats (n = 3 at the highest dose of TCA and n = 4 or 5 for the control and the lower TCA dose), one study in $B6C3F_1$ mice (n = 4 or 5 for all groups), and one study in F344 rats (n = 4 for all groups).

The body weight of the controls was reported to range from 269 to 341 g in the differing strains of rats (1.27-fold) and 21–28 g in the differing strains of mice (1.33-fold, age not reported). For percent liver/body weight ratios, the range was 4.4–5.6% in control rats (1.27-fold) and 5.1–6.8% in control mice (1.33-fold).

As discussed in other studies, the determination of PCO activity appears to be highly variable. This enzyme activity is often used as a proxy for peroxisome proliferation. For PCO activity, the range of activity in controls was much greater than for either body weight or percent liver/body weight. For rats, there was a 2.8-fold difference in PCO control activity, and in mice, there was a 4.6-fold difference in PCO activity. Between the two studies performed in the same strain of rat (F344), there was a 2.83-fold difference in PCO activity between controls, and for the two studies in the same strain of mouse ($B6C3F_1$) there was a 3.14-fold difference in PCO activity between controls. Not only were there differences between strains and experiments in the same strain, but also differences in control values between species with a wider range of values in the mice. The lowest level of PCO activity in control rats, expressed as nanomoles NAD reduced/minute/mg/protein, was 3.34, and for control mice, was 1.40. The highest level reported in control in rats was 9.46, and for control mice, was 6.40.

These groups of rats and mice were exposed to 2 g/L sodium chloride, or 2 or 5 g/L TCA in drinking water for 14 days and their PCO activity was assayed. These doses of TCA did not affect body weight except for the Sprague-Dawley rats, which lost ~16% of their body weight. This was also the same group in which only three rats survived treatment. The Osborne-Mendel and F344 strains did not exhibit loss of body weight or mortality due to TCA exposure.

There was a large variation in response to TCA exposure between the differing strains of rats and mice with a much larger difference between the strains of mice. For the three rat strains tested, there was a range between 0% change and 2.38-fold of control for PCO activity at the 5 g/L TCA exposure. For the 2 g/L TCA exposure, there was a range of 0% change to 1.54-fold of control for PCO activity. The Osborne-Mendel rats had 1.54-fold of control value for PCO activity at 2 g/L TCA and 2.38-fold of control value for PCO activity reported at 5 g/L, exhibiting the most consistent increase in PCO with increased dose of TCA. Two experiments were reported for F344 rats with one reporting a 1.63-fold of control and the other a 1.79-fold of control value for 5 g/L TCA. Only one of the F334 experiments also exposed rats to 2 g/L TCA and reported no change from control values.

For the four strains of mice tested, there was a range of 7.44–22.13-fold of control values reported at the 5 g/L TCA exposures and 3.76–25.92-fold of control values at the 2 g/L TCA exposures for PCO activity. For the C57BL/6 strain of mice, there was little difference between the 5 and 2 g/L TCA exposures and a generally threefold higher induction of PCO activity by TCA at the 5 g/L TCA exposure level than for the other mouse strains. Although there was a 2.5-fold difference between the 5 and 2 g/L TCA exposure dose, the difference in magnitude of PCO activity between these doses ranged from 0.85- to 2.23-fold for all strains of mice. For the

$B6C3F_1$ mice, there was a difference between reported increases of PCO activity in the text (i.e., reported as 9.59-fold of control) for one of the experiments and that presented graphically in Figure 2 (i.e., 8.70-fold of control). Nevertheless in the two studies of $B6C3F_1$ mice, 5 g/L TCA was reported to induce 7.78-fold of control and 8.70-fold of control for PCO activity, and 2 g/L TCA was reported to induce 5.56-fold of control and 4.70-fold of control for PCO activity.

For the two F344 rat studies in which ~200 mg/kg or 5 g/L TCA was administered for 10 or 14 days, there was 1.63-fold of control and 1.79-fold of control values reported for PCO activity. Thus, for experiments in which the same strain and dose of TCA were administered, there was not as large a difference in PCO response than between strains and species.

Whether increases in percent liver/body weight ratios were similar in magnitude to increased PCO activity can be assessed by examination of the differences in magnitude of increase over control for the 5 and 2 g/L TCA treatments in the varying rat and mouse strains. The relationship in exposure concentration was a 2.5:1 ratio for the 5 and 2 g/L doses. For rats treatment of 5 g/L TCA to Sprague-Dawley rats resulted in a significant decrease in body weight, and therefore, affected the magnitude of increase in percent liver/body weight ratio for this group. However, for the rest of the rat and mouse data, this dose was not reported to affect body weight so that there is more confidence in the dose-response relationship.

For the Sprague-Dawley rat, there was no change in the percent liver/body weight ratio at 2 g/L but a 10% decrease at 5 g/L TCA exposure with no change in PCO activity for either. However, for the Osborne-Mendel rats, there was no change in percent liver/body weight ratios for either exposure concentration of TCA, but PCO activity was reported to be 1.54-fold of control at 2 g/L and 2.38-fold of control at 5 g/L TCA. Thus, there was a ratio of 2.5-fold increase in PCO activity between the 5 and 2 g/L treatment groups. For the F344 rats, there was a 2-fold difference in liver weight increases (i.e., 12 vs. 6% increase over control) between the two exposure concentrations but 1.6-fold of control value for PCO activity at the 5 g/L TCA exposure concentration and no increase in PCO activity at the 2 g/L level. Thus, for the three strains of rats, there did not appear to be a consistent correlation between liver weight induction by TCA and PCO activity.

For differing strains of mice, similar concentrations of TCA were reported to vary in the induction of liver weight increases. The range of liver weight induction was 1.26–1.66-fold of control values between the four strains of mice at 5 g/L TCA and 1.16–1.63-fold at 2 g/L TCA. In general, for mice the magnitudes of the difference in the increase in dose between the 5 g/L and 2 g/L TCA exposure concentration (2.5-fold) was generally higher than the increase percent liver/body weight ratios at these doses. The differences in liver weight induction between the 2 and 5 g/L doses were ~40% for the Swiss-Webster, C3H, and for one of the $B6C3F_1$ mouse experiments. For the C57BL/6 mouse, there was no difference in liver weight induction between the 2 and 5 g/L TCA exposure groups. For the other $B6C3F_1$ mouse experiments, there was a

2.5-fold greater induction of liver weight increase for the 5 g/L TCA group than for the 2 g/L exposure group (1.39- vs. 1.16-fold of control for percent liver/body weight, respectively).

For PCO activity, the Swiss-Webster, C3H, and one of the $B6C3F_1$ mouse experiments were reported to have approximately twofold difference in the increase in PCO activity between the two doses. For the other $B6C3F_1$ mouse experiment, there was only about a 50% increase and for the C57BL/6 mouse data, there was 15% less PCO activity induction reported at the 5 g/L TCA dose that at the 2 g/L dose. None of the difference in increases in liver weight or PCO activity in mice from the 2 or 5 g/L TCA exposures were of the same magnitude as the difference in TCA exposure concentration (i.e., 2.5-fold) except for liver weight from the one experiment in $B6C3F_1$ mice. These are also the data used for comparisons with the Sprague-Dawley rat discussed below.

In regard to strain differences for TCA response in mice, there did not appear to be correlations of the magnitude of 5 g/L TCA-induced changes in percent liver/body weight ratio or PCO activity with the body weights reported for control mice for each strain. The control weights between the four strains of mice varied from 21 to 28 g. The strain with the greatest response (C57Bl/6) for TCA-induced changes in percent liver/body weight ratio (i.e., 1.66-fold of control) and PCO activity (22.13-fold of control) had a mean body weight reported to be 26 g for controls. At this dose, the range of percent liver/body weight for the other strains was reported to be 1.26–1.39-fold of control and the range of PCO activity reported to be of 7.48–8.71-fold of control.

Of note is that in the literature, this study has been cited as providing evidence of differences between rats and mice for peroxisomal response to TCA and DCA. Generally, the PCO data from the Sprague-Dawley rats and $B6C3F_1$ mice at the highest dose of TCA and DCA have been cited. However, the Sprague-Dawley strain was reported to have greater mortality from TCA at this exposure than the other strains tested (i.e., only three rats survived and provided PCO levels) and a lower PCO response (no change in PCO activity over control) that the other two strains tested in this study (i.e., Osborne-Mendel rats was reported to have had 2.38-fold of control and the F344- had a 1.63–1.79-fold of control for PCO activity after exposure to 5 g/L TCA with no mortality). The $B6C3F_1$ mouse was reported to have a 7.78- or 8.71-fold of control for PCO activity from 5 g/L TCA exposure. Certainly, the male mouse is more responsive to TCA induction of PCO activity. However, as discussed above, there are large variations in control levels of PCO activity and in the magnitude and dose-response of TCA-induction of PCO activity between rat and mouse strains and between species. It is not correct to state that the rat is refractory to TCA-induction of peroxisome activity.

Unfortunately, the authors chose the Sprague-Dawley rat (i.e., the most unresponsive strain for PCO activity and most sensitive to toxicity) for studies for comparative studies between DCA and TCA effects. The authors also tested for carnitine acetyl CoA transferase (CAT) activity as a marker of peroxisomal enzyme response and took morphometric analysis of

peroxisome number and cytoplasmic volume for one liver section for each of two $B6C3F_1$ mice or Sprague-Dawley rats from the 5 g/L TCA and 5 g/L DCA treatment groups. Only six electron micrograph fields were analyzed from each section (12 fields total) were analyzed without identification as to what area of the liver lobules they were being taken from. Hence, there is a question as to whether the areas that are known to be peroxisome rich were assayed of not. Also as noted above, previous studies have indicate that such high concentration of DCA and TCA inhibit drinking water consumption and therefore, raising issues not only about toxicity, but also the dose that rats and mice received.

The number of peroxisomes per 100 μm^3 and cytoplasmic volume of peroxisomes was reported to be 6.60 and 1.94%, respectively, for control rats, and 6.89 and 0.61% for control mice, respectively. For 5 g/L TCA and 5 g/L DCA, the numbers of peroxisomes were reported to be increased to 7.14 and 16.75, respectively, in treated Sprague-Dawley rats. Thus, there was 2.5- and 1.08-fold of control reported in peroxisome numbers for 5 g/L DCA and TCA, respectively. The cytoplasmic volume of peroxisomes was reported to be 2.80 and 0.89% for 5 g/L DCA and 5 g/L TCA, respectively (i.e., a 1.44-fold of control and ~60% reduction for 5 g/L DCA and 5 g/L TCA, respectively). Thus, 5 g/L TCA was reported to slightly increase the number of peroxisomes, but decrease the percent of the cytoplasmic volume occupied by peroxisome by half. For DCA, the reported pattern was for both to increase. PCO activity was reported to increase by a similar magnitude as peroxisome numbers but not volume in the 5 g/L TCA treated Sprague-Dawley rats. However, although peroxisomal volume was reported to be cut nearly in half and for peroxisome number to be similar, 5 g/L TCA treatment was not reported to change PCO activity in the Sprague-Dawley rat.

For comparisons between DCA and TCA, $B6C3F_1$ mice were examined at 1, 2, and 5 g/L concentrations. DCA was reported to induce a higher percent liver/body weight ratio that did TCA at every concentration (i.e., 1.55-, 1.27-, and 1.21-fold of control for DCA and 1.39-, 1.16-, and 1.08-fold of control for TCA at 1, 2, and 5 g/L concentrations, respectively). As noted above, for other strains of mice tested and a second experiment with $B6C3F_1$ mice, there was $\leq$40% difference in percent liver/body weight ratio between the 2 and 5 g/L exposures to TCA, but for this experiment, there was a 2.5-fold difference. Thus, at 5 g/L, there was ~40% greater induction of liver weight for DCA than TCA.

In the $B6C3F_1$ mice, 5 g/L TCA was reported to increase peroxisome number to 30.75 and cytoplasmic volume to 4.92% (i.e., 4.4- and 8.1-fold of control, respectively). For 5 g/L DCA treatment, the peroxisome number was reported to be 30.77 and 3.75% (i.e., 4.5- and 6.1-fold of control, respectively). While there was no difference in peroxisome number and ~40% difference in cytoplasmic volume at the 5 g/L exposures of DCA and TCA, there was a greater difference in the magnitude of PCO activity increase. The 5 g/L TCA exposure was reported to induce 4.3-fold of control for PCO activity, while 5 g/L DCA induced as 9.6-fold of control PCO activity (although a figure in the report shows 8.7-fold of control), which is a

~2.5-fold difference between DCA and TCA at this exposure concentration. Thus, for one of the $B6C3F_1$ mouse studies, 5 g/L DCA and TCA treatments were reported to give a similar increase peroxisome number, TCA to induce a 40% greater increase in peroxisomal cytoplasmic volume than DCA and a 2.5-fold greater increase in PCO activity, but DCA to induce ~40% greater liver weight induction than TCA.

Not only were PCO activity, peroxisome number, and cytoplasmic volume occupied by peroxisomes analyzed, but also CAT activity as a measure of peroxisome proliferation. For TCA and DCA, the results were opposite those reported for PCO activity. In Sprague-Dawley rats, control levels of CAT were reported to be 1.81 nmoles of carnitine transferred/min/mg/protein. Exposure to 5 g/L TCA was reported to increase CAT activity by 3.21-fold of control, while 5 g/L DCA was reported to induce CAT activity to 10.33-fold of control levels in Sprague-Dawley rats. However, while PCO activity was reported to be the same as controls and peroxisomal volume decreased, 5 g/L TCA increased CAT activity 3.21-fold of control in these rats. The level of CAT induced by 5 g/L DCA was over 10-fold of control in the rat while peroxisome number was only 2.5-fold of control and cytoplasmic volume 1.4-fold of control. Thus, the fold increases for these three measures were not the same for DCA treatment and for TCA in rats. Nevertheless for CAT, DCA was a stronger inducer in rats than was TCA.

In $B6C3F_1$ mice, 5 g/L TCA and 5 g/L DCA induced CAT activity to a similar extent (4.50- and 5.61-fold of control, respectively). The magnitude of CAT induction was similar to that of peroxisome number for both 5 g/L DCA and 5 g/L TCA and lower than PCO activity in DCA-treated mice and cytoplasmic volume in TCA-treated mice by about half. Thus, using CAT as the marker of peroxisome proliferation, the rat was more responsive than the mouse to DCA and nearly as responsive to TCA as the mouse at this high dose in these two specific strains. These data illustrate the difficulty of using only one measure for peroxisome proliferation and show that the magnitude of increased PCO activity is not necessarily predictive of the peroxisome number or cytoplasmic volume or CAT activity. The difficulty of interpretation of the data from so few animals and sections for the electron microscopy analysis, and the low number of animals for PCO activity and CAT activity (n = 3–6), the high dose studied (5 g/L), and the selection of a rat strain that appears to be more resistant to this activity but more susceptible to toxicity than the others tested, should be taken into account before conclusions can be made about differences between these chemicals for peroxisome activity between species.

Of note is that PCO activity was also shown to be increased by corn oil alone in F344 rats and to potentiate the induction of PCO activity of TCA. After 10 days of exposure to either water, corn oil, 200 mg/kg-day TCA in corn oil, or 200 mg/kg TCA in water via gavage dosing, there was 1.40-fold PCO activity from corn oil treatment alone in comparison to water, a 1.79-fold PCO activity from TCA in water treatment in comparison to water, and a 3.14-fold PCO activity from TCA in corn oil treatment in comparison to water.

The authors provided data for three concentrations of DCA and TCA for Sprague-Dawley and for one experiment in the $B6C3F_1$ mouse for examination of changes in body and percent liver/body weight ratios (1, 2, or 5 g/L DCA or TCA) after 14 days of exposure. As noted above, not only did the 5 g/L exposure concentration of DCA result in mortality in the Sprague-Dawley strain of rat, but the 5 and 2 g/L concentrations of DCA were reported to decrease body weight (~20 and 25%, respectively). The 5 g/L dose of TCA was also reported to induce a statistically significant decrease in body weight in the Sprague-Dawley rat. There were no differences in final body weight in any of the mice exposed to TCA or DCA.

As noted above, no TCA or DCA exposure group of Sprague-Dawley rats was reported to have a statistically significant increase in percent liver/body weight ratio over control. For the $B6C3F_1$ male mice, the percent liver/body weight ratio was 1.22-, 1.27-, and 1.55-fold of control after exposure to 1, 2, and 5 g/L DCA, respectively, and 1.08-, 1.16-, and 1.39-fold of control after exposure to 1, 2, and 5 g/L TCA, respectively. Thus, for DCA, there was only a 20% increase in liver weight corresponding to the twofold increase between the 1 and 2 g/L exposure levels of DCA. Between the 2 and 5 g/L exposure concentrations of DCA, there was a 2-fold increase in liver weight corresponding to a 2.5-fold increase in exposure concentration. For TCA, the magnitude of increase in dose was reported to be proportional to the magnitude of increase in percent liver/body weight ratio in the $B6C3F_1$ male mouse. As stated above, the correspondence between magnitude of dose and percent liver weight for TCA exposure in this experiment differed from the other experiment reported for this strain of mouse and also differed from the other three strains of mice examined in this study where the magnitude in liver weight gain was much less than exposure concentration.

E.2.3.2. Subchronic and Chronic Studies of DCA and TCA

Several experiments have been conducted with exposure to DCA and TCA, generally at very high levels with a limited dose range, for less periods of time than standard carcinogenicity bioassays, and with very limited information on any endpoints other than the liver tumor induction. Caldwell and Keshava (2006) and Caldwell et al. (2008b) have examined these studies for inferences of modes of action for TCE. Key studies are briefly described below for comparative purposes of results reported in TCE studies.

E.2.3.2.1. Snyder et al. (1995)

Studies of TCE have reported either no change or a slight increase in apoptosis only after a relatively high exposure level (Channel et al., 1998; Dees and Travis, 1993). Inhibition of apoptosis, which has been suggested to prevent removal of "initiated" cells from the liver and lead to increased survival of precancerous cells, has been proposed as part of the mode of action for peroxisome proliferators (see Section E.3.4). The focus of this study was to examine whether DCA, which has been shown to inhibit DNA synthesis after an initial transient increase (see

Section E.2.3.1.1), also alters the frequency of spontaneous apoptosis in mice. This study exposed 28-day-old male B6C3F$_1$ male mice (n = 5) to 0, 0.5 or 5.0 g/L buffered DCA in drinking water for up to 30 days (Phase I = 5–15 days exposure and Phase II = 20–30 days treatment).

Portions of the left lobe of the liver were prepared for histological examination after H&E staining. Hepatocyte number was determined by counting nuclei in 50 fields with nonparenchymal cell nuclei excluded on the basis of nuclear size. Multinucleate cells were counted as one cell. Apoptotic cells were visualized by in situ TDT nick end-labeling assay from 2 to 4 different liver sections from each control or treated animal. The average number of apoptotic cells was then determined for each animal in each group. The authors reported that in none of the tissues examined were necrotic foci observed, there was no any indication of lymphocyte or neutrophil infiltration indicative of an inflammatory response, and suggested that no necrotic cells contributed to the responses in their analysis.

Control animals were reported to exhibit apoptotic frequencies ranging from ~0.04 to 0.085% and that over the 30-day period the frequency rate declined. The authors suggested that this result is consistent with reports of the livers of these young animals undergoing rapid changes in cell death and proliferation. They note that animals receiving 0.5 g/L DCA also had a similar trend of decreasing apoptosis with age, supportive of the decrease being a physiological phenomenon. The 0.5 g/L exposure level of DCA was reported to decrease the percentage of apoptotic hepatocytes as the earliest time point studied and to remain statistically significantly decreased from controls from 5 to 30 days of exposure. The rate of apoptosis ranged from ~0.025 to 0.060% after 0.5 g/L DCA exposure during the 30-day period (i.e., and ~30–40% reduction). Animals receiving the 5.0 g/L DCA dose exhibited a significant reduction at the earliest time point that was sustained at a similar level and statistically significant throughout the time-course of the experiment (percent apoptosis ranged from 0.015 to 0.030%).

The results of this study not only provide a baseline of apoptosis in the mouse liver, which is very low, but also show the importance of taking into account the effects of age on such determinations. The authors reported that the for rat liver, the estimated frequency of spontaneous apoptosis to be ~0.1%, and therefore, greater than that of the mouse. The significance of the DCA-induced reduction in apoptosis, of a level that is already inherently low in the mouse, for the mode of action for induction of cancer is difficult to discern.

E.2.3.2.2. **Mather et al. (1990)**

This 90-day study in male Sprague-Dawley rats examined the body and organ weight changes, liver enzyme levels, and PCO activity in livers from rats treated with estimated concentrations of 3.9, 35.5, 345 mg/kg-day DCA or 4.1, 36.5, or 355 mg/kg-day TCA from drinking water exposures (i.e., 0, 50, 500, and 5,000 ppm or 0.05, 0.5, or 5.0 g/L DCA or TCA in the drinking water). All dose levels of DCA and TCA were reported to result in a dose-

dependent decrease in fluid intake at 2 months of exposure. The rats were 9 (DCA) or 10 (TCA) weeks old at the beginning of the study (n = 10/group). Animals with body weights that varied >20% of mean weights were discarded from the study. The DCA and TCA solutions were neutralized. The mean values for initial weights of the animals in each test group varied <3%.

DCA treatment induced a dose-related decrease in body weight that was statistically significant at the two highest levels (i.e., a 6, 9.5, and 17% decrease from control). TCA treatment also resulted in lower body weights that were not statistically significant (i.e., 2.1, 4.4, and 5.9%). DCA treatments were reported to result in a dose-related increase in absolute liver weights (1.01-, 1.13-, and 1.36-fold of control that were significantly different at the highest level) and percent liver/body weight ratios (1.07-, 1.24-, and 1.69-fold of control that were significant at the two highest dose levels). TCA treatments were reported to not result in changes in either absolute liver weights or percent liver/body weight ratios with the exception of statistically significant increase in percent liver/body weight ratios at the highest level of treatment (1.02-fold of control).

Total serum protein levels were reported to be significantly depressed in all animals treated with DCA with animals in the two highest dose groups also exhibiting elevations of ALP. Alanine-aminotransferase levels were reported to be elevated only in the highest treatment group. No consistent treatment-related effect on serum chemistry was reported to be observed for the TCA-treated animals with data not shown.

In terms of PCO activity, there was only a mild increase at the highest dose of 15% for TCA and a 2.5-fold level of control for DCA treatment that were statistically significant. The difference in PCO activity between control groups for the DCA and TCA experiments was reported to be 33%. No treatment effect was reported to be apparent for hepatic microsomal enzymes, or measures of immunotoxicity for either DCA or TCA, but data were not shown. Focal areas of hepatocellular enlargement in both DCA- and TCA-treated rats were reported to be present with intracellular swelling more severe with the highest dose of DCA treatment. Livers from DCA treated rats were reported to stain positively for PAS, indicating significant amounts of glycogen with TCA treated rats reported to display “less evidence of glycogen accumulation.” Of note is that, in this study of rats, DCA was reported to induce a greater level of PCO activity than did TCA.

E.2.3.2.3. **Parrish et al. (1996)**

Parrish et al. (1996) exposed male B6C3F$_1$ mice (8 weeks old and 20–22 g upon purchase) to TCA or DCA (0, 0.01, 0.5, and 2.0 g/L) for 3 or 10 weeks (n = 6). Livers were excised and nuclei isolated for examination of 8-OHdG and homogenates examined for cyanide insensitive acyl-CoA oxidase (ACO) and laurate hydroxylase activity. The authors noted that control values between experiments varied as much as a factor of twofold for PCO activity and that data were presented as percent of concurrent controls. Initial body weights for treatment

groups were not presented and thus, differences in mean values between the groups cannot be ascertained.

Final body weights were reported to not be statistically significantly changed by DCA or TCA treatments at 21 or 71 days of treatment (all were within ~8% of controls). The mean percent liver/body ratios were reported to be 5.4, 5.3, 6.1, and 7.2% for control, 0.1, 0.5, and 2.0 g/L TCA, respectively, and 5.4, 5.5, 6.7, and 7.9% for control, 0.1, 0.5, and 2.0 g/L DCA, respectively, after 21 days of exposure. This represents 0.98-, 1.13-, and 1.33-fold of control levels with these exposure levels of TCA and 1.02-, 1.24-, and 1.46-fold of control levels with DCA after 21 days of exposure. For 71 days of exposure, the mean percent liver/body ratios were reported to be 5.1, 4.6, 5.8, and 6.9% for control, 0.1, 0.5, and 2.0 g/L TCA, respectively and 5.1, 5.1, 5.9, and 8.5% for control, 0.1, 0.5, and 2.0 g/L DCA, respectively. This represents 0.90-, 1.14-, and 1.35-fold of control with TCA exposure and 1.0-, 1.15-, and 1.67-fold of control with DCA exposure after 71 days of exposure. The magnitude of difference between the 0.1 and 0.5 g/L TCA doses is 5, and between 0.5 and 2.0 g/L doses is fourfold.

For the 21- and 71-day exposures the magnitudes of the increases in percent liver/body weight over control values were greater for DCA than TCA exposure at same concentration with the exception of 0.5 g/L doses at 71 days in which both TCA and DCA induced similar increases. For TCA, the 0.01 g/L dose produces a similar 10% decrease in percent liver/body weight. Although there was a fourfold increase in magnitude between the 0.5 and 2.0 g/L TCA exposure concentrations, the magnitude of increase for percent liver/body weight increase was 2.5-fold between them at both 21 and 71 days of exposure. For DCA, the 0.1 g/L dose was reported to have a similar value as control for percent liver/body weight ratio. Although there was a 4-fold difference in dose between the 0.5 and 2.0 g/L DCA exposure concentrations, there was a ~2-fold increase in percent liver/body weight increase at 21 days and ~4.5-fold increase at 71 days.

As a percentage of control values, TCA was reported to induce a dose-related increase in PCO activity at 21 days (~1.5-, 2.2-, and ~4.1-fold of control, for 0.1, 0.5, and 2 g/L TCA exposures). Only the 2.0 g/L dose of DCA was reported to induce a statistically significant increase at 21 days of exposure of PCO activity over control (~1.8-fold of control) with the 0.1 and 0.5 g/L exposure PCO activity to be slightly less than control values (~20% less). Thus, although there was no increase in percent liver/body weight at 0.1 g/L TCA, the PCO activity was reported to be increased by ~50% after 21 days. A 13% increase in liver weight at 0.5 g/L TCA was reported to be associated with 2.2-fold of control level of PCO activity and a 33% increase in liver weight after 2.0 g/L TCA to be associated with 4.1-fold of control level of PCO activity.

Thus, increases in PCO activity were not necessarily correlated with concurrent TCA-induced increases in liver weight and the magnitudes of increase in liver weight between 0.5 and 2.0 g/L TCA (2.5-fold) was greater than the corresponding increase in PCO activity

(1.8-fold of control). Although there was a 20-fold difference in TCA dose, the magnitude of increase in PCO activity between 0.1 and 2.0 g/L TCA was ~2.7-fold. As stated above, the 4-fold difference in TCA dose at the two highest levels resulted in a 2.5-fold increase in liver weight. For DCA, the increases in liver weight at 0.1 and 0.5 g/L DCA exposures were not associated with increased PCO activity after 21 days of exposure. The 2.0 g/L DCA exposure concentration was reported to induce 1.8-fold of control PCO activity.

After 71 days of treatment, TCA induced a dose-related increase in PCO activity that was approximately twice the magnitude as that reported at 21 days (i.e., ~9-fold greater at 2.0 g/L). After 71 days, for DCA the 0.1 and 0.5 g/L doses produced a statistically significant increase in PCO activity (~1.5- and 2.5-fold of control, respectively). The administration of 1.25 g/L clofibric acid in drinking water was used as a positive control and reported to induce approximately six- to sevenfold of control PCO activity at 21 and 71 days of exposure.

Laurate hydroxylase activity was reported to be elevated significantly only by TCA at 21 days (2.0 g/L TCA dose only) and to increased to approximately the same extent (~1.4–1.6-fold of control values) at all doses tested. For 0.1 g/L DCA, the laurate hydroxylase activity was reported to be similar to that of 0.1 g/L TCA (~1.4-fold of control) but to be ~1.2-fold of control at both the 0.5 and 2.0 g/L DCA exposures. At 71 days, both the 0.5 and 2.0 g/L TCA exposures induced a statistically significant increase in laurate hydroxylase activity (i.e., 1.6- and 2.5-fold of control, respectively) with no change after DCA exposure. The actual data rather than percent of control values were reported for laurate hydroxylase activity. The control values for laurate hydroxylase activity varied 1.7-fold between 21 and 71 days experiments.

The results for 8-OHdG levels are discussed in Section E.3.4.2.3. Of note is that the increases in PCO activity noted for DCA and TCA were not associated with 8-OHdG levels (which were unchanged, see Section E.3.4.2.3) and also not with changes laurate hydrolase activity or percent liver/body weight ratio increases observed after either DCA or TCA exposure. A strength of this study is that it examined exposure concentrations that were lower than those examined in many other short-term studies of DCA and TCA.

E.2.3.2.4. **Bull et al. (1990)**

The focus of this study was the determination of "dose-response relationships in the tumorigenic response to these chemicals [sic DCA and TCA] in B6C3F_1 mice, determine the nature of the nontumor pathology that results from the administration of these compounds in drinking water, and test the reversibility of the response." Male and female B6C3F_1 mice (age 37 days) were treated from 15 to 52 weeks with neutralized TCA and TCA. A highly variable number and generally low number of animals were reported to be examined in the study with n = 5 for all time periods except for 52 weeks where in males the n = 35 for controls, n =11 for 1 g/L DCA, n = 24 for 2 g/L DCA, n = 11 for 1 g/L TCA, and n = 24 for 2 g/L TCA exposed

mice. Female mice were only examined after 52 weeks of exposure and the number of animals examined was n = 10 for control, 2 g/L DCA, and 2 g/L TCA exposed mice.

"Lesions to be examined histologically for pathological examination were selected by a random process" with lesions reported to be selected from 31 of 65 animals with lesions at necropsy. 73 of 165 lesions identified in 41 animals were reported to be examined histologically. All hyperplastic nodules, adenomas, and carcinomas were lumped together and characterized as hepatoproliferative lesions. Accordingly, there were only exposure concentrations available for dose-response analyses in males and only "multiplicity of hepatoproliferative lesions" were reported from random samples. Thus, these data cannot be compared to other studies and are unsuitable for dose-response with inadequate analysis performed on random samples for pathological examination.

The authors state that some of the lesions taken at necropsy and assumed to be proliferative were actually histologically normal, necrotic, or an abscess as well. It is also limited by a relatively small number of animals examined in regard to adequate statistical power to determine quantitative differences. Similar concerns were raised by Caldwell et al. (2008b) with a subsequent study (eg., Bull et al., 2002). For example, the authors report that 5/11 animals had "lesions" at 1 g/L TCA at 52 weeks and 19/24 animals had lesions at 2 g/L TCA at 52 weeks. However, while 7 lesions were examined in 5 mice bearing lesions at 1 g/L TCA, only 16 of 30 lesions from 11 of the 19 animals bearing lesions examined in the 2 g/L TCA group. Therefore, almost half of the mice with lesions were not examined histologically in that group along with only half of the "lesions."

The authors reported the effects of DCA and TCA exposure on liver weight and percent liver/body changes ($m \pm$ SEM) and these results gave a pattern of hepatomegaly generally consistent with short-term exposure studies. The authors report "no treatment produced significant changes in the body weight or kidney weight of the animals (data not shown)."

In male mice (n = 5) at 37 weeks of exposure, liver weights were reported to be 1.6 ± 0.1, 2.5 ± 0.1, and 1.9 ± 0.1 g for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. The percent liver/body weights were reported to be 4.1 ± 0.3, 7.3 ± 0.2, and 5.1 ± 0.1% for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. In male mice at 52 weeks of exposure, liver weights were reported to be 1.7 ± 0.1, 2.5 ± 0.1, 5.1 ± 0.1, 2.2 ± 0.1, and 2.7 ± 0.1 g for control (n = 35), 1 g/L DCA (n = 11), 2 g/L DCA (n = 24), 1 g/L TCA (n = 11), and 2 g/L TCA (n = 24) exposed mice, respectively. In male mice at 52 weeks of exposure, percent liver/body weights were reported to be 4.6 ± 0.1, 6.5 ± 0.2, 10.5 ± 0.4, 6.0 ± 0.3, and 7.5 ± 0.5% for control, 1 g/L DCA, 2 g/L DCA, 1 g/L TCA, and 2 g/L TCA exposed mice, respectively. For female mice (n = 10) at 52 weeks of exposure, liver weights were reported to be 1.3 ± 0.1, 2.6 ± 0.1, and 1.7 ± 0.1 g for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. The percent liver/body weights were reported to be 4.8 ± 0.3, 9.0 ± 0.2, and 6.0 ± 0.3% for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively.

Although the number of animals examined varied threefold between treatment groups in male mice, the authors reported that all DCA and TCA treatments were statistically increased over control values for liver weight and percent body/liver weight in both genders of mice. In terms of percent liver/body weight ratio, female mice appeared to be as responsive as males at the exposure concentration tested. Thus, hepatomegaly reported at these exposure levels after short-term exposures appeared to be further increased by chronic exposure with equivalent levels of DCA inducing greater hepatomegaly than TCA.

Interestingly, after 37 weeks of treatment and then a cessation of exposure for 15 weeks, liver weights were assessed in control male mice, 2 g/L DCA treated mice, and 2 g/L TCA treated mice (n = 11 for each group but results for controls were pooled and therefore, n = 35). Liver weights were reported to be 1.7 ± 0.1, 2.2 ± 0.1, and 1.9 ± 0.1 g for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. The percent liver/body weights were reported to be 4.6 ± 0.1, 5.7 ± 0.3, and 5.4 ± 0.2% for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. After 15 weeks of cessation of exposure, liver weight and percent liver/body weight were reported to still be statistically significantly elevated after DCA or TCA treatment.

The authors partially attributed the remaining increases in liver weight to the continued presence of hyperplastic nodules in the liver. The authors stated that because of the low incidence of lesions in the control group and the two groups that had treatments suspended, all of the lesions from these groups were included for histological sectioning. However, the authors presented a table indicating that, of the 23 lesions detected in seven mice exposed to DCA for 37 weeks, 19 were examined histologically. Therefore, groups that were exposed for 52 weeks had a different procedure for tissue examination as those at 37 weeks.

In terms of liver tumor induction, the authors stated that "statistical analysis of tumor incidence employed a general linear model ANOVA with contrasts for linearity and deviations from linearity to determine if results from groups in which treatments were discontinued after 37 weeks were lower than would have been predicted by the total dose consumed." The multiplicity of tumors observed in male mice exposed to DCA or TCA at 37 weeks and then sacrificed at 52 weeks were reported by the authors to have a response in animals that received DCA very close to that which would be predicted from the total dose consumed by these animals. The response to TCA was reported by the authors to deviate significantly ($p = 0.022$) from the linear model predicted by the total dose consumed.

Multiplicity of lesions per mouse and not incidence was used as the measure. Most importantly, the data used to predict the dose response for "lesions" used a different methodology at 52 weeks than those at 37 weeks. Not only were not all animal's lesions examined but foci, adenomas, and carcinomas were combined into one measure. Therefore, foci, of which a certain percentage have been commonly shown to spontaneously regress with time, were included in the calculation of total "lesions." Pereira and Phelps (1996) note that in initiated mice treated with DCA, the yield of altered hepatocytes decreases as the tumor yields

increase between 31 and 51 weeks of exposure suggesting progression of foci to adenomas. Initiated and noninitiated control mice also had fewer foci/mouse with time.

Because of differences in methodology and the lack of discernment between foci, adenomas, and carcinomas for many of the mice exposed for 52 weeks, it is difficult to compare differences in composition of the "lesions" after cessation of exposure. For TCA treatment, the number of animals examined for determination of which "lesions" were foci, adenomas, and carcinomas was 11/19 mice with "lesions" at 52 weeks, while all 4 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined.

For DCA treatment, the number of animals examined was only 10/23 mice with "lesions" at 52 weeks while all 7 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. Most importantly, when lesions were examined microscopically, they did not all turn out to be preneoplastic or neoplastic. Two lesions appeared "to be histologically normal" and one necrotic. Not only were a smaller number of animals examined for the cessation exposure than continuous exposure, but only the 2 g/L exposure levels of DCA and TCA were studied for cessation. The number of animals bearing "lesions" at 37 and then 15 week cessation weeks was 7/11 (64%) while the number of animals bearing lesions at 5 weeks was 23/24 (96%) after 2 g/L DCA exposure. For TCA, the number of animals bearing lesions at 37 weeks and then 15 weeks cessation was 4/11 (35%), while the number of animals bearing lesions at 52 weeks was 19/24 (80%). While suggesting that cessation of exposure diminished the number of "lesions," conclusions regarding the identity and progression of those lesions with continuous vs. noncontinuous DCA and TCA treatment are tenuous.

Macroscopically, the "livers of many mice receiving DCA in their drinking water displayed light colored streaks on the surface" at every sacrifice period and "corresponded with multi-focal areas of necrosis with frequent infiltration of lymphocytes." At the light microscopic level, the lesions were described to also be present in the interior of the liver as well. For TCA-treated mice, "similar necrotic lesions were also observed… but at a much lower frequency, making it difficult to determine if they were treatment-related." Control animals were reported not to show degenerative changes. "Marked cytomegaly" was reported for mice treated with either 1 or 2 g/L DCA "throughout the liver." In regard to cell size, the authors did not give any description in the methods section of the paper as to how sections were selected for morphometric analysis or what areas of the liver acinus were examined but reported after 52 weeks of treatment the long axis of hepatocytes measured (mean ± S.E.) 24.9 ± 0.3, 38.5 ± 1.0, and 29.3 ± 1.4 μm in control, DCA-, and TCA-treated mice, respectively.

Mice treated with TCA (2 g/L) for 52 weeks were reported to have livers with "considerable dose-related accumulations of lipofuscin." However, no quantitative analyses were presented. A series of figures representative of treatment showed photographs (1,000×) of lipofuscin fluorescence indicating greater fluorescence in TCA treated liver than control or DCA treated liver.

A series of photographs of H&E sections in the report (see Figures 2a, b, and c) were shown as representative histology of control mice, mice treated with 2 g/L DCA and 2 g/L TCA. The area of the liver from which the photographs were taken did not include either portal tract or central veins and the authors did not give the zone of the livers from which they were taken. The figure representing TCA treatment shows only a mild increase in cell volume in comparison to controls, while for DCA treatment, the hepatocyte diameter was greatly enlarged, pale stained so that cytoplasmic contents appear absent, nuclei often pushed to the cell perimeter, and the sinusoids appearing to be obscured by the swollen hepatocytes. The apparent reduction of sinusoidal volume by the enlarged hepatocytes raises the possibility of decreased blood flow through the liver, which may have been linked to focal areas of necrosis reported for this high exposure level.

In a second set of figures, glycogen accumulation was shown with PAS staining at the same level of power (400×) for the same animals. In control animals, PAS-positive material was not uniformly distributed between or within hepatocytes but tended to show a zonal pattern of moderate intensity. PAS positive staining (which the authors reported to be glycogen) appeared to be slightly less than controls but with a similar pattern in the photograph representing TCA exposure. However, for DCA, the photograph showed a uniform and heavy stain within each hepatocyte and across all hepatocytes.

The authors stated in the results section of the paper that "the livers of TCA-treated animals displayed less evidence of glycogen accumulation and it was more prominent in periportal than centrilobular portions of the liver acinus." In their abstract they state "TCA produced small increases in cell size and a much more modest accumulation of glycogen." Thus, the statement in the text, which is suggestive that TCA induced an increase in glycogen over controls that was not as much as that induced by DCA, and the statement in the abstract, which concludes TCA exposure increased glycogen is not consistent with the photographs. In the photograph shown for TCA, there is less not more PAS-positive staining associated with TCA treatment in comparison to controls.

In Sanchez and Bull (1990), the authors report that "TCA exposure induced a much less intense level of PAS staining that was confined to periportal areas" but do not compare PAS staining to controls but only to DCA treatment. In the discussion section of the paper, the authors state "Except for a small increase in liver weight and cell size, the effects produced by DCA were not observed with TCA." Thus, there seems to be a discrepancy with regard to what the effects of TCA are in relation to control animals from this report that has caused confusion in the literature. Kato-Weinstein et al. (2001) reported that in male mice exposed to DCA and TCA the DCA increased glycogen and TCA decreased glycogen content of the liver using chemical measurement of glycogen in liver homogenates and using ethanol-fixed sections stained with PAS, a procedure designed to minimize glycogen loss.

E.2.3.2.5. **Nelson et al. (1990)**

Nelson et al. (1990) reported that they used the same exposure paradigm as Herren-Freund et al. (1987), with little description of methods used in treatment of the animals. Male B6C3F_1 mice were reported to be exposed to DCA (1 or 2 g/L) or TCA (1 or 2 g/L) for 52 weeks. The number of animals examined for nontumor tissue was 12 for controls. The number of animals varied from two to eight for examination of nontumor tissue, hyperplastic nodules, and carcinoma tissues for c-Myc expression. There was no description for how hyperplastic nodules were defined and whether they included adenomas and foci. For the 52-week experiments, the results were pooled for lesions that had been obtained by exposure to the higher or lower concentrations of DCA or TCA (i.e., the TCA results are for lesions induced by either 1.0 or 2.0 g/L TCA).

A second group of mice were reported to be given either DCA or TCA for 37 weeks and then normal drinking water for the remaining time until 52 weeks with no concentrations given for the exposures to these animals. Therefore, it is impossible to discern what dose was used for tumors analyzed for c-Myc expression in the 37-week treatment groups and if the same dose was used for 37 and 52 week results.

Autoradiography was described for three different sections per animal in five different randomly chosen high power fields per section. The number of hyperplastic nodules or the number of carcinomas per animal induced by these treatments was not reported nor the criteria for selection of lesions for c-Myc expression. Apparently, a second experiment was performed to determine the expression of c-H-ras. Whereas in the first experiment, there were no hyperplastic nodules, in the second, one control animal was reported to have a hyperplastic nodule. The number of control animals reported to be examined for nontumor tissue in the second group was 12. The numbers of animals in the second group was reported to vary from one to seven for examination of nontumor tissue, hyperplastic nodules, and carcinoma tissues for c-H-ras expression. The number of animals per group for the investigation of H-ras did not match the numbers reported for that of c-Myc. The number of animals treated to obtain the "lesion" results was not presented (i.e., how many animals were tested to get a specific number of animals with tumors that were then examined). The number of lesions assessed per animal was not reported.

At 52 weeks of exposure, hyperplastic nodules (n = 8 animals) and carcinomas (n = 6 animals) were reported to have approximately twofold expression of c-Myc relative to nontumor tissue (n = 6 animals) after DCA treatment. After 37 weeks of DCA treatment and cessation of exposure, there was a ~30% increase in c-Myc in hyperplastic nodules (n = 4 animals) that was not statistically significant. There were no carcinomas reported at this time.

After 52 weeks of TCA exposure, there was approximately twofold of nontumor tissue reported for c-Myc in hyperplastic nodules (n = 6 animals) and approximately threefold reported for carcinomas (n = 6 animals). After 37 weeks of TCA exposure, there was ~2-fold c-Myc in

hyperplastic nodules (n = 2 animals) that was not statistically significant and ~2.6-fold increase in carcinomas (n = 3 animals) that was reported to be statistically significant over nontumor tissue. There was no difference in c-Myc expression between untreated animals and nontumor tissue in the treated animals.

The authors reported that c-Myc expression in TCA-induced carcinomas was "almost 6 times that in control tissue (corrected by subtracting nonspecific binding)," and concluded that c-Myc in TCA-induced carcinomas was significantly greater than in hyperplastic nodules or carcinomas and hyperplastic nodules induced by DCA. However, the c-Myc expression reported as the number of grains per cells was ~2.6-fold in TCA-induced carcinomas and ~2-fold in DCA-induced carcinomas than control or nontumor tissue at 52 weeks. The hyperplastic nodules from DCA and TCA treatments at 52 weeks gave identical ratios of approximately twofold. In three animals per treatment, c-Myc expression was reported to be similar in "selected areas of high expression" for either DCA or TCA treatments of 52 weeks.

There did not appear to be a difference in c-H-ras expression between control and nontumor tissue from DCA- or TCA-treated mice. The levels of c-H-ras transcripts were reported to be "slightly elevated" in hyperplastic nodules induced by DCA (~67%) or TCA (~43%) but these elevations were not statistically significant in comparison to controls. However, carcinomas "derived from either DCA- or TCA-treated animals were reported to have significantly increased c-H-ras levels relative to controls." The fold increase of nontumor tissue at 52 weeks for DCA-induced carcinomas was ~2.5-fold, and for TCA induced carcinomas, ~2.0-fold. Again, the authors stated that "if corrected for nonspecific hybridization, carcinomas expressed approximately 4 times as much c-H-ras than observed in surrounding tissues" Given that control and nontumor tissue results were given as the controls for the expression increases observed in "lesions," it is unclear what the usefulness of this "correction" is. The authors reported that "focal areas of increased expression of c-H-ras were not observed within carcinomas."

The limitations of this experiment include uncertainty as to what doses were used and how many animals were exposed to produce animals with tumors. In addition, results of differing doses were pooled and the term hyperplastic nodule was undefined. The authors state that c-Myc expression in itself is not sufficient for transformation and that its overexpression commonly occurs in malignancy. They also state that "Unfortunately, the limited amount of tissue available prevented a more serious pursuit of this question in the present study." In regard to the effects of cessation of exposure, the authors do not present data on how many animals were tested with the cessation protocol, what doses were used, and how many lesions comprised their results and thus, comparisons between these results and those from 52 weeks of continuous exposure are hard to make. Quantitatively, the small number of animals, whose lesions were tested, was n = 2–4 for the cessation groups. Bull et al. (1990) is given as the source of data for the cessation experiment (see Section E.2.3.2.4).

E.2.3.2.6. **DeAngelo et al. (1999)**

The focus of this study was to "determine a dose response for the hepatocarcinogenicity of DCA in male mice over a lifetime exposure and to examined several modes of action that might underlie the carcinogenic process." As DeAngelo et al. (1999) pointed out, many studies of DCA had been conducted at high concentrations and were less-than-lifetime studies, and therefore, were of suspect relevance to environmental concentrations. This study is one of the few that examined DCA at a range of exposure concentrations to determine a dose-response in mice. The authors concluded that DCA-induced carcinogenesis was not dependent on peroxisome proliferation or chemically sustained proliferation. The number of HCCs/animals was reported to be significantly increased over controls at all DCA treatments including 0.05 g/L and a NOEL was not observed. Peroxisome proliferation was reported to be significantly increased at 3.5 g/L DCA only at 26 weeks and did not correlate with tumor response. No significant treatment effects on labeling of hepatocytes (as a measure of proliferation) outside proliferative lesions were reported, and thus, the DCA-induced liver cancer was not dependent on peroxisome proliferation or chemically sustained cell proliferation.

Male B6C3F_1 mice were 28–30 days of age at the start of study and weighed 18–21 g (or ~14% range). They were exposed to 0, 0.05, 0.5, 1.0, 2.0, and 3.5 g/L DCA via drinking water as a neutralized solution. The time-weighted mean daily water consumption calculated over the 100-week treatment period was reported to be 147, 153, 158, 151, 147, and 124 (84% of controls) mL/kg/day for 0, 0.05, 0.5, 1, 2, and 3.5 g/L DCA, respectively. The number of animals used for interim sacrifices was 35, 30, 30, 30, and 30 for controls, 0.5, 1.0, 2.0, and 3.5 g/L DCA-treated groups respectively (i.e., 10 mice per treatment group at interim sacrifices of 26, 52, and 78 weeks). The number of animals at final sacrifice was reported to be 50, 33, 24, 32, 14 and 8 for controls, 0.05, 0.5, 1.0, 2.0, and 3.5 g/L DCA-treated groups respectively. The number of animals with unscheduled deaths before final sacrifice was reported to be 3, 2, 1, 9, 11, and 8 for controls, 0.05, 0.5, 1.0, 2.0, and 3.5 g/L DCA-treated groups respectively. The Authors reported that early mortality tended to occur from liver cancer.

The number of animals examined for pathology were reported to be 85, 33, 55, 65, 51, and 41 for controls, 0.05, 0.5, 1.0, 2.0, and 3.5 g/L DCA treated groups, respectively. The experiment was conducted in two parts with control, 0.5, 1.0, 2.0, and 3.5 g/L groups treated and then 1 months later, a second group consisting of 30 control group mice and 35 mice in a 0.05 g/L DCA exposure group were studied.

The authors reported no difference in prevalence and multiplicity of hepatocellular neoplasms in the two groups so that data were summed and reported together. The number of animals reported as examined for tumors were n = 10 animals, with controls reported to be 35 animals split among three interim sacrifice times—exact number per sacrifice time is unknown. The number of animals reported "with pathology" and assumed to be included in the

tumor analyses from Table 1, and the sum of the number of animals "scheduled for sacrifice that survived until 100 weeks" and "interim sacrifices" do not equal each other. For the 1 g/L DCA exposure group, 30 animals were sacrificed at interim periods 32 animals were sacrificed at 100 weeks and 9 animals were reported to have unscheduled deaths, but of those 71 animals, only 65 animals were reported to have pathology for the group. Therefore, some portion of animals with unscheduled deaths must have been included in the tumor analyses. The exact number of animals that may have died prematurely but included in analyses of pathology for the 100-week group is unknown.

In Figure 3 of the study, the authors reported prevalence and multiplicity of HCCs following 79–100 weeks of DCA exposure in their drinking water. The number of animals in each dose group used in the tumor analysis for 100 weeks was not given by the authors. Given that the authors included animals that survived past the 78-week interim sacrifice period but died unscheduled deaths in their 100-week results, the number must have been greater than those reported as present at final sacrifice. A comparison of the data for the 100-week data presented in Table 3a and Figure 3 shows that the data reported for 100 weeks is actually for animals that survived from 79 to 100 weeks.

The authors report a dose-response that is statistically significant from 0.5 to 3.5 g/L DCA for HCC incidence and a dose-response in HCC multiplicity that is significantly increased over controls from 0.05 to 0.5 g/L DCA that survived 79–100 weeks of exposure (i.e., 0, 8-, 84-, 168-, 315-, and 429 mg/kg-day dose groups with prevalences of 26, 33, 48, 71, 95, and 100%, respectively, and multiplicities of 0.28, 0.58, 0.68, 1.29, 2.47, and 2.90, respectively). Hepatocellular adenoma incidence or multiplicity was not reported for the 0.05 g/L DCA exposure group.

In Table 3 of the report, the time course of HCCs and adenoma development are given and summarized in Table E-2.

Table E-2. Prevalence and multiplicity data from DeAngelo et al. (1999)

Prevalence	Multiplicity (lesions/animal *m* ± SEM)	
	Carcinomas	Adenomas
52 wks control = 0% carcinomas, 0% adenoma	0	0
0.5 g/L DCA = 0/10 carcinoma, 1/10 adenomas	0	0.10 ± 0.09
1.0 g/L DCA = 0/10 carcinomas, 1/10 adenomas	0	0.10 ± 0.09
2.0 g/L DCA = 2/10 carcinomas, 0/10 adenomas	0.20 ± 0.13	0
3.5 g/L DCA = 5/10 carcinomas, 5/10 adenomas	0.70 ± 0.25	0.80 ±0.31
78 wks control = 10% carcinomas, 10% adenomas	0.10 ± 0.10	0.10 ± 0.09
0.5 g/L DCA = 0/10carcinoma, 1/10 adenomas	0	0.10 ± 0.09
1.0 g/L DCA = 2/10 carcinomas, 2/10 adenomas	0.20 ± 0.13	0.20 ± 0.13
2.0 g/L DCA = 5/10 carcinomas, 5/10 adenomas	1.0 ± 0.47	1.00 ± -0.42
3.5 g/L DCA = 7/10 carcinomas, 5/10 adenomas	1.20 ± 0.37	1.00 ± 0.42
100 wks control = 26% carcinoma, 10% adenoma	0.28 ± 0.07	0.12 ± 0.05
0.5 g/L DCA = 48% carcinoma, 20% adenomas	0. 68 ± 0.17	0.32 ± 0.14
1.0 g/L DCA = 71% carcinomas, 51.4% adenomas	1.29 ± 0.17	0.80 ± 0.17
2.0 g/L DCA = 95% carcinomas, 42.9% adenomas	2.47 ± 0.29	0.57 ± 0.16
3.5 g/L DCA = 100% carcinomas, 45% adenomas	2.90 ± 0.40	0.64 ± 0.23

The authors reported HCCs and number of lesions/animal in mice that survived 79–100 weeks of exposure. They combined exposure groups to be animals after the week 78 sacrifice time that did and did not make it to 100 weeks. These are the same data reported above for the 100-week exposure with the inclusion of the 0.05 g/L DCA data. The difference between number of animals at interim and final sacrifices and those "with pathology" and used in the tumor analysis but most likely coming from unscheduled deaths is reported in Table E-3 as "extra" and varied across treatment groups.

Table E-3. Difference in pathology by inclusion of unscheduled deaths from DeAngelo et al. (1999)

Dose = prevalence of hepatocellular carcinoma	Number hepatocellular carcinoma/animal	n = at 100 wks	Extra added in
Control = 26%	0.28	50	0
0.05 g/L = 33%	0.58	33	0
0.5 g/L = 48%	0.68	24	1
1 g/L = 71%	1.29	32	3
2 g/L = 95%	2.47	14	7
3.5 g/L = 100%	2.9	8	3

These data show a dose-related increase in tumor formation and decrease in time-to-tumor associated with DCA exposure at the lowest levels examined. These findings are limited

by the small number of animals examined at 100 weeks but especially those examined at "interim sacrifice" periods (n = 10). The data illustrate the importance of examining multiple exposure levels at lower concentrations at longer durations of exposure and with an adequate number of animals to determine the nature of a carcinogenic response.

Preneoplastic and non-neoplastic hepatic changes were reported to have been described previously and summarized as large preneoplastic foci observed at 52 weeks with multiplicities of 0.1, 0.1, 0.2 and 0.16 for 0.5, 1, 2, and 3.5 g/L DCA exposure, respectively. At 100 weeks, all values were reported to be significant (0.03, 0.06, 0.14, 0.27 for 0.5, 1, 2, and 3.5 g/L DCA exposure respectively). Control values were not reported by the authors.

The authors reported that the prevalence and severity of hepatocellular cytomegaly and of cytoplasmic vacuolization with glycogen deposition to be dose-related and considered significant in all dose groups examined when compared to control liver. However, no quantitative data were shown.

The authors reported a severity index of 0 = none, 1 = ≤25%, 2 = 50–75%, and 4 = 75% of liver section for hepatocellular necrosis and report, at 26 weeks, scores (n = 10 animals) of 0.10 ± 0.10, 0.20 ± 0.13, 1.20 ± 0.38, 1.20 ± 0.39, and 1.10 ± 0.28 for control, 0.5, 1, 2, and 3.5 g/L DCA treatment groups, respectively. Thus, there appeared to be a treatment-related, but not dose-related, increase in hepatocellular necrosis that does not involve most of the liver from 1 to 3.5 g/L DCA at this time point. At 52 weeks of exposure, the score for hepatocellular necrosis was reported to be 0, 0, 0.20 ± 0.13, 0.40 ± 0.22, and 1.10 ± 0.43 for control, 0.5, 1, 2, and 3.5 g/L DCA treatment groups, respectively. At 78 weeks of exposure, the score for hepatocellular necrosis was reported to be 0, 0, 0, 0.30 ± 0.21, and 0.20 ± 0.13 for control, 0.5, 1, 2, and 3.5 g/L DCA treatment groups, respectively. Finally, at the final sacrifice time when more animals were examined, the extent of hepatocellular necrosis was reported to be 0.20 ± 0.16, 0.20 ± 0.08, 0.42 ± 0.15, 0.38 ± 0.20, and 1.38 ± 0.42 for control, 0.5, 1, 2, and 3.5 g/L DCA treatment groups, respectively.

Thus, there was no reported increase in hepatocellular necrosis at any exposure period for 0.5 g/L DCA treatment, and the mild hepatocellular necrosis seen at the three highest exposure concentrations at 26 weeks had diminished with further treatment except for the highest dose at up to100 weeks of treatment. Clearly, the pattern of hepatocellular necrosis did not correlate with the dose-related increases in HCCs reported by the authors and was not increased over control at the 0.5 g/L DCA level where there was a DCA-related tumor increase.

The authors cited previously published data and state that CN-insensitive palmitoyl CoA oxidase activity (a marker of peroxisome proliferation) data for the 26-week time point plotted against 100-week HCC prevalence of animals bearing tumors was significantly enhanced at concentrations of DCA that failed to induce "hepatic PCO" activity. The authors reported that neither 0.05 nor 0.5 g/L DCA had any marked effect on PCO activity and that it was "only significantly increased after 26 weeks of exposure to 3.5 g/L DCA and returned to control level

at 52 weeks (data not shown)." In regards to hepatocyte labeling index after treatment for 5 days with tritiated thymidine, the authors reported that animals examined in the dose-response segment of the experiment at 26 and 52 weeks were examined but no details of the analysis were reported. The authors commented on the results from this study and a previous one that included earlier time points of study and stated that there were "no significant alterations in the labeling indexes for hepatocytes outside of proliferative lesions at any of the DCA concentrations when compared to the control values with the exception of 0.05 g/L DCA at 4 weeks (4.8 ± 0.6 vs. 2.7 ± 0.4 control value; data not shown)."

The effects of DCA on body weight, absolute liver weight, and percent liver/body weight were given in Table 2 of the paper for 26, 52, 78, and 100 weeks of exposure. For 52- and 78-week studies, 10 animals per treatment group were examined. Liver weights were not determined for the lowest exposure concentration (0.05 g/L DCA) except for the 100-week exposure period. At 26 weeks of exposure, there was not a statistically significant change in body weight among the exposure groups (i.e., 35.4 ± 0.7, 37.0 ± 0.8, 36.8 ± 0.8, 37.9 ± 0.6, and 34.6 ± 0.8 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). Absolute liver weight was reported to have a dose-related significant increase in comparison to controls at all exposure concentrations examined, with liver weight reaching a plateau at the 2 g/L concentration (i.e., 1.86 ± 0.07, 2.27 ± 0.10, 2.74 ± 0.08, 3.53 ± 0.07, and 3.55 ± 0.1 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). The percent liver/body weight ratio increases due to DCA exposure were reported to have a similar pattern of increase (i.e., 5.25 ± 0.11, 6.12 ± 0.16, 7.44 ± 0.12, 9.29 ± 0.08, and 10.24 ± 0.12% for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). This represented a 1.17-, 1.41-, 1.77-, and 1.95-fold of control percent liver/body weight at these exposures at 26 weeks.

At 52 weeks of exposure, there was not a statistically significant change in body weight among the exposure groups except for the 3.5 g/L exposed group in which there was a significant decrease in body weight (i.e., 39.9 ± 0.8, 41.7 ± 0.8, 41.7 ± 0.9, 40.8 ± 1.0, and 35.0 ± 1.1 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). Absolute liver weight was reported to have a dose-related significant increase in comparison to controls at all exposure concentrations examined with liver weight reaching a plateau at the 2 g/L concentration (i.e., 1.87 ± 0.13, 2.39 ± 0.04, 2.92 ± 0.12, 3.47 ± 0.13, and 3.25 ± 0.24 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). The percent liver/body weight ratio increases due to DCA exposure were reported to have a similar pattern of increase (i.e., 4.68 ± 0.30, 5.76 ± 0.12, 7.00 ± 0.15, 8.50 ± 0.26, and 9.28 ± 0.64% for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively).

For liver weight and percent liver/body weight, there was much larger variability between animals within the treatment groups compared to controls and other treatment groups. There were no differences reported for patterns of change in body weight, absolute liver weight, or percent liver/body weight between animals examined at 26 weeks and those examined at 52 weeks.

At 78 weeks of exposure, there was not a statistically significant change in body weight among the exposure groups except for the 3.5 g/L exposed group in which there was a significant decrease in body weight (i.e., 46.7 ± 1.2, 43.8 ± 1.5, 43.4 ± 0.9, 42.3 ± 0.8, and 40.2 ± 2.2 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). Absolute liver weight was reported to have a dose-related increase in comparison to controls at all exposure concentrations examined, but none were reported to be statistically significant (i.e., 2.55 ± 0.14, 2.16 ± 0.09, 2.54 ± 0.36, 3.31 ± 0.63, and 3.93 ± 0.59 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). The percent liver/body weight ratio increases due to DCA exposure were reported to have a similar pattern of increase over control values but only the 3.5 g/L exposure level was reported to be statistically significant (i.e., 5.50 ± 0.35, 4.93 ± 0.09, 5.93 ± 0.97, 7.90 ± 1.55, and 10.14 ± 1.73% for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively).

Finally, for the animals reported to be sacrificed between 90 and 100 weeks, there was not a statistically significant change in body weight among the exposure groups except for the 2.0 and 3.5 g/L exposed groups in which there was a significant decrease in body weight (i.e., 43.9 ± 0.8, 43.3 ± 0.9, 42.1 ± 0.9, 43.6 ± 0.7, 36.1 ± 1.2, and 36.0 ± 1.3 g for control, 0.05, 0.5, 1, 2, and 3.5 g/L DCA, respectively). Absolute liver weight did not show a dose-response pattern at the two lowest exposure levels but was elevated with the three highest doses with the two highest being statistically significant (i.e., 2.59 ± 0.26, 2.74 ± 0.20, 2.51 ± 0.24, 3.29 ± 0.21, 4.75 ± 0.59, and 5.52 ± 0.68 g for control, 0.05, 0.5, 1, 2, and 3.5 g/L DCA, respectively). The percent liver/body weight ratio increases due to DCA exposure were reported to have a similar pattern of increase over control values but only the 2.0 and 3.5 g/L exposure levels were reported to be statistically significant (i.e., 6.03 ± 0.73, 6.52 ± 0.55, 6.07 ± 0.66, 7.65 ± 0.55, 13.30 ± 1.62, and 15.70 ± 2.16% for control, 0.05, 0.5, 1, 2, and 3.5 g/L DCA, respectively).

It must be recognized that liver weight increases, especially in older mice, will reflect increased weight due to tumor burden and thus, DCA-induced hepatomegaly will be somewhat obscured at the longer treatment durations. However, by 100 weeks of exposure, there did not appear to be an increase in liver weight at the 0.05 and 0.5 g/L exposures, while there was an increase in tumor burden reported. Examination of the 0.5 g/L exposure group from 26 to 100 weeks shows that slight hepatomegaly, reported as either absolute liver weight increase over control or change in percent liver/body ratio, was present by 26 weeks (i.e., 22% increase in liver weight and 17% increase in percent liver/body weight), decreased with time, and while similar at 52 weeks, was not significantly different from control values at 78- or 100-week durations of exposure. However, tumor burden was increased at this low concentration of DCA.

The authors present a figure comparing the number of HCCs per animal at 100 weeks compared with the percent liver/body weight at 26 weeks and show a linear correlation (r^2 = 0.9977). Peroxisome proliferation and DNA synthesis, as measured by tritiated thymidine, were reported to not correlate with tumor induction profiles and were also not correlated with early liver weight changes induced by DCA exposure. Most importantly, in a paradigm that

examined tumor formation after up to 100 weeks of exposure, DCA-induced tumor formation was reported to occur at concentrations that did not also cause cytotoxicity and at levels 20–40 times lower than those used in "less than lifetime" studies reporting concurrent cytotoxicity.

E.2.3.2.7. Carter et al. (2003)

The focus of this study was to present histopathological analyses that included classification, quantification, and statistical analyses of hepatic lesions in male B6C3F$_1$ mice receiving DCA at doses as low as 0.05 g/L for 100 weeks and at 0.5, 1.0, 2.0, and 3.5 g/L for between 26 and 100 weeks. This analysis used tissues from the DeAngelo et al. (1999) (two blocks from each lobe and all lesions found at autopsy).

This study used the following diagnostic criteria for hepatocellular changes. Altered hepatic Foci (AHF) were defined as histologically identifiable clones that were groups of cells smaller than a liver lobule that did not compress the adjacent liver. Large foci of cellular alteration (LFCA) were defined as lesions larger than the liver lobule that did not compress the adjacent architecture [previously referred to as hyperplastic nodules by Bull et al. (1990)] but had different staining. These are not non-neoplastic proliferative lesions termed "hepatocellular hyperplasia" that occur secondary to hepatic degeneration or necrosis. Adenomas showed growth by expansion resulting in displacement of portal triad and had alterations in both liver architecture and staining characteristics. Carcinomas were composed of cells with a high nuclear-to-cytoplasmic ration and with nuclear pleomorphism and atypia that showed evidence of invasion into the adjacent tissue. They frequently showed a trabecular pattern characteristic of mouse hepatocellular carcinomas.

The report grouped lesions as eosinophilic, basophilic and/or clear cell, and dysplastic. "Eosinophilic lesions included lesions that were eosinophilic but could also have clear cell, spindle cell or hyaline cells. Basophilic lesions were grouped with clear cell and mixed cell (i.e., mixed basophilic, eosinophilic, hyaline, and/or clear cell) lesions." The authors reported that:

> this grouping was necessary because many lesions had both a basophilic and clear cell component and a few <10 % had an eosinophilic or hyaline component…Lesions with foci of cells displaying nuclear pleomorphism, hyperchromasia, prominent nucleoli, irregular nuclear borders and/or altered nuclear to cytoplasmic ratios were considered dysplastic irrespective of their tinctorial characteristics.

Therefore, Carter et al. (2003) lumped mixed phenotype lesions into the basophilic grouping so that comparisons with the results of Bull et al. (2002) or Pereira (1996), which segregate mixed phenotype from those without mixed phenotype, cannot be done.

This report examined type and phenotype of preneoplastic and neoplastic lesions pooled across all time points. Therefore, conclusions regarding what lesions were evolving into other

lesions have left out the factor of time. Bannasch (1996) reported that examining the evolution of foci through time is critical for discerning neoplastic progression and described foci evolution from eosinophilic or basophilic lesions to more basophilic lesions. Carter et al. (2003) suggested that size and evolution into a more malignant state are associated with increasing basophilia, a conclusion consistent with those of Bannasch (1996). The analysis presented by Carter et al. (2003) also suggested that there was more involvement of lesions in the portal triad, which may give an indication where the lesions arose. Consistent with the results of DeAngelo et al. (1999), Carter et al. (2003) reported that "DCA (0.05 – 3.5 g/L) increased the number of lesions per animal relative to animals receiving distilled water and shortened the time to development of all classes of hepatic lesions." They also concluded that:

> although this analysis could not distinguish between spontaneously arising lesions and additional lesions of the same type induced by DCA, only lesions of the kind that were found spontaneously in control liver were found in increased numbers in animals receiving DCA…Development of eosinophilic, basophilic and/or clear cell and dysplastic AHF was significantly related to DCA dose at 100 weeks and overall adjusted for time.

The authors concluded that the presence of isolated, highly dysplastic hepatocytes in male B6C3F$_1$ mice chronically exposed to DCA suggested another direct neoplastic conversion pathway other than through eosinophilic or basophilic foci.

It appears that the lesions being characterized as carcinomas and adenomas in DeAngelo et al. (1999) were not the same as those by Carter et al. (2003) at 100 weeks even though they were from the same tissues (see Table E-4). Carter et al. (2003) identified all carcinomas as dysplastic despite tincture of lesion and subdivided adenomas by tincture. If the differing adenoma multiplicities are summed for Carter et al. (2003), they do not add up to the same total multiplicity of adenoma given by DeAngelo et al. (1999).

Table E-4. Comparison of data from Carter et al. (2003) and DeAngelo et al. (1999)

Exposure level of DCA at 79–100 wks (g/L)	Total adenoma multiplicity (Carter)	Total adenoma multiplicity (DeAngelo)	Total carcinoma multiplicity (Carter)	Total carcinoma multiplicity (DeAngelo)	Sum of adenomas and carcinoma multiplicity (Carter)	Sum of adenomas and carcinoma multiplicity (DeAngelo)
0	0.22	0.12	0.05	0.28	0.27	0.40
0.05	0.48	-	<0.025	0.58	~0.50	-
0.5	0.44	0.32	0.20	0.68	0.64	1.0
1.0	0.52	0.80	0.30	1.29	0.82	2.09
2.0	0.60	0.57	1.55	2.47	2.15	3.27
3.5	1.48	0.64	1.30	2.90	2.78	3.54

It is unclear how many animals were included in the differing groups in both studies for pathology. The control and high-dose groups differ in respect to "animals with pathology" between DeAngelo et al. (1999) and the "number of animals in groups" examined for lesions in Carter et al. (2003). Neither report gave how many animals with unscheduled deaths were treated in regards to how the pathology data were included in presentation of results. Given that DeAngelo et al. (1999) represents animals at 100 weeks as also animals from 79 to 100 weeks of exposure, it is probable that the animals that died after 79 weeks were included in the group of animals sacrificed at 100 weeks. However, the number of animals affecting that result (which would be a mix of exposure times) for either DeAngelo et al. (1999) or Carter et al. (2003) is unknown from published reports.

In general, it appears that Carter et al. (2003) reported more adenomas/animal for their 100 week animals than DeAngelo et al. (1999) did, while DeAngelo et al. (1999) reported more carcinomas/animal.

In order to compare these data with others (eg., Pereira and Phelps, 1996) for estimates of multiplicity by phenotype or tincture it would be necessary to add foci and LFCA together as foci, and adenomas and carcinomas together as tumors. It would also be necessary to lump mixed foci together as "basophilic" from other data sets as was done for Carter et al. (2003) in describing "basophilic lesions." If multiplicity of carcinomas and adenomas are summed from each study to control for differences in identification between adenoma and carcinoma, there are still differences in the two studies in multiplicity of combined lesions/animal with DeAngelo et al. (1999) giving consistently higher estimates. However, both studies show a dose response of tumor multiplicity with DCA and a difference between control values and the 0.05 DCA exposure level. Error is introduced by having to transform the data presented as a graph in Carter et al. (2003). Also no SEM is given for the Carter data.

In regard to other histopathological changes, the authors report that:

> necrosis was found in 11.3% of animals in the study and the least prevalent toxic or adaptive response. No focal necrosis was found at 0.5 g/L. The incidence of focal necrosis did not differ from controls at 52 or 78 weeks and only was greater than controls at the highest dose of 3.5 g/L at 100 weeks. Overall necrosis was negatively related to the length of exposure and positively related to the DCA dose. Necrosis was an early and transitory response. There was no difference in necrosis 0 and 0.05 g/L or 0.5 g/L. There was an increase in glycogen at 0.5 g/L at the perioportal area. There was no increase in steatosis but a dose-related decrease in steatosis. Dysplastic LFCA were not related to necrosis indicating that these lesions do not represent, regenerative or reparative hyperplasia. Nuclear atypia and glycogen accumulation were associated with dysplastic adenomas. Necrosis was not related to occurrence of dysplastic adenomas. Necrosis was of borderline significance in relation to presence of hepatocellular carcinomas. Necrosis was not associated with dysplastic LFCAs or Adenomas.

They concluded that "the degree to which hepatocellular necrosis underlies the carcinogenic response is not fully understood but could be significant at higher DCA concentrations (≥1 g/L)."

E.2.3.2.8. Stauber and Bull (1997)

This study was designed to examine the differences in phenotype between altered hepatic foci and tumors induced by DCA and TCA. Male B6C3F_1 mice (7 weeks old at the start of treatment) were treated with 2.0 g/L neutralized DCA or TCA in drinking water for 38 or 50 weeks, respectively. They were then treated with additional exposures (n = 12) of 0, 0.02, 0.1, 0.5, 1.0, or 2.0 g/L DCA or TCA for an additional 2 weeks. Three days prior to sacrifice in DCA-treated mice or 5 days for TCA-treated mice, animals had miniosmotic pumps implanted and administered BrdU.

Immunohistochemical staining of hepatocytes from randomly selected fields (minimum of 2,000 nuclei counter per animal) from five animals per group were reported for 14- and 28-day treatments. It was unclear how many animals were examined for 280- and 350-day treatments from the reports. The percentage of labeled cells in control livers was reported to vary between 0.1 and 0.4% (i.e., fourfold).

There was a reported ~3.5-fold of control level for TCA labeling at a 14-day time period and a ~5.5-fold for DCA. At 28 days, there was ~2.5-fold of control for TCA, but a ~2.3-fold decrease of control for DCA. At 280 days, there was no data reported for TCA, but for DCA, there was a ~2-fold decrease in labeling over control. At 350 days, there were no data for DCA, but a reported ~2.3-fold decrease in labeling of control with TCA. The authors reported that the increases at day 14 for TCA and DCA exposure and the decrease at day 28 for DCA exposure were statistically significant, although a small number of animals were examined. Thus, although there may be some uncertainty in the exact magnitude of change, there was, at most, ~5-fold of control labeling for DCA within after 14 days of exposure that was followed by a decrease in DNA synthesis by day 28 of treatment. These data show that hepatocytes undergoing DNA synthesis represented a small population of hepatocytes with the highest level with either treatment <1% of hepatocytes. Rates of cell division were reported to be less than control for both DCA and TCA by 40 and 52 weeks of treatment.

In this study, the authors reported that there was no necrosis with the 2.0 g/L DCA dose for 52 weeks and concluded that necrosis is a recurring but inconsistent result with chronic DCA treatment. Histological examination of the livers involved in the present study found little or no evidence of such damage or overt cytotoxicity. It was assumed that this effect has little bearing on data on replication rates.

Foci and tumors were combined in reported results, and therefore, cannot be compared the results Bull et al. (2002) or to DeAngelo et al. (1999). Prevalence rates were not reported.

Data were reported in terms of "lesions" with DCA-induced "lesions" containing a number of smaller lesions that were heterogeneous and more eosinophilic with larger "lesions" tending to less numerous and more basophilic. For TCA results using this paradigm, the "lesions" were reported to be less numerous, more basophilic, and larger than those induced by DCA. The DCA-induced larger "lesions" were reported to be more "uniformly reactive to c-Jun and c-Fos but many nuclei within the lesions displaying little reactivity to c-Jun." The authors stated that while most DCA-induced "lesions" were homogeneously immunoreactive to c-Jen and C-Fos (28/41 lesions), the rest were stained heterogeneously. For TCA-induced lesions, the authors reported not difference in staining between "lesions" and normal hepatocytes in TCA-treated animals. Again, of note is that not only were "lesions" comprised of foci and tumors at different stages of progression reported in these results, but that also DCA and TCA results were reported for different durations of exposure.

E.2.3.2.9. **Pereira (1996)**

The focus of this study was to report the dose-response relationship for the carcinogenic activity of DCA and TCA in female B6C3F_1 mice and the characteristics of the lesions. Female B6C3F_1 mice (7–8 weeks of age) were given drinking water with either DCA or TCA at 2.0, 6.67, or 20 mmol/L and neutralized with sodium hydroxide to a pH or 6.5–7.5. The control received 20 mmol/L sodium chloride. Conversion of mmol/L to g/L was as follows: 20.0 mmol/L DCA = 2.58 g/L, 6.67 mmol/L DCA = 0.86 g/L, and 2.0 mmol/L = 0.26 g/L; 20.0 mmol/L TCA = 3.27 g/L, 6.67 mmol/L TCA = 1.10 g/L, and 2.0 mmol/L TCA = 0.33 g/L. The concentrations were reported to be chosen so that the high concentration was comparable to those previously used by us to demonstrate carcinogenic activity. The mice were exposed until sacrifice at 360 (51 weeks), or 576 days (82 weeks) of exposure.

Whole liver was reported to be cut into ~3 mm blocks and along with representative sections of the visible lesions, fixed and embedded in paraffin and stained with H&E for histopathological evaluation of foci of altered hepatocytes, hepatocellular adenomas, and HCCs. The slides were reported to be evaluated blind. Foci of altered hepatocytes in this study were defined as containing six or more cells and hepatocellular adenomas were distinguished from foci by the occurrence of compression at >80% of the border of the lesion.

Body weights were reported to be decreased only the highest dose of DCA from 40 weeks of treatment onward. For TCA, there were only two examination periods (weeks 51 and 82) that had significantly different body weights from control and only at the highest dose. Liver/body weight percentage was reported in comparison to concentration graphically and shows a dose-response for DCA with steeper slope than that of TCA at 360 and 576 days of exposure. The authors reported that all three concentrations of DCA resulted in increased vacuolation of hepatocytes. Such vacuolization was probably due to glycogen removal from tissue processing. Using a score of 1–3 (with 0 indicating the absence of vacuolization,

+1 indicating vacuolated hepatocytes in the periportal zone, + 2 indicating distribution of vacuolated hepatocytes in the midzone, and +3 indicating maximum vacuolization of hepatocytes throughout the liver), the authors also reported that "the extent of vacuolization of the hepatocytes in the mice administered 0, 2.0, 6.67 or 20.0 mmol/l DCA was scored as 0.0, 0.80 ± 0.08, 2.32 ± 0.11, or 2.95 ± 0.05, respectively."

Cell proliferation was reported to be determined in treatment groups containing 10 mice each and exposed to either DCA or TCA for 5, 12, or 33 days with animals implanted with miniosmotic pumps 5 days prior to sacrifice and administered BrdU. Tissues were immunohistochemically stained for BrdU incorporation. At least 2,000 hepatocytes/mouse were reported to be evaluated for BrdU-labeled and unlabeled nuclei and the BrDU-labeling index was calculated as the percentage of hepatocytes with labeled nuclei.

Pereira (1996) reported a dose-related increase in BrDU labeling in 2,000 hepatocytes that was statistically significant at 6.67 and 20.mmol/L DCA at 5 days of treatment but that labeling at all exposure concentrations decreased to control levels by days 12 and 33 of treatment. The largest increase in BrdU labeling was reported to be twofold of controls at the highest concentration of DCA after 5 days of exposure. For TCA, all doses (2.0, 6.67, and 20 mmol/L) gave a similar and statistically significant increase in BrDU labeling by 5 days of treatment (~3-fold of controls) but by days 12 and 33, there were no increases above control values at any exposure level. Given the low level of hepatocyte DNA synthesis in quiescent control liver, these results indicate a small number of hepatocytes underwent increased DNA synthesis after DCA or TCA treatment and that by 12 days of treatment, these levels were similar to control levels in female B6C3F_1 mice.

Incidence of foci and tumors in mice administered DCA or TCA (prevalence or number of animals with tumors of those examined at sacrifice) in this report are given in Tables E-5 and E-6.

Table E-5. Prevalence of foci and tumors in mice administered NaCl, DCA, or TCA from Pereira (1996)

Treatment	N	Foci		Adenomas		Carcinomas	
		Number	%	Number	%	Number	%
82 wks							
20.0 mmol NaCl	90	10	11.1	2	2.2	2	2.2
20.0 mmol DCA	19	17	89.5[a]	16	84.2[a]	5	26.3[a]
6.67 mmol DCA	28	11	39.3[a]	7	25.0[a]	1	3.6
2.0 mmol DCA	50	7	14.0	3	6.0	0	0
20.0 mmol TCA	18	11	61.1[a]	7	38.9[a]	5	27.8[a]
6.67 mmol TCA	27	9	33.3[a]	3	11.1	5	18.5[a]
2.0 mmol TCA	53	10	18.9	4	7.6	0	0
51 wks							
20.0 mmol NaCl	40	0	0	1	2.5	0	0
20.0 mmol DCA	20	8	40.0[a]	7	35[a]	1	5
6.67 mmol DCA	20	1	5	3	15	0	0
2.0 mmol DCA	40	0	0	0	0	0	0
20.0 mmol TCA	20	0	0	2	15.8	5	25[a]
6.67 mmol TCA	19	0	0	3	7.5	0	0
2.0 mmol TCA	40	3	7.5	3	2.5	0	0

[a]$p < 0.05$.

NaCl = sodium chloride control

Table E-6. Multiplicity of foci and tumors in mice administered NaCl, DCA, or TCA from Pereira (1996)

Treatment	N	Foci/mouse	Adenomas/mouse	Carcinomas/mouse
82 wks				
20.0 mmol NaCl	90	0.11 ± 0.03	0.02 ± 0.02	0.02 ± 0.02
20.0 mmol DCA	19	7.95 ± 2.00[a]	5.58 ± 1.14[a]	0.37 ± 0.17[b]
6.67 mmol DCA	28	0.39 ± 0.11[b]	0.32 ± 0.13[b]	0.04 ± 0.04
2.0 mmol DCA	50	0.14 ± 0.05	0.06 ± 0.03	0
20.0 mmol TCA	18	1.33 ± 0.31[a]	0.61 ± 0.22[b]	0.39 ± 0.16[b]
6.67 mmol TCA	27	0.41 ± 0.13[b]	0.11 ± 0.06	0.22 ± 0.10[b]
2.0 mmol TCA	53	0.26 ± 0.08	0.08 ± 0.04	0
51 wks				
20.0 mmol NaCl	40	0	0.03 ± 0.03	0
20.0 mmol DCA	20	0.60 ± 0.22[a]	0.45 ± 0.17[a]	0.10 ± 0.10
6.67 mmol DCA	20	0.05 ± 0.05	0.20 ± 0.12	0
2.0 mmol DCA	40	0	0	0
20.0 mmol TCA	20	0	0.15 ± 0.11	0.50 ± 0.18[b]
6.67 mmol TCA	19	0	0.21 ± 0.12	0
2.0 mmol TCA	40	0.08 ± 0.04	0.08 ± 0.04	0

[a]$p < 0.01$.
[b]$p < 0.05$.

These data show the decreased power of using fewer than 50 mice, especially at shorter durations of exposure. By 82 weeks of exposure, increased adenomas and carcinomas induced by TCA or DCA treatment are readily apparent.

The foci of altered hepatocytes and the tumors obtained from this study were reported to be basophilic, eosinophilic, or mixed containing both characteristics and are shown in Tables E-7 and E-8. DCA was reported to induce a predominance of eosinophilic foci and tumors, with over 80% of the foci and 90% of the tumors in the 6.67 and 20.0 mmol/L concentration groups being eosinophilic. Only approximately half of the lesions were characterized as eosinophilic with the rest being basophilic in the group administered 2.0 mmol/L DCA. The eosinophilic foci and tumors were reported to consistently stained immunohistochemically for the presence of GST-π, while basophilic lesions did not stain for GST-π, except for a few scattered cells or small areas comprising <10% of foci.

Table E-7. Phenotype of foci reported in mice exposed to NaCl, DCA, or TCA by Pereira (1996)

Treatment at 51 and 82 wks	N	% Foci		
		Basophilic	Eosinophilic	Mixed
20.0 mmol NaCl	10	70	30	0
20.0 mmol DCA	150	3.3	96.7	0
6.67 mmol DCA	11	18.2	81.8	0
2.0 mmol DCA	7	42.8	57.2	0
20.0 mmol TCA	22	36.4	54.6	9.1
6.67 mmol TCA	11	45.5	54.5	0
2.0 mmol TCA	13	38.5	61.5	0

Table E-8. Phenotype of tumors reported in mice exposed NaCl, DCA, or TCA by Pereira (1996)

Treatment at 51 and 82 wks	N	Tumors		
		Basophilic	Eosinophilic	Mixed
20.0 mmol NaCl	4	50	25	25.5
20.0 mmol DCA	105	2.9	96.1	1
6.67 mmol DCA	10	10	90	0
2.0 mmol DCA	3	0	100	0
20.0 mmol TCA	18	61.1	22.2	16.7
6.67 mmol TCA	6	100	0	0
2.0 mmol TCA	4	100	0	0

The foci of altered hepatocytes in the TCA treatment groups were approximately equally distributed between basophilic and eosinophilic in tincture. However, the tumors were predominantly basophilic, lacking GST-pi (21 of 28 or 75%) including all 11 HCCs. The limited numbers of lesions (i.e., 14) in the sodium chloride (vehicle control) group were characterized as 64.3, 28.6, and 7.1% basophilic, eosinophilic, and mixed, respectively.

These data for female B6C3F_1 mice show that DCA and TCA treatment induced a mixture of basophilic or eosinophilic foci. The pooling of the data between time and adenoma vs. carcinoma decreases the ability to ascertain the phenotype of tumor due to treatment or the progression of phenotype with time as well as the small number of tumor examined at lower exposure concentrations. Foci that occurred at 51 and 82 weeks were presented as one result. Adenoma and carcinoma data were pooled as one endpoint (n = number of total foci or tumors examined). Therefore, evolution of phenotype between less to more malignant stages of tumor were lost.

E.2.3.2.10. Pereira and Phelps (1996)

The focus of this study was to determine tumor response and phenotype in methyl nitrosourea (MNU)-treated mice after DCA or TCA exposure. The concentrations of DCA or TCA were the same as Pereira (1996). For Pereira (1996), the animals were reported to be 7–8 weeks of age when started on treatment and sacrificed after 360 or 576 days of exposure (51 or 82 weeks). For this study and Tao et al. (2004b), animals were reported to be 6 weeks of age when exposed to DCA or TCA via drinking water and to be 31 or 52 weeks of age at sacrifice. Thus, exposure time would be ~24 or 45 weeks. A control group of non-MNU treated animals was presented for female B6C3F_1 mice treated for 31 or 52 weeks and are discussed in Table E-9.

Table E-9. Multiplicity and incidence data (31 week treatment) from Pereira and Phelps (1996)

Treatment	Number	Foci/mouse	incidence %	Adenomas/mouse	incidence %
20.0 mmol NaCl	15	0.13 ± 0.13	6.7	0.13 ± 0.13	not reported
20.0 mmol DCA	10	0.40 ± 0.16	40	0	0
6.67 mmol DCA	10	0.10 ± 0.10	10	0	0
2.0 mmol DCA	15	0	0	0	0
20.0 mmol TCA	10	0	0	0	0
6.67 mmol TCA	10	0	0	0	0
2.0 mmol TCA	15	0	0	0	0

Although this paradigm appears to be the same paradigm as those reported in Pereira (1996), fewer animals were studied. The number of animals in each group varied between 8 controls and 14 animals in the 2.0 mmol/L treatment groups. In mice that were not treated with MNU but were treated with either DCA or TCA at 31 weeks, there were no reported statistically significant treatment-related effects upon the yield of foci or altered hepatocytes and liver tumors but the number of animals examined was small and therefore, of limited power to detect a response. The results below indicate a DCA-related increase in foci and percentage of mice with foci.

See Section E.4.2.3 for further discussion of the results of co-exposures to MNU and DCA or TCA from this study.

E.2.3.2.11. Ferreira-Gonzalez et al. (1995) HCCs induced by TCA or DCA in male B6C3F_1 mice. Mice (28-day

The focus of this study was the investigation of differences in H-ras mutation spectra in old) were exposed for 104 weeks to 0. 1.0, or 3.5 g/L DCA or 4.5 g/L TCA that was pH adjusted. Tumors observed from this treatment were diagnosed as either hepatocellular adenomas or carcinomas. DNA was extracted from either spontaneous, DCA- or TCA-induced HCCs.

Samples for analysis were chosen randomly in the treatment groups, of which 19% of untreated mice had spontaneous liver HCCs (0.26 carcinomas/animal).

DCA treatment induced 100% prevalence at 3.5 g/L (5.06 carcinomas/animal) and 70.6% carcinomas at 1.0 g/L (1.29 carcinomas/animal). TCA treatment was reported to induce 73.3% prevalence at 4.5 g/L (1.5 carcinomas/animal). The number of samples analyzed was 32 for spontaneous carcinomas, 33 for mice treated with 3.5 g/L DCA, 13 from mice treated with 1.0 g/DCA, and 11 from mice treated with 4.5 g/L TCA.

This study has the advantage of comparison of tumor phenotype at the same stage of progression (HCC), for allowance of the full expression of a tumor response (i.e., 104 weeks), and an adequate number of spontaneous control lesions for comparison with DCA or TCA treatments. However, tumor phenotype at an endstage of tumor progression reflects of tumor progression and not earlier stages of the disease process.

There were no ras mutations detected except at H-61 in DNA from spontaneously arising tumors of control mice. Only 4/57 samples from carcinogen-treated mice were reported to demonstrate mutation other than in the second exon of H-ras. In spontaneous liver carcinomas, 58% were reported to show mutations in H-61 as compared with 50% of tumor from 3.5 g/L DCA-treated mice and 45% of tumors from 4.5.g/L TCA-treated mice. Thus, there was a heterogeneous response for this phenotypic marker for the spontaneous, DCA-, and TCA-treatment induced HCCs.

All samples positive for mutation in the exon 2 of H-ras were sequenced for the identification of the base change responsible for the mutation. The authors noted that H-ras mutations occurring in spontaneously developing HCCs from $B6C3F_1$ male mice are largely confined to codon 61 and involve a change from CAA to either AAA or CGA or CTA in a ratio of 4:2:1. They noted that in this study, all of the H-ras second codon mutations involved a single base substitution in H-61 changing the wild-type sequence from CAA to AAA (80%), CGA (20%), or CTA for the 18 HCCs examined.

In the 16 HCCs from 3.5 g/L DCA treatment with mutations, 21% were AAA transversions, 50% were CGA transversions, and 29% were CTA transversions. For the six HCCs from 1.0 g/L DCA with mutations, 16% were an AAA transversion, 50% were a CGA transversion, and 34% were a CTA transversion. For the five HCCs from 4.5 g/L TCA with mutations, 80% were AAA transversions, 20% CGA tranversions, and 0% were CTA transversions. The authors note that the differences in frequency between DCA and TCA base substitutions did not achieve statistical significance due to the relatively small number of tumors from TCA-treated mice. They note that the finding of essentially equal incidence of H-ras mutations in spontaneous tumors and in tumors of carcinogen-treated mice did not help in determining whether DCA and TCA acted as “genotoxic” or “nongenotoxic” compounds.

E.2.3.2.12. **Pereira et al. (2004a)**

Pereira et al. (2004a) exposed 7–8-week-old female B6C3F$_1$ mice treated with "AIN-76A diet" to neutralized 0 or 3.2 g/L DCA in the drinking water and 4.0 or 8.0 g/kg L methionine added to their diet. The final concentration of methionine in the diet was estimated to be 11.3 and 15.3 g/kg. Mice were sacrifice 8 and 44 weeks after exposure to DCA, with body and liver weights evaluated for foci, adenomas, and HCCs. No histological descriptions were given by the authors other than tinctorial phenotype of foci and adenomas for a subset of the data. The number of mice examined was 36 for the DCA + 8.0 g/kg methionine or 4.0 g/kg methionine group sacrificed at 44 weeks. However, for the DCA-only treatment group, the number of animals examined was 32 at 44 weeks and for those groups that did not receive DCA but either methionine at 8.0 or 4.0 g/kg, there were only 16 animals examined. All groups examined at 8 weeks had eight animals per group.

Liver glycogen was reported to be isolated from 30 to 50 mg of whole liver. Peroxisomal acyl-CoA oxidase activity was reported to be determined using lauroyl-CoA as the substrate and was considered a marker of peroxisomal proliferation. Whole-liver DNA methylation status was analyzed using a 5-MeC antibody.

Methionine (8.0 g/kg) and DCA co-exposure was reported to result in the death of three mice, while treatment with methionine (4.0 g/kg) and DCA or methionine (8.0 g/kg) alone was reported to kill one mouse in each group. The authors reported that "There was an increased in body weight during weeks 12 to 36 in the mice that received 8.0 g/kg methionine without DCA. There was no other treatment-related alteration in body weight." However, the authors do not present the data and initial or final body weights were not presented for the differing treatment groups.

DCA treatment was reported to increase percent liver/body weight ratios at 8 and 44 weeks to about the same extent (i.e., ~2.4-fold of control at 8 weeks and 2.2-fold of control at 44 weeks). Methionine co-exposure was reported to not affect that increase (~2.4-, 2.2-, and 2.1-fold of control after DCA treatment alone, DCA/4 g/kg methionine, and DCA/8 mg/kg methionine treatment for 8 weeks, respectively). There was a slight increase in percent liver/body weight ratio associated with 8.0 g/kg methionine treatment alone in comparison to controls (~7%) at 8 weeks with no difference between the two groups at 44 weeks.

After 8 weeks of only DCA exposure, the amount of glycogen in the liver was reported to be ~2.09-fold of the value for untreated mice (115 vs. 52.5 mg/g glycogen in treated vs. control, respectively, at 8 weeks). Both 4 and 8 g/kg methionine co-exposure reduced the amount of DCA-induced glycogen increase in the liver (~1.64-fold of control for DCA/4.0 g/kg methionine and ~1.54-fold of control for DCA/8.0 mg/kg methionine). Thus, for treatment with DCA alone or with the two co-exposure levels of methionine, the magnitude of the increase in liver weight was greater than that of the increase in liver glycogen (i.e., 2.42- vs. 2.09-fold of control percent liver/body weight vs. glycogen content for DCA alone, 2.20- vs. 1.64-fold of control percent

liver/body weight vs. glycogen content for DCA/4.0 g/kg methionine, 2.10- vs. 1.54-fold of control percent liver/body weight vs. glycogen content for DCA/8.0 g/kg methionine). Thus, the magnitudes of treatment-related increases were higher for percent liver/body weight than for glycogen content in these groups.

In regard to percentage of liver mass that glycogen represented, the control value for this study is similar to that presented by Kato-Weinstein et al. (2001) in male mice (~60 mg glycogen/g liver) and represents ~6% of liver mass. Therefore, a doubling of the amount of glycogen is much less than the twofold increases in liver weight observed for DCA exposure in this paradigm. These data suggest that DCA-related increases in liver weight gain are not only the result of increased glycogen accumulation, and that methionine co-exposure is affecting glycogen accumulation to a much greater extent than the other underlying processes that are contributing to DCA-induced hepatomegaly after 8 weeks of exposure. The authors reported that 8-weeks of DCA exposure alone did not result in a significant increase in cell proliferation as measured by PCN index (neither data nor methods were shown). This is consistent with other data showing that DCA effects on DNA synthesis were transient and had subsided by 8 weeks of exposure.

The levels of lauroyl-CoA oxidase activity were reported to be increased (~1.33-fold of control) by DCA treatment alone at 8 weeks and to be slightly reduced by 8 g/kg methionine treatment alone (~0.83-fold of control). Methionine co-exposure was reported to have little effect on DCA-induced increases in lauroyl-CoA oxidase activity. The levels of DNA methylation were reported to be increased by 8.0 g/kg methionine only treatment at 8 weeks ~1.32-fold of control, and reduced by DCA only treatment to ~0.44-fold of control. DCA and 4.0 g/kg methionine co-exposure gave similar results as controls (within 2%). Co-exposures of DCA and 8.0 g/kg methionine treatments were reported to increase DNA methylation 1.22-fold of controls after 8 weeks of co-exposure.

In the 44-week study, the authors reported that foci and hepatocellular adenomas were found. However, the authors do not report the incidences of these lesions in their study groups (how many of the treated animals developed lesions). As noted above, the numbers of animals in these groups varied widely between treatments (e.g., n = 36 for DCA and co-exposure to 8.0 g/kg methionine but only n = 16 for 8 g/kg methionine treatment alone). Although reporting unscheduled deaths in the 8.0 g/kg methionine and DCA co-exposure groups, the authors did not indicate whether these mortalities occurred in the 44- or 8-week study groups.

Multiplicities of foci and adenoma data were presented. DCA was reported to induce 2.42 ± 0.38 foci/mouse and 1.28 ± 0.31 adenomas/mouse (mean ± SE) after 44 weeks of treatment. The DCA-induced foci and adenomas were reported to stain as eosinophilic with "relatively large hepatocytes and nuclei." The authors did not present data on the percent of foci and adenomas that were eosinophilic using this paradigm. The addition of 4.0 or 8.0 g/kg methionine to the AIN-76A diet was reported to reduce the number of DCA-induced

adenomas/mouse to 0.167 ± 0.093 and 0.028 ± 0.028, respectively. However, the addition of 4.0 g/kg methionine to the DCA treatment was reported to increase the number of foci/mouse (3.4 ± 0.46 foci/mouse). The addition of 8.0 g/kg methionine to the DCA treatment was reported to yield 0.94 ± 0.24 foci/mouse. There were no foci or tumors in the 16 mice that received either the control diet or the 8.0 g/kg methionine treatment without DCA. The authors did not report whether methionine treatment had an effect on the tincture of the foci or adenomas induced by DCA.

Therefore, a very high level of methionine supplementation to an AIN-760A diet, was shown to affect the number of foci and adenomas (i.e., decrease them) after 44 weeks of co-exposure to very high exposure concentration of DCA. However, a lower level of methionine co-exposure increased the incidence of foci at the same concentration of DCA. Methionine treatment alone at the 8 g/kg level was reported to increase liver weight, decrease lauroyl-CoA activity, and increase DNA methylation.

No histopathology was given by the authors to describe the effects of methionine alone. Co-exposure of methionine with 3.2 g/L DCA was reported to decrease by ~25% DCA-induced glycogen accumulation and increase mortality, but not to have much of an effect on peroxisome enzyme activity (which was not elevated by >33% over control for DCA exposure alone). The authors suggested that their data indicate that methionine treatment slowed the progression of foci to tumors. Whether these results would be similar for lower concentrations of DCA and lower concentrations of methionine that were administered to mice for longer durations of exposure cannot be ascertained from these data. It is possible that in a longer-term study, the number of tumors would be similar. Whether methionine treatment co-exposure had an effect on the phenotype of foci and tumors was not presented by the authors in this study. Such data would have been valuable to discern if methionine co-exposure at the 4.0 mg/kg level that resulted in an increase in DCA-induced foci, resulted in foci of a differing phenotype or resulted in a more heterogeneous composition than DCA treatment alone.

E.2.3.2.13. **DeAngelo et al. (2008)**

In this study, neutralized TCA was administered in drinking water to male B6C3F$_1$ mice (28–30 days old) in three studies. In the first study, control animals received 2 g/L sodium chloride while those in the second study were given 1.5 g/L neutralized acetic acid (HAC) to account for any taste aversion to TCA dosing solutions. In a third study, deionized water served as the control.

No differences in water uptake were reported. Mean initial weights were reported to not differ between the treatment groups (19.5 ± 2.5 g – 21.4 ± 1.6 g or ~10% difference). The first study was reported to be conducted at the U.S. EPA laboratory in Cincinnati, Ohio in which mice were exposed to 2 g/L sodium chloride, or 0.05, 0.5, or 5 g/L TCA in drinking water for 60 weeks. There were five animals at each concentration that were sacrificed at 4, 15, 31, and

45 weeks with 30 animals sacrificed at 60 weeks of exposure. There were 3 unscheduled deaths in the 0.05 g/L TCA group leaving 27 mice at final necropsy. For the other exposure groups, there were 29 or 30 animals at final necropsy.

In the second study, also conducted in the same laboratory, mice were reported to be exposed to 1.5 g/L neutralized acetic acid or 4.5 g/L TCA for 104 weeks. Serial necropsies were conducted (5 animals per group) at 15, 30, and 45 weeks of exposure and 10 animals in the control group at 60 weeks. For this study, a total of 25 animals were sacrificed in interim necropsies in the 1.5 g/L HAC group and 15 in the 4.5 g/L TCA group. There were 7 unscheduled deaths in the HAC group and 12 in the 4.5 g/L TCA group, leaving 25 and 30 animals in the final necropsy groups, respectively.

Study 3 was conducted at the U.S. EPA laboratory in Research Triangle Park, North Carolina. Mice were exposed to deionized water or 0.05 or 0.5 g/L TCA in the drinking water for 104 weeks with serial necropsies (n = 8 per group) conducted at 26, 52, and 78 weeks. There were 19–21 animals reported at interim sacrifices and 17 unscheduled deaths in the deionized water group, 24 unscheduled deaths in the 0.05 g/L TCA group, and 24 unscheduled deaths in the 0.5 g/L TCA group. This left 34 mice at final necropsy in the control group, 29 mice in the 0.05 g/L TCA group, and 27 mice in the 0.5 g/L group.

At necropsy, liver, kidneys, spleen, and testes weights were reported to be taken and organs examined for gross lesions. Tissues were prepared for light microscopy and stained with H& E. At termination of the exposure periods, a complete rodent necropsy was reported to be performed. Representative blocks of tissue were examined only in five mice from the high-dose and control groups with the exception of gross lesions, liver, kidney, spleen, and testis at interim and terminal sacrifices. If the number of any histopathologic lesions in a tissue was "significantly increased above that in control animals," then that tissue was reported to be examined in all TCA dose groups.

For Study #3, a second contract pathologist reviewed 10% of the described hepatic lesions. No "major differences" were reported between the two pathologic diagnoses.

The prevalence and multiplicity of hepatic tumors were reported to be derived by performing a histopathologic examination of surface lesions and four sections cut from each of four tissue blocks excised from each liver lobe. Tumor prevalence was reported to be calculated as the percentage of the animals with a neoplastic lesion compared to the number of animals examined. Tumor multiplicity was reported to be calculated by dividing the number of each lesion or combined adenomas and carcinomas by the number of animals examined. Preneoplastic large foci of cellular alteration were also observed over the course of the study.

The prevalence and severity of hepatocellular cytoplasmic alterations, inflammation, and necrosis were reported to be determined using a scale based on the amount of liver involved of 1 = minimal (occupying 25%), 2 = mild (occupying 25–50%), 3 = moderate (occupying 50–

75%), and 4 = marked (occupying >75%). The only "significant change outside of the liver" was reported to be testicular degeneration.

LDH was determined in arterial blood collected at 30 and 60 weeks (Study 1) and 4, 30, and 104 weeks (Study 2). Cyanide insensitive PCO was also reported to be measured. Five days prior to sacrifice, tritiated thymidine (Studies 1 and 2) or BrdU (Study 3) was administered via miniosmotic pumps and the number of hepatocyte nuclei with grain counts >6 were scored in 1,000 cells or chromogen pigment over nuclei (BrdU). The labeling index was calculated by dividing the number of labeled hepatocyte nuclei by the total number of hepatocytes scored.

Total neoplastic and preneoplastic lesions (multiplicity) were counted individually or combined (adenomas and carcinomas) for each animal. The analysis of tumor prevalence data was reported to include only those animals examined at the scheduled necropsies or animals surviving to week 60 (Study 1) or longer than 78 weeks (Studies 2 and 3). The data from all of the scheduled necropsies were combined for an overall test of treatment-related effect.

For Study #1 (60-week exposure), all TCA-treated groups experienced a decrease in drinking water consumption, with the decreases in drinking water for the 0.5 and 5 g/L TCA exposure groups reported as statistically significant by the authors. The water consumption in mL/kg-day was reported to be reduced by 11, 17, and 30% in the 0.05, 0.5, and 5 g/L TCA treated groups compared to 2 g/L sodium chloride control animals as measured by time-weighted mean daily water consumption measured over the study. The control value was reported to be 171 mL/kg/day. Although the 0.05 g/L exposure concentrations were not measured, the 0.5 and 5 g/L solutions were within 4% of target concentrations. The authors estimated that the mean daily doses were 0, 8, 68, and 602 mg/kg-day.

For the 102-week studies, the mean water consumption with deionized water was reported to be 112 and 132 mL/kg-day for control animals given 1.5 g/L HAC. Therefore, there appeared to be a 35% decrease in water consumption between the controls in Study #1 given 2 g/L sodium chloride and controls in Study #3 given deionized water but conducted at a different laboratory. There appeared to be a 23% reduction in water consumption between animals given 2 g/L sodium chloride and those given 1.5 g/L HAC at the same laboratory (Study #2).

As the concentrations of TCA were increased, there would be a corresponding increase in the amount of sodium hydroxide needed to neutralize the solutions and a corresponding increase in salts in the solution as well as TCA. The authors did not address nor discuss the differences in drinking water consumption between the differing control solutions between the studies.

DeAngelo et al. (1999) reported mean drinking water consumption of 147 mL/kg/day in control mice of over 100 weeks and that the highest dose of DCA (3.5 g/L) reduced drinking water consumption by 26%. Carter et al. (1995) reported that DCA at 5 g/L decreased drinking water consumption by 64 and 46%, but 0.5 g/L DCA did not affect drinking water consumption. In this study, while reporting that Study #1 showed that increasing TCA concentration decreased

drinking water consumption, the drinking water consumption in Studies #2 and #3 were similar between controls and TCA exposure groups with both being less than the control and low TCA concentration values reported in Study #1 (i.e., in Study #2, the 1.5 g/L HAC and 4.5 g/L TCA drinking water consumption was ~130 mL/kg/day and in Study #3, the drinking water consumption was ~112 mL/kg/day for the deionized water control and 0.05 and 0.5 g/L TCA exposure groups). Thus, the drinking water concentrations for Study #3 was ~35% less than for the control values for Study #1 and was also ~25% less than for DeAngelo et al. (1999). The reasons for the apparently lower drinking water averages for Study #3 and the lack of effect of the addition of 0.5 g/L TCA that was reported in Study #1 and in other studies, was not discussed by the authors.

In Study #1, there was little difference between exposure groups (n = 5) noted for the final body weights (mean range of 27.6–28.1 g) in mice sacrificed after 4 weeks of exposure. However, absolute liver weight and percent liver/body weight ratios increased with TCA dose. The percent liver/body weight ratios were 5.7 ± 0.4, 6.2 ± 0.3, 6.6 ± 0.4, and 7.7 ± 0.6% for the 2 g/L sodium chloride control, 0.05, 0.5, and 5 g/L TCA exposure groups, respectively. These represent 1.09-, 1.16-, and 1.35-fold of control levels that were statistically significant.

At 15 weeks of exposure the fold increases in percent liver/body weight ratios were 1.14-, 1.16-, and 1.47-fold of controls for 0.05, 0.5, and 5 g/L TCA. At 31 weeks of exposure, the fold increases in percent liver/body weight ratios were 0.98-, 1.09-, and 1.59-fold of controls for 0.05, 0.5, and 5 g/L TCA. At 45 weeks of exposure, the fold increases in percent liver/body weight ratios were 1.13-, 1.45-, and 1.98-fold of controls for 0.05, 0.5, and 5 g/L TCA. At 60 weeks of exposure, the percent liver/body weight ratios were 0.94-, 1.25-, 1.60-fold of controls for 0.05, 0.5, and 5 g/L TCA.

Thus, the range of increase at the lowest level of TCA exposure (i.e., 0.05 g/L) was 0.94–1.14-fold of controls. These data consistently show TCA-induced increases in liver weight from 4 to 60 weeks of the study that were dose-related. For the 0.5 g/L exposure group, the magnitude of the increase compared to control was reported to be about the same between weeks 4 and 30, with the highest increase reported to be at week 45 (1.45-fold of control). In regard to the correspondence with magnitude of difference in dose of TCA and liver weight increase, there was ~2-fold increase in liver weight gain corresponding to 10-fold increases in TCA concentration at 4 weeks of exposure. For the 4- and 15-week exposures, there were ~3.3- and 3.9-fold difference in liver weight that corresponded to a 100-fold difference in exposure concentration of TCA (i.e., 0.05 vs. 5.0 g/L TCA).

The small number of animals examined, n = 5, limit the power of the study to determine the change in percent liver/body weight up to 45 weeks, especially at the lowest dose. However, the 0.05 g/L TCA exposure groups at 4 and 15 weeks were reported to significantly increased percent liver/body weight ratios.

The percent liver/body weight ratios for all of the treatment groups and the ability to detect significant changes were affected by changes in final body weight and changing numbers of animals. After 4–30 weeks of exposure, the final body weights of mice increased in control animals but were within 11% of each other between weeks 31 and 60. The percent liver/body weight ratios in controls decreased from 4 to 31 weeks and were slightly elevated by 60 weeks compared to the 31-week level. Although control values were changing, there appeared to be no difference between control values and treated values in final body weight for any duration of exposure with the exception of the 5 g/L TCA exposure group after 60 weeks of exposure, which was decreased by ~15%. At the 31- and 60-week exposure durations, the 0.05 g/L TCA groups did not have increased percent liver/body weight ratios over controls.

In Study #2, conducted in the same laboratory but with a 1.5 g/L HAC solution used for control groups, there was <5% difference in final body weights between control mice give HAC and those treated with 4.5 g/L TCA up to 45 weeks. However, final body weight was reduced by TCA treatment by 104 weeks by ~15%. Between the interim sacrifices of 15, 30, and 45 weeks, the percent liver/body weight ratios in control mice were similar at 15 and 45 weeks (~4.8%) but greater in the 30-week control group (5.3 or ~10% greater than other interim control groups). The TCA-induced increases in body weight were 1.60-, 1.40-, and 1.79-fold of control for the 15-, 30-, and 45-week groups exposed to 4.5 g/L TCA in Study #2. The smaller magnitude of TCA-induced liver weight increase at 30 weeks than that for 15 and 45 weeks, was a reflection of the increased percent liver/body weight ratio reported for the HAC control at that time point.

Comparisons can be made between Studies #1 and #2 for 4.5 or 5.0 g/L TCA exposure levels and controls for 15, 30/31, and 45 weeks of exposure to ascertain the consistency of response from the same laboratory. Although the two studies had differing control solutions and reported different drinking water consumption overall, they were exposing the TCA groups to almost the same concentration of TCA in the same buffered solutions for the same periods of time with the same number of mice per group.

Between Studies #1 and #2, there were consistent percent liver/body weight ratios induced by either 5.0 or 4.5 g/L TCA at weeks 15 and 30/31 (i.e., within 3% of each other). The percent liver/body ratios for these exposure groups ranged from 7.3 to 7.7% between weeks 15 and 30/31 for the ~5.0 g/L TCA exposure in both studies. Final body weights were within 10%. While the percent liver/body weight ratios induced by ~5.0 g/L TCA were similar, the magnitude of increase in comparison to the controls was 1.47- and 1.59-fold of control for Study #1, and 1.60- and 1.40-fold of control for Study #2 after 15 and 30/31 weeks of exposure, respectively. At 45 weeks, the percent liver/body weight ratios were within 11% of each other (9.4 vs. 8.4%) and final body weights were within 2% of each for this exposure concentration between the two studies giving a 1.98- and 1.79-fold of control percent liver/body weight, respectively. Thus, the apparent magnitude of TCA-induced increase in percent liver/body weight was affected by control values used as the basis for comparison. The percent liver/body weights reported for

either 4.5 or 5.0 g/L TCA exposure groups for weeks 15 and 30/31 was similar between the two studies conducted in the same laboratory.

Study #3 was conducted in a separate laboratory, interim sacrifice times were not the same as for Study #1, the number of animals examined differed (n = 5 for Study #1 and n = 8 for Study #3), and control animals studied for comparative purposes were given different drinking water solutions (deionized water vs. 2 g/L sodium chloride). Most importantly, the body weights reported at 52 weeks were much greater than that reported at 45 weeks for Studies #1 and #2.

However, a comparison of TCA-induced liver weight gain and the effects of final body weight can be made between the 0.05 and 0.5 g/L TCA exposure groups at 30 weeks (Study #1) and 26 weeks (Study #3), at 45 weeks and 60 weeks (Study #1), and 52 weeks (Study #3). At 31 weeks, there was <2% difference in mean final body weights between control and the two TCA-treatment groups in Study #1. There was also little difference between the TCA-treated groups at week in Study #3 at week 26 and the TCA treatment groups in at week 31 in Study #1 (i.e., range of 42.6–43.5 g for 0.05 and 0.5 g/L TCA treatments in Studies #1 and #3). However, in Study #3, the control value was 12% lower than that of Study #1 for mean final body weight. Based on final body weights, there would be an expectation of similar results between the two studies at the 26- and 30-week time points.

At the 45-week (Study #1), 52-week (Study #3), and 60-week (Study #1) durations of exposure, the mean final body weights varied little between their corresponding control groups at each sacrifice time (<4% variation between control and TCA-treated groups). However, there was variation in mean final body weights between the differing sacrifice times. Control and TCA-treated groups were reported to have lower mean final body weights at 45 weeks of exposure in Study #1 than at either 30 or 60 weeks. The 45-week mean final body weights in Study #1 were also reported to be lower than those at 52 weeks in Study #3. Control mean body weight values were 28% higher at 52 weeks in Study #3 than 45 weeks in Study #1 and 15% higher for 60 weeks in Study #1. In essence, for Study #1, mean final body weights went down between 31 and 45 weeks of exposure and then went back up at 60 weeks of exposure for control mice (~43, ~40, and ~44 g for 31, 45, and 60 weeks, respectively) as well as for both TCA concentrations. However, for Study #3, final mean body weights went up between 26 and 52 weeks of exposure for control mice (~39 vs. ~51 g) and for both TCA concentrations.

While for Study #1, the percent liver/body weight ratios were 0.98- and 1.09-fold of control at 31 weeks of exposure, at week 45, the ratios were 1.13- and 1.45-fold of control, and at week 60, they were 0.94- and 1.25-fold of controls for the 0.05 and 0.5 g/L TCA exposure levels, respectively. For Study #3, the pattern differed than that of Study #1. There was a 1.07- and 1.18-fold of control percent liver/body weight for 26 weeks but a 0.92- and 1.04-fold of control percent liver/body weight change at 52 weeks of exposure at 0.05 and 0.5 g/L TCA exposure, respectively.

Thus, there appeared to be differences in control and the treatment groups at the 26-week sacrifice groups in Study #3 that was not apparent at the 52-week sacrifice time. Overall, the final body weights appeared to be similar between controls and TCA treatment groups at the 52-week sacrifice time in Study #3 and at the 31-, 45-, and 60-week sacrifice times in Study #1. However, although consistent within sacrifice times, the final body weights differed between the various sacrifice times in Studies #1 and #3. The patterns of percent liver/body weight at differing and similar sacrifice times appeared to differ between the Studies #1 and #3 at the same concentrations of TCA. The largest difference appeared to be between week 45 group in Study #1 and week 52 group in Study #3 where both concentrations of TCA were reported to induce increases in percent liver/body weight in one study but to have little difference in the other. The differences in mean final body weights between these two sacrifice times were also the largest although control and TCA-treatment groups had little difference on this parameter. Similar to the work of Kjellstrand and colleagues with TCE (Kjellstrand et al., 1983a), the groups with the lower body weight appeared to have the greatest response in liver weight increase.

These data illustrate the variability in findings of percent liver weight induction between laboratories, studies, choice of controls solutions, and the effects of final body weights on this parameter. They also illustrate the limitations for determining either the magnitude or pattern of liver weight increases using a small number of test animals. As animals age, the size of their liver changes, but also during the latter parts of the lifespan, foci and spontaneously occurring liver tumors can affect liver weight. The results of Study #1 show a consistent dose-response in TCA liver weight increases at 4- and 15-week time periods over a range of concentration from 0.05 to 5 g/L TCA.

In regard to non-neoplastic pathological changes, the authors reported that:

> Increased incidences and severity of centrilobular cytoplasmic alterations, inflammation, and necrosis were the only nonproliferative changes seen in livers of animals exposed to TCA for 60 weeks (Tables 7-9; Study 1. Incidences were between 21 and 93%; severity ranged from minimal to mild; and some lesions were transient. Centrilobular cytoplasmic alterations (Table 7) were the most prominent nonproliferative lesion. The incidence and severity were dose related and significantly increased at all TCA concentrations. Centrilobular alterations are a low-grade degeneration of the hepatocytes characterized by an intense eosinophilic cytoplasm with deep basophilic granularity (microsomes) and slight hepatomegaly. The distribution ranged from centrilobular to diffuse. The incidence of inflammation was increased significantly in the 5 g/L TCA treatment group (Table 8), but was significantly lower in the 0.05- and 0.5 g/L groups between 31 and 45 weeks, but abated by 60 weeks. There was a significant dose-related trend, but a significant increase in severity was only found at 5 g/L. No alteration in the severity of this lesion was observed. The occurrence and severity of nonproliferative lesions in animals exposed to 0.5 and 4.5 g/L TCA for 104 weeks were similar to those observed at 60 weeks (data not shown). No

> pathology outside the liver was observed except for a significant dose-related trend and incidence of testicular tubular degeneration at 0.5 and 5 g/L TCA.

The results shown in Table 7 by the authors for the 60-week TCA-exposed mice did not show a dose-response for either incidence or severity of centrilobular cytoplasmic alterations. They reported a 7, 48, 21, and 93% incidence and a 0.10 ± 0.40, 0.70 ± 0.82, 0.34 ± 0.72, and 1.60 ± 0.62 mean severity score for control, 0.05, 0.5, and 5.0 g/L TCA exposure groups, respectively. Thus, for control, 0.05, and 0.5 g/L TCA exposure, there was less than minimal (i.e., score of 1 or occupying <25% of the microscopic field) severity of this finding for the 27–30 mice examined in each group. Only slight hepatomegaly is noted by the authors to be included in their description of the centrilobular cytoplasmic alteration. Interestingly, the elevation of this parameter for both incidence and severity in the 0.05 g/L TCA exposed group compared to 0.5 g/L exposure group did not correspond to an increase in percent liver/body weight for this same exposure group. While the percent liver/body weight ratio was 32% higher, the incidence and severity of this lesion were reported to be half that in the 0.5 vs. 0.05 g/L exposure groups after 60 days of TCA exposure. Thus, TCA-induced hepatomegaly did not appear to be associated with this centrilobular cytoplasmic change.

Similarly the incidence of hepatic inflammation was reported to be 10, 0, 7, and 24% and severity, 0.11 ± 0.40, 0.09 ± 0.30, 0.12 ± 0.33, and 0.29 ± 0.48 for control, 0.05, 0.5, and 5.0 g/L TCA exposure groups, respectively. Thus, at no TCA exposure concentration was the incidence >24%, and the severity was considerably less than minimal. The reported results for hepatic necrosis were pooled from data from the five mice exposed for either 30 or 45 weeks (n = 10 total). No incidences of necrosis were reported for either control or 0.05 g/L TCA exposed mice. At 0.5 g/L, TCA 3/10 mice were reported to have necrosis but at a severity level of 0.50 ± 0.97. At 5.0 g/L, TCA 5/10 mice were reported to have necrosis but at a severity level of 1.30 ± 1.49. The limitations of the small number of animals pooled in these data are obvious. However, there does not appear to be much more than minimal necrosis at the highest dose of TCA between 30 and 45 weeks and this response is reported by the authors to be transient.

Serum LDH activity was reported by the authors for 31- and 60-week TCA exposures in Study #1. They state that:

> There was a dose-related trend at 31 weeks; serum LDH was significantly increased at 0.5 and 5 g/L TCA (161 ± 39 and 190 ± 44, respectively vs. 100 ± 28 IU for the control). LDH activity returned to control levels at 60 weeks. Similarly, elevated LDH levels were observed at early time periods for 0.5 and 4.5 g/L TCA during the 104 week exposure (data not shown: Studies 2 and 3).

The data presented by the author for Study #1 are from 5 animals/group for the 30-week results and 30 animals/group for the 60-week results. Of interest is for the 60-week data, there appears to be 50% decreased in LDH activity at 0.05 and ~25% decrease in LDH activity at

0.5 g/L TCA treatment with the LDH level reported to be the same as control for the 5 g/L TCA exposure group. For the 31-week data, in which only five animals were tested in each treatment group, there appeared to be a slight increase at the 0.5 g/L (60% increase over control) and 5 g/L (90% increase over control) treatment groups. The data for necrosis detected by light microscopy and by LDH level is consistent with no changes from control detected at the 0.05 g/L TCA treatment group and less than minimal necrosis of on a 60% increase in LDH level over control reported for 0.5 g/L TCA treatment. Even at the highest dose of 5.0 g/L TCA, there is still little necrosis or LDH release reported over control.

Data for testicular tubular degeneration was reported for Study #1 after 60 weeks of TCA exposure. The incidence of testicular tubular degeneration was reported to be 7, 0, 14, and 21% for mice exposed to 2.0 g/L sodium chloride, 0.05, 0.5, and 5.0 g/L TCA. The severity of the lesions was reported to be 0.10 ± 0.40, 0, 0.17 ± 0.47, and 0.21 ± 0.41 with a significant trend with dose reported by the authors for severity and for the 0.5 and 5 g/L treatment groups to be significantly increased over control incidence levels. Of note, similar to the percent liver/body weight ratios and hepatic inflammation values for this data set, the values for testicular tubular degeneration were slightly higher in control mice than 0.05 g/L TCA exposed mice. In regard to mean severity levels for testicular degeneration, although still minimal, there was little difference between the results for reported for the 0.5 and 5.0 g/L TCA exposed mice.

In regard to peroxisome proliferation, liver PCO activity was presented for up to 60 weeks (Study #1) and 104 weeks (Study #2). Similar to the data for LDH activity, ~30 animals were examined at the 60-week time point but only 5 animals per exposure group were examined for 4-, 15-, 31-, and 45-week results. The data are presented in a figure, and in some instances, it is hard to determine the magnitude of change.

Similar to other reports, the baseline level of PCO activity was variable between control groups and ranged 2.7-fold (~1.49–4.06 nmol NAD reduced/minute/mg protein given by the authors). There appeared to be little change in PCO activity between the 0.05 g/L TCA exposure and control levels for up to 45 weeks of exposure (i.e., the groups with n = 5) in Study #1. For the 60-week group, the 0.05 g/L TCA group PCO activity was ~1.7-fold of control but was not statistically significant. For the 0.5 g/L TCA treatment groups, the increase ranged from ~1.3- to 2.7-fold of control after 4, 15, 31, and 45 weeks of exposure with the largest differences reported at 4 and 60 weeks (i.e., 2.2- and 2.7-fold of control, respectively). For the 5.0 g/L TCA exposure groups, the increase ranged from ~3.2- to ~5.7-fold of control after 4, 15, 31, and 45 weeks of exposure.

While the data at 60 weeks had the most animals examined (~30 vs. 5) with ~1.7-, 2.7-, and 4.5-fold of control PCO activity, at this time period, the authors report the occurrence of tumors had already occurred. At the earlier time points of 4 and 15 weeks, there was a difference in the magnitude of TCA-induced increases in PCO activity. As displayed graphically, at 4 weeks, the PCO increases were ~1.3-, 2.4-, and 5.3-fold of control for 0.05, 0.5, and 5.0 g/L

TCA, respectively, while at 15 weeks, the PCO levels were decreased by 5%, increased to 1.3-fold, and increased to 3.2-fold of control with only the 5.0 g/L treatment group difference to be statistically significant.

For Study #2, the authors present a figure (Figure #4) that states that PCO values were given for mice given HAC or 4.5 g/L TCA for 4–60 weeks. However, the data presented in #4 appears to be for 15-, 30-, 45-, and 104-week exposures. The number of mice is not given in the figure but the methods section states that serial sections were conducted on 5 mice/group for these interim sacrifice periods. The number of mice examined for PCO activity at 104 weeks was not given by the authors but the number of mice at final sacrifice was given as 25. The levels of PCO in the control tissues varied by ~33% for weeks 15–45 but there was a ~5-fold difference between the level reported at 104 weeks and that for the earlier time periods in control mice shown in the figures (~2.23 vs. 0.41 nmol NAD reduced/min/mg protein as given by the authors). The increase over control induced by 4.5 g/L TCA in Study #2 was shown to be ~6.9-, 4.8-, 3.6-, and 19-fold of controls for 15, 30, 45 and 104 weeks, respectively.

Therefore, at a comparable level of TCA exposure (~5.0 g/L), number of mice examined (n = 5), and durations of exposure (15, 30, and 45 weeks), the increase in PCO activity induced by ~5.0 g/L TCA varied between 3.2- and 5.7-fold of control in Study #1 and between 3.6- and 6.9-fold of control in Study #2. There was not a consistent pattern between the two studies in regard to level of PCO induction from ~5 g/L TCA and duration of exposure. The lowest TCA-induced PCO activity increase was recorded at 15 weeks in Study #1 (i.e., 3.2-fold of control) and highest PCO activity increase was recorded at 15 weeks in Study #2 (i.e., 6.9-fold of control). No PCO data were reported for data in Study #3 with the exception of the authors stating that "PCO activity was significantly elevated for the 0.5 g/L TCA exposure over the 104 weeks (study 3). The extent of the increases was similar to those measured for 0.5 g/L TCA (200–375%: data not shown) in Study 1." No other details are given for PCO activity in Study #3.

Hepatocyte proliferation was reported by the authors to be assessed by either incorporation of tritiated thymidine (Studies #1 and #2) or BrdU (Study #3) into hepatocyte nuclei. As noted previously, these techniques measure DNA synthesis and not necessarily hepatocyte proliferation. The authors did not report whether specific areas of the liver were analyzed by autoradiographs or how many autoradiographs were examined in the analyses they conducted. For later time points of examination (60–104 weeks), the authors did not indicate whether hepatocytes in foci or adenomas were excluded from DNA synthesis reports. The authors present data for what are clearly, 31-, 45-, and 60-week exposures for Study #1 as the percent tritiated thymidine labeled nuclei. An early time point that appears to be 8 weeks is also given.

However, for Study #1, only 4- and 15-week durations were tested, so it cannot be established what time period the earlier time point represents. What is very apparent from the

data presented for Study #1 is that the baseline level of tritiated thymidine incorporation was relatively high and was highly variable for the five animals examined (~8% of hepatocytes were labeled). There did not appear to be an apparent pattern of TCA treatment groups at this timepoint, with the 0.05 and 5.0 g/L TCA groups having a similar percentage of labeled hepatocytes and for 0.5 g/L TCA reported to have a 60% reduction in labeled hepatocytes.

After 31 weeks of exposure, the control values were reported to be 2% of hepatocytes labeled. The authors report that only the 5.0 g/L TCA group had a statistically significant increase of control and was elevated to ~6% of hepatocytes. The two lower exposure concentrations of TCA had similar reported incidences of labeled hepatocytes of 4.5% that were not reported to be statistically significant.

For the 45-week exposure period in Study #1, the control value was reported to be 1.2%, with only the 5.0 g/L TCA value reported to be statistically significantly increased at 3.2% and the other two TCA groups to be similar to control. Finally, for the 60-week group from Study #1, the control value was reported to be 0.6% of hepatocytes labeled and only the 0.5 g/L TCA dose reported to be statistically significantly increased over control at 3.2%.

What is clear from this study is that the control value for the unidentified early time point is much higher than the other values. There should not be such a large difference in mature mice nor such a high level. The difference in control values between the earlier time point and the 31-week time point was fourfold. The difference between the earlier time point and the 45-week time point was approximately sevenfold. There did not appear to be an increase in hepatocyte tritiated thymidine labeling due to any concentration of TCA at the early unidentified time point (approximately week 10 from the figure) from Study #1. There was no dose-response apparent for the other study periods and the percent of hepatocytes labeled were ≤3%. These results indicated that DNA synthesis was not increased by 10–60-week exposures to TCA exposure that induced increased liver tumor response.

For Study #2, results were reported for tritiated thymidine incorporation into hepatocytes in a figure that was labeled as 4.5 g/L TCA and control tissue for 104 weeks but showed data for 15, 30, and 45 weeks of exposure. Of note is that the control values for this study were much lower than that reported for Study #1. The percent of hepatocytes labeled with tritiated thymidine was reported to be ~2% for the 15-week exposure period and <1% for the 30- and 45-week exposure periods. For the 4.5 g/L TCA exposures, the percent hepatocytes labeled with tritiated thymidine were ~2–4% at all time points with only the 45-week period identified by the authors as statistically significant.

For Study #3, rather than tritiated thymidine, BrdU was used as a measure of DNA synthesis. The results are presented in Figure #8 of the report in which the 0.5 g/L TCA concentration is mislabeled as 0 g/L and the figure is mislabeled as having a duration of 104 weeks, but the data are presented for 26, 52, and 78 weeks of exposure. The percent of hepatocytes at 26 weeks was reported to be ~1–2% for the control, 0.05, and 0.5 g/L TCA

groups. At 52 weeks, the control value was ~1%, the 0.05 g/L TCA value was <0.1% and the 0.5 g/L TCA value was ~3.5% but was not statistically significant. At 78 weeks of exposure, the control value was reported to be ~0.2% with only the 0.05 g/L TCA group having a statistically significant increase over control.

From these data, the estimated control values for DNA synthesis at similar time points of exposure ranged from 0.4 to 2% at 26–31 weeks and ~0.1–1.2% at 45–52 weeks. The results for Studies #1 and #2 were inconsistent in regard to the magnitude of tritiated thymidine incorporation, but were consistent in that there was a lot of variability in these measurements, not a consistent pattern with time that was TCA-dose related, and, even at the highest dose of TCA, did not indicate much of an increase in cell proliferation at 15–45 weeks of exposure. Similarly, the results for Studies #1 and #3 indicate that at the two lower doses of TCA, there were not generally statistically significant increases in DNA synthesis from 15 to 45 weeks of exposure, although there was an increase in liver tumor response at later time points.

The authors reported that "all gross and microscopic histopathological alterations were consistent across the three studies." However, the histological descriptions that follow were focused on the liver for both neoplastic and non-neoplastic parameters. As stated above, only a few animals (n = 5) from the control and high TCA dose level were examined for lesions other than liver, kidneys, spleen, and testes. Thus, whether other neoplastic lesions were induced by TCA exposure cannot be determined from this set of studies.

Study #1 was conducted for 60 weeks. Although of short duration and using ≤30 animals, the authors reported in the text that:

> a significant trend with dose was found for liver cancer. The prevalence and multiplicity of adenomas (38%; 0.55 ± 0.15) or carcinoma (38%; 0.42 ± 0.11) were statistically significant at 602 mg/kg-day TCA compared to control (7%; 0.07 ± 0.05) [sic for both adenoma and carcinoma the same value was given, mean ± SD]. When either an adenoma or a carcinoma was present, statistical significant was seen at both 5 g/L (55%; 1.00 ± 0.19) and 0.5 g/L (38%: 0.52 ± 0.14 TCA exposure groups compared to control (13%; 0.13 ± 0.06).

No significant changes in liver neoplasia were reported to be observed by the authors at 0.05 g/L TCA. Preneoplastic large foci of cellular alteration (24%) were seen in the 5 g/L TCA group compared to control.

Although not statically significant, there was an incidence of 15% adenoma in the 0.05 g/L TCA treatment group (n = 27) and a multiplicity of 0.15 ± 0.07 adenomas/mouse reported, with both values being twice that of the values given for the controls (n = 30). The incidence and multiplicity for carcinomas was approximately the same for the 0.05 g/L TCA treatment group and the control group. Given the small number of animals examined, the study was limited in its ability to determine statistical significance for the lower TCA exposure level.

The fold increases of incidence and multiplicity of adenomas at 60 weeks was 2.1-, 3.0-, and 5.4-fold of control incidence and 2.1-, 3.4-, and 7.9-fold of control multiplicity for 0.05, 0.5, and 5 g/L exposure to TCA. For multiplicity of adenomas and carcinomas combined, there was a 1.46-, 4.0-, and 7.68-fold of control values. Analysis of tumor prevalence data for this study included only animals examined at scheduled necropsy. Since most animals survived until 60 weeks, most were included and a consistent time point for tumor incidence was reported.

There are significant discrepancies for reporting of data for tumor incidences in this report for the 104-week data. While the methods section and table describing the dose calculation and animal survival indicate that Study #3 control animals were administered deionized water and those from Study#2 were given HAC, Table 6 of the report gives 2 g/L sodium chloride as the control solution given for Study #2 and 1.5 g/L HAC for Study #3. A comparison of the descriptions of animal survival and tumor incidence and multiplicity between the results given in DeAngelo et al. (2008) and George et al. (2000) (see Table E-10) shows not only that the control data presented in DeAngelo et al. (2008) for Study #3 are the same data as that presented by George et al. (2000) previously, but also indicates that rather than 1.5 g/L HAC, the tumor data presented in DeAngelo et al. (2008) is for mice exposed to deionized water. DeAngelo et al. (2008) did not report that these data were from a previous publication.

Table E-10. Comparison of descriptions of control data between George et al. (2000) and DeAngelo et al. (2008)

Descriptor	George et al. (2000)	DeAngelo et al. (2008)
Species	Mouse	Mouse
Strain	$B6C3F_1$	$B6C3F_1$
Gender	Male	Male
Age	28–30 d	28–30 d
Source	Charles River, Portage	Charles River, Portage
Mean initial body weight	19.5 ± 2.5 g	19.5 ± 2.5 g
Water consumption	111.7 mL/kg-d	112 mL/kg-d
Laboratory	Research Triangle Park, North Carolina	Research Triangle Park, North Carolina
Number of animals at start	72	72
Number of animals at interim sacrifice	22	21
Number of unscheduled deaths	16	17
Number of animals at final sacrifice	34	34
Number of animals for pathology	65	63
Adenoma incidence	21.40%	21%
Adenoma multiplicity	0.21 ± 0.06	0.21 ± 0.06
Carcinoma incidence	54.80%	55%
Carcinoma multiplicity	0.74 ± 0.12	0.74 ± 0.12

For Studies #2 and #3, tumor prevalence data were reported in the methods section of the report to include necropsies of animals that survived >78 weeks and thus, included animals that were scheduled for necropsy but also those that were moribund and sacrificed at differing times.

Thus, for the longer times of study, there was a mixture of exposure durations that included animals that were ill and sacrificed early and those that survived to the end of the study. Animals that were allowed to live for longer periods or did not die before scheduled sacrifice times had a greater opportunity to develop tumors. However, animals that died early may have died from tumor-related causes.

The mislabeling of the tumor data in DeAngelo et al. (2008) has effects on the interpretation of results; if the tumor results table was not mislabeled, it would indicate that 17 animals were included in the liver tumor analysis that were not included in the final necropsy and that the seven unscheduled deaths could not account for the total number of "extra" mice included in the tumor analysis, so some of the animals had to have come from interim sacrifice times (≤78 weeks) and that for Study #3, the data from 9 animals at terminal sacrifice were not used in the tumor analysis. Not only does it appear that the control data was mislabeled for Study #3, but the control data were also apparently mislabeled for Study #2 as being 2.0 g/L sodium chloride rather than 1.5 g/L HAC. Of the 42 animals used for the tumor analysis in Study #3, only 34 were reported to have survived to interim sacrifice so that 8 animals were included from unscheduled deaths. However, the authors report that there were 17 unscheduled deaths in the study, but not all were included in the tumor analysis. The basis for the selection of the eight animals for tumor analysis was not given by the authors.

Not only are the numbers of control animals used in the tumor analysis different between two studies (25 mice in Study #2 and 42 mice in Study #3), but the liver tumor results reported for Study #2 and Study #3 were very different. Of the 42 "control" mice examined from Study #3, the incidence and multiplicity of adenomas were reported to be 21% and 0.21 ± 0.06, respectively. For carcinomas, the incidence and multiplicity were reported to be 55% and 0.74 ± 0.12, respectively, and the incidence and multiplicity of adenomas and carcinomas combined were reported to be 64% and 0.93 ± 0.12, respectively. For the 25 mice reported by the authors for Study #2 to have been treated with "2.0g/L NaCl" but were probably exposed to 1.5 g/L HAC, the incidence and multiplicity of adenomas was 0%. For carcinomas, the incidence and multiplicity were reported to be 12% and 0.20 ± 0.12, respectively, and the incidence and multiplicity of adenomas and carcinomas combined were reported to be 12% and 0.20 ± 0.12, respectively. Therefore, while ~64% the 42 control mice in Study #3 were reported to have adenomas and carcinomas, only 12% of the 25 mice were reported to have adenomas and carcinomas in Study #2 for 104 weeks.

While the effect of using fewer mice in one study vs. the other will be to reduce the power of the study to detect a response, there are additional factors that raise questions regarding the tumor results. Not only were the tumor incidences reported to be higher in control mice from

Study #3 than Study #2, but the number of unscheduled deaths was reported to also be twofold higher. The age, gender, and strain of mouse were reported to be the same between Studies #2 and #3 with only the vehicles differing and weight of the mice to be reported to be different. Although the study by George et al. (2000) described the same control data set as for Study #3 as being for animals given deionized water, there is uncertainty as to the identity of the vehicle used for the tumor results reported for Study #3 and there are some discrepancies in reporting between the two studies. As discussed below in Section E.2.5, the differences in the weight of the mice between Studies #1, #2, and #3 is critical to the issue of differences in background tumor rate and hence interpretability of the study.

As noted by Leakey et al. (2003a), the greatest correlation with liver tumor incidence and body weight appears between the ages of 20 and 60 weeks in male mice. As reported in Section E.2.5, the mean 45-week body weight reported for control male $B6C3F_1$ mice in the George et al. (2000) study, which is the same control data as DeAngelo et al. (2008) was ~50 g. This is a much greater body weight than reported for Study #1 at 45 weeks (i.e., 39.6 g) and for Study #2 at 45 weeks (i.e., 39.4 g). Using probability curves presented by Leakey et al. (2003a), the large background rate of 64% of combined adenomas and carcinomas for Study #3 is in the range predicted for such a large body weight (i.e., ~65%). Such a high background incidence compromises a 2-year bioassay, as it prevents demonstration of a positive dose-response relationship. Thus, Study #3 of DeAngelo et al. (2008) is not comparable to the results in Studies #1 and #2 for the determination of the dose-response for TCA.

The accurate determination of the background liver tumor rate is very important in determining a treatment-related effect. The very large background level of tumor incidence reported for Study #3 makes the detection of a TCA-related change in tumor incidence at low exposure levels very difficult to determine. Issues also arise as to what the source of the tumor data were in the TCA-treatment and control groups in Study #3. While 29 mice exposed to 0.05 g/L TCA were reported to have been examined at terminal sacrifice, 35 mice were used for liver tumor analysis. Similarly, while 27 mice exposed to 0.5 g/L TCA were reported to have been examined at terminal sacrifice, 37 mice were used for tumor analysis. Finally, for the 42 control animals examined for tumor pathology in the control group, 34 were examined at terminal sacrifice. Clearly, more animals were included in the analyses of tumor incidence and multiplicity than were sacrificed at the end of the experiment. What effect differential addition of the results from mice not sacrificed at 104 weeks and the selection bias that may have resulted from their inclusion on these results cannot be determined. Not only were the background levels of tumors reported to be increased in the control animals in Study #3 compared to Study #2 at 104 weeks, but the rate of unscheduled deaths was doubled. This is also an expected consequence of using much larger mice (Leakey et al., 2003a).

For the 35 mice examined after 0.05 g/L TCA in Study #3, the incidence and multiplicity of adenomas were reported to be 23% and 0.34 ± 0.12, respectively. For carcinomas, the

incidence and multiplicity were reported to be 40% and 0.71 ± 0.19, respectively, and the incidence and multiplicity of adenomas and carcinomas combined were reported to be 57% and 1.11 ± 0.21, respectively. For the 37 mice examined after 0.5 g/L TCA in Study #3, the incidence and multiplicity of adenomas were reported to be 51% and 0.78 ± 0.15, respectively. For carcinomas, the incidence and multiplicity were reported to be 78% and 1.46 ± 0.21, respectively, and the incidence and multiplicity of adenomas and carcinomas combined were reported to be 87% and 2.14 ± 0.26, respectively.

Thus at 0.5 g/L TCA, the results presented for this study for the "104 week" liver tumor data were significantly increased over the reported control values. However, these results are identical to those reported in Study #3 for a 10-fold higher concentration of TCA (4.5 g/L TCA) for the same 104 weeks of exposure but in the much larger mice. Of the 36 animals exposed to 4.5 g/L TCA in Study #2 and included in the tumor analysis, 30 animals were reported to be examined at 104 weeks. The incidence and multiplicity of adenomas were reported to be 59% and 0.61 ± 0.16, respectively. For carcinomas, the incidence and multiplicity were reported to be 78% and 1.50 ± 0.22, respectively, and the incidence and multiplicity of adenomas and carcinomas combined were reported to be 89% and 2.11 ± 0.25, respectively.

The importance of selection and determination of the control values for comparative purposes of tumor induction are obvious from these data. The very large difference in control values between Study #2 and Study #3 is the determinant of the magnitude of the dose response for TCA after 104 weeks of exposure. The tumor response for 0.5 and 4.5 g/L TCA exposure between the two experiments was identical. Therefore, only the background tumor rate determined the magnitude of the response to treatment. If similar control values (i.e., a historical control value) were used in these experiments, there would appear to be no difference in TCA-tumor response between 0.5 and 4.5 g/L TCA at 104 weeks of exposure. DeAngelo et al. (1999) report for male B6C3F_1 mice exposed only water for 79–100 weeks the incidence of carcinomas to be 26% and multiplicity to be 0.28 lesions/mouse. For 100-week data, the incidence and prevalence of adenomas were reported to be 10% and 0.12 ± 0.05 and to be 26% and 0.28 ± 0.07 for carcinomas.

Issues with reporting for that study have already been discussed in Section E.2.3.2.6. However, the data for DeAngelo et al. (1999) are more consistent with the control data for "1.5 g/L HAC" for Study #2 in which there were 0% adenomas and 12% carcinomas with a multiplicity of 0.20 ± 0.12, than for the control data for Study #3 in which 64% of the control mice were reported to have adenomas and carcinomas and the multiplicity was 0.93 ± 0.12. If either the control data from DeAngelo et al. (1999) or Study #2 were used for comparative purposes for the TCA-treatment results of Study #2 or #3, there would be a dose-response between 0.05 and 0.5 g/L TCA but no difference between 0.5 and 4.5 g/L TCA after 100 weeks of exposure. The tumor incidence would have peaked at ~90% in the 0.5 and 4.5 g/L TCA exposure groups. These results would be more consistent with the 60-week results in Study #1

in which 0.5 and 5 g/L TCA exposure groups already had incidences of 38 and 55% of adenomas and carcinomas combined, respectively, compared to the 13% control level. With increased time of exposure, the differences between the two highest TCA exposure concentrations may diminish as tumor progression is allowed to proceed further. However, the use of the larger and more tumor prone mice in Study #3 also increases the tumor incidence at the longer period of study.

The authors also presented data for multiplicity of combined adenomas or carcinomas for mice sacrificed at weeks 26, 52, and 78 for Study #3 (n = 8 per group). No indication of variability of response, incidence data, statistical significance, or data for adenomas vs. carcinomas, or the incidence of adenomas was reported. The authors reported that "neoplastic lesions were first found in the control and 0.05 g/L TCA groups at 52 weeks. At 78 weeks, adenomas or carcinomas were found in all groups (0.29, 0.20, and 0.57 tumors/animals for control, 0.05 g/L TCA, and 0.5 g/L TCA, respectively)." Because no other data were presented at the 52 and 78 week time points in this study, these results cannot be compared to those presented for Study #1, which was conducted for 60 weeks. Of note, the results presented from Study #1 for 60 weeks of exposure to control, 0.05, or 0.5 g/L TCA exposure in 27–30 mice show a 13, 15, and 38% incidence of hepatocellular adenomas and carcinomas and a multiplicity of 0.13 ± 0.06, 0.19 ± 0.09, and 0.52 ± 0.14, respectively. Both the incidence and multiplicity of adenomas were twofold higher in the 0.05 g/L TCA treatment group than for the control. However, the interim data presented by the authors from Study #3 for 52 weeks of exposure in only eight mice per group gives a higher multiplicity of adenomas and carcinomas for control animals (~0.25) than for either 0.05 or 0.5 g/L TCA treatments. Again, comparisons between Studies #2 and #3 are difficult due to difference in mouse weight.

Of note, there are no descriptions given in this report in regard to the phenotype of the tumors induced by TCA or for the liver tumors reported to occur spontaneously in control mice. Such information would have been of value, as this study reports results for a range of TCA concentration and for 60 and 100 weeks of exposure. Insight could have been gained as to the effects of differing concentrations of TCA exposure and whether TCA-induced liver tumors had a similar phenotype as those occurring spontaneously, as well as information in regard to effects on tumor progression and heterogeneity.

Although only examining tissues from five mice from the control and high-dose groups only at 104 weeks at organ sites other than the liver, the authors report that:

> neoplastic lesions at 104 weeks (Studies #2 and #3) at organ sites other than the liver were found in the lung, spleen, lymph nodes, duodenum (lymphosarcoma), seminal vesicles, skin, and thoracic cavity of control and treated animals. All were considered spontaneous for the male B6C3F_1 mouse and did not exceed the tumor incidences when compared to a historical control database (NIEHS, 1998; Haseman et al., 1984).

No data were shown. The limitations involved in examining only five animals in the control and high-dose groups, and the need to examine the concurrent control data in each experiment, especially given the large variation in liver tumor response between long-term studies carried out in the two different laboratories used for Study #2 and Study #3 using the same strain and gender of mouse, make assertions regarding extrahepatic carcinogenicity of TCA from this study impossible to support.

A key issue raised from this study is whether changes in any of the parameters measured in interim sacrifice periods before the appearance of liver tumors (i.e., 4–15 weeks) corresponded to the induction of liver tumors. The first obstacle for determining such a relationship is the experimental design of these studies in which only a full range of TCA concentrations is treated for 60 weeks of exposure with a small number of animals available for determination of a carcinogenic response (i.e., ≤30 animals in Study #1) and a very small number of animals (n = 5 group) examined for other parameters. Also as stated above, PCO activity was highly variable between controls and between treatment groups (e.g., the PCO activity for Studies #1 and #2 at ~5 g/L exposure for 15 weeks).

On the other hand, most of the animals that were examined at terminal sacrifice were also utilized for the tumor results without the differential deletion or addition of "extra" animals for the tumor analysis. For the 60-week data in Study #1, there appeared to be a consistent dose-related increase in the incidence and multiplicity of tumors after TCA exposure (Table E-11). The TCA-induced increases in liver tumor responses can be compared with both increased liver weight and PCO activity that were also reported to be increased with TCA dose as earlier events. Although the limitations of determining the exact magnitude of responses has already been discussed, as shown below, the incidence and multiplicity of adenomas show a dose-related increase at 60 weeks. However, the magnitude of differences in TCA concentrations was not similar to the magnitude of increased liver tumor induction by TCA after 60 weeks of exposure.

First of all, the greater occurrence of TCA-induced increases in adenomas than carcinomas reported after 60 weeks of exposure would be expected for this abbreviated duration of exposure as they would be expected to occur earlier than carcinomas. For adenoma induction, there was an approximately twofold increase between the 0.05 g/L dose of TCA and the control group for incidence (7 vs. 15%) and multiplicity (0.07 vs. 0.15 tumors/animals). However, an additional 10-fold increase in TCA dose (0.5 g/L) only resulted in a reported 1.8-fold greater incidence (15 vs. 21%) and 2.2-fold increase in multiplicity (0.15 vs. 0.24 tumors/animal) of control adenoma levels. An additional 10-fold increase in dose (5.0 vs. 0.5 g/L TCA) resulted in a 2.2-fold increase in incidence (21 vs. 38%) and 2.9-fold increase in multiplicity (0.24 vs. 0.55 tumors/animal) of control adenoma levels.

Thus, a 100-fold difference in TCA exposure concentration resulted in differences of fourfold of control incidence and sixfold of control multiplicity for adenomas. For adenomas or carcinomas combined (a parameter that included carcinomas for which only the two highest

exposure levels of TCA were reported to increase incidence and multiplicity), the incidences were reported to be 13, 15, 38, and 55%, and the multiplicity was reported to be 0.13, 0.19, 0.52, and 1.00 for control, 0.05, 0.5, and 5.0 g/L TCA at 60 weeks. For multiplicity of adenomas or carcinomas, the 0.05 g/L TCA exposure induced a 1.5-fold increase over control. An additional 10-fold increase in TCA (0.5 g/L) induced a 6-fold increase in tumors/animal. An additional 10-fold increase in TCA (5.0 vs. 0.5 g/L) induced an additional 2.2-fold increase in tumors/animal. Therefore, using combinations of adenomas or carcinomas, there was a 13-fold increase in multiplicity that corresponded with a 100-fold increase in dose.

Table E-11. TCA-induced increases in liver tumor occurrence and other parameter over control after 60 weeks (Study #1)

Dose TCA g/L	Adenomas		Adenomas or carcinomas		% liver/body weight		PCO activity	
Sodium chloride	Incidence 7%	Multiplicity 0.07	Incidence 13%	Multiplicity 0.13	4-wk	15-wk	4-wk	15-wk
0.05	15% (2.1-fold)	0.15 (2.1-fold)	15% (1.2-fold)	0.19 (1.5-fold)	1.09-fold	1.14-fold	1.3-fold	1.0 -fold
0.5	21% (3.0-fold)	0.24 (3.4-fold)	38% (2.9-fold)	0.52 (4.0-fold)	1.16-fold	1.16-fold	2.4-fold	1.3-fold
5.0	38% (5.4-fold)	0.55 (7.9-fold)	55% (4.2-fold)	1.00 (7.7-fold)	1.35-fold	1.47-fold	5.3-fold	3.2-fold

The results for adenoma induction at 60 weeks of TCA exposure (i.e., ~2-fold increased incidences and 2–3-fold increases in multiplicity with 10-fold increases in TCA dose) are similar to the ~2-fold increase in liver weight gain resulting from 10-fold differences in dose reported at 4 weeks of exposure. For PCO activity, there was a ~30% increase in PCO activity from control at 0.05 g/L TCA. A 10-fold increase in TCA exposure concentration (0.5 g/L) resulted in an additional ~5-fold increase in PCO activity. However, another 10-fold increase in TCA concentration (0.5 vs. 5 g/L) resulted in a 3-fold increase in PCO activity. The 100-fold increase in TCA dose (0.05 vs. 5 g/L TCA) was correlated with a 14-fold increase in PCO activity. For 15 weeks of TCA exposure, there was no difference in 0.05 g/L and control PCO activity and only a 30% difference between the 0.05 and 0.5 g/L TCA exposures. There was a sevenfold difference in PCO activity between the 0.5 and 5.0 g/L TCA exposure concentrations. The increases in PCO activity and liver weight data at 15 weeks did not fit the magnitude of increases in tumor multiplicity or incidence data at 60 weeks as well as did the 4-week data. However, the TCA-induced increase in tumors at 60 weeks (especially adenomas) seemed to correlate more closely with the magnitude of liver weight increase than for PCO activity at both 4 and 15 weeks.

In regard to Studies #1 and #2, there were consistent periods of study for percent liver/body weight with the consistency of the control values being a large factor in the magnitude of TCA-induced liver weight increases. As discussed above, there were differences in the magnitude of percent liver/body weight increase at the same concentration between the two studies (e.g., a 1.47-fold of control percent liver/body weight in the 5 g/L TCA exposed group in Study #1 and 1.60-fold of control in Study #2 at 15 weeks). For the two studies that had extended durations of exposure (Studies #2 and #3), the earliest time period for comparison of percent liver/body weight is 26 weeks (Study #3) and 30 weeks (Study #2). If those data sets (26 weeks for Study #3 and 30 weeks for Study #2) are combined, then 0.05, 05, and 4.5 g/L TCA gives a percent liver body/weight increase of 1.07-, 1.18-, and 1.40-fold over concurrent control levels. Using this parameter, there appears to be a generally consistent pattern as that reported for Study #1 at weeks 4 and 15. Generally, a 10-fold increase in TCA exposure concentration resulted in ~2.5-fold increased in additional liver weight observed at ~30 weeks of exposure, which correlated more closely with adenoma induction at 60 weeks than did changes in PCO activity. A similar comparison between Studies of longer duration (Studies #2 and #3) could not be made for PCO activity as data were not reported for Study #3.

For 104-week studies of TCA-tumor induction (Studies #2 and #3), the lower TCA exposure levels (0.05 and 0.5 g/L TCA) were assayed in a separate experiment and by a separate laboratory than the high dose (5.0 g/L TCA) and most importantly in larger, more tumor prone mice. The total lack of similarity in background levels of tumors in Studies #2 and #3, the differences in the number of animals included in the tumor analyses, and the low number of animals examined in the tumor analysis at 104 weeks (<30 for the TCA treatment groups) makes the determination of a dose-response TCA-induced liver tumor formation after 104-weeks of

exposure problematic. The correlation of percent liver/body weight increases with incidence and multiplicity of liver tumors in Study #1 and the similarity of dose-response for early induction of percent liver/body weight gain between Study #1 suggest that there should be a similarity in tumor response. However, as noted above, the 104-week studies had very difference background rates of spontaneous tumors reported in the control mice between Studies #2 and #3.

Table E-12 shows the incidence and multiplicity data for Studies #2 and #3 along with the control data for DeAngelo et al. (1999) for the same paradigm. It also provides an estimate of the magnitude of increase in liver tumor induction by TCA treatments if the control values from the DeAngelo et al. (1999) data set were used as the background tumor rate. As shown below, the background rates for Study #2 are more consistent with those of DeAngelo et al. (1999). Whereas there was a 2:1 ratio of multiplicity for adenomas and adenomas and carcinomas between 0.5 and 5.0 g/L TCA after 60 weeks of exposure, there was no difference in any of the data (i.e., adenoma, carcinoma, and combinations of adenoma and carcinoma incidence and multiplicity) for these exposure levels in Studies #2 and #3 for 104 weeks. The difference in the incidences and multiplicities for all tumors was twofold between the 0.05 and 0.5 g/L TCA exposure groups in Study #2. These results are consistent with the two highest exposure levels, reaching a plateau of response with a long enough duration of exposure (~90% of animals having liver tumors) and with the 2-fold difference in liver tumor induction between concentrations of TCA that differed by 10-fold, reported in Study #1.

Table E-12. TCA-induced increases in liver tumor occurrence after 104 weeks (Studies #2 and #3)

Dose TCA	Adenomas		Carcinomas		Adenomas or carcinomas	
	Incidence	Multiplicity	Incidence	Multiplicity	Incidence	Multiplicity
Study #3						
1.5 g/L HAC (H_2O?)	21%	0.21	55%	0.74	64%	0.93
0.05 g/L TCA	23%	0.34	40%	0.71	57%	1.11
	(1.1-fold)	(1.6-fold)	(0.7-fold)	(1.0-fold)	(0.9-fold)	(1.2-fold)
0.5 g/L TCA	51%	0.78	78%	1.46	87%	2.14
	(2.4-fold)	(3.7-fold)	(1.4-fold)	(2.0-fold)	(1.4-fold)	(2.3-fold)
Study #2						
2.0 g/L NaCl (HAC?)	0%	0	12%	0.20	12%	0.20
4.5 g/L TCA	59%	0.61	78%	1.50	89%	2.14
	(?)	(?)	(6.5-fold)	(7.5-fold)	(7.4-fold)	(11-fold)
DeAngelo et al. (1999)						
H_2O	10%	0.12	26%	0.28		
0.05 g/TCA (S #3)	(2.3-fold)	(2.8-fold)	(1.5-fold)	(2.5-fold)		
0.5 g/L TCA (S #3)	(5.1-fold)	(6.5-fold)	(3.0-fold)	(5.2-fold)		
5.0 g/L TCA (S #2)	(5.9-fold)	(6.5-fold)	(3.0-fold)	(5.4-fold)		

H_2O = water

If either the control values for Study #2 or the control values from DeAngelo et al. (1999) were used for as the background rate of spontaneous liver tumor formation, the magnitude of liver tumor induction by the 0.05 g/L TCA over control levels differs dramatically from that reported as control tumor rates in Study #3. To put the 64% incidence data for carcinomas and adenomas reported in DeAngelo et al. (2008) for the control group of Study #3 in context, other studies cited in this review for $B6C3F_1$ mice show a much lower incidence in liver tumors in that: (1) the NCI (1976) study of TCE reports a colony control level of 6.5% for vehicle and 7.1% incidence of HCCs for untreated male $B6C3F_1$ mice (n = 70–77) at 78 weeks; (2) Herren-Freund et al. (1987) report a 9% incidence of adenomas in control male $B6C3F_1$ mice with a multiplicity of 0.09 ± 0.06 and no carcinomas (n = 22) at 61 weeks; (3) NTP (1990) reports an incidence of 14.6% adenomas and 16.6% carcinomas in male $B6C3F_1$ mice after 103 weeks (n = 48); and (4) Maltoni et al. (1986) report that $B6C3F_1$ male mice from the "NCI source" had a 1.1% incidence of "hepatoma" (carcinomas and adenomas) and those from "Charles River Co." had a 18.9% incidence of "hepatoma" during the entire lifetime of the mice (n = 90 per group). The importance of examining an adequate number of control or treated animals before confidence can be placed in those results in illustrated by Anna et al. (1994) in which at 76 weeks 3/10 control male $B6C3F_1$ mice that were untreated and 2/10 control animals given corn oil were reported to have adenomas but from 76 to 134 weeks, 4/32 mice were reported to have adenomas (multiplicity of 0.13 ± 0.06) and 4/32 mice were reported to have carcinomas (multiplicity of 0.12 ± 0.06).

Using concurrent control values reported in Study #3, there is no increase in incidence of multiplicity of adenomas and carcinomas for the 0.05 g/L exposure group. However, compared to either the control data from DeAngelo et al. (1999) or the control data from Study #3, there is a ~2–3- or ~5-fold increased in incidence or multiplicity of liver tumors, respectively. Thus, trying to determine a correspondence with either liver weight increases or increases in PCO activity at earlier time points will depend on the confidence placed in the concurrent control data reported in Study #3 in the 104 week studies. As noted previously, the use of larger, tumor prone mice in Study #3 limits its usefulness to determine the dose-response for TCA.

The authors provided a regression analysis for "tumors/animal" or multiplicity as a percent of control values and PCO activity for the 60- and 104-week data. Whether adenomas and carcinomas combined or individual tumor type were used was not stated. In addition, comparing PCO activity at the end of the experiments, when there was already a significant tumor response rather than at earlier time points, may not be useful as an indicator of PCO activity as a key event in tumorigenesis. A regression analysis of these data are difficult to interpret because of the dose spacing of these experiments as the control and 5 g/L exposure levels will basically determine the shape of the dose-response curve. The 0.05 and 0.5 g/L exposure groups in the regression were so close to the control value in comparison to the 5 g/L exposure, that the dose response will appear linear between control and the 5.0 g/L value with

the two lowest doses not affecting the slope of the line (i.e., "leveraging" the regression). The value of this analysis is limited by: (1) the use of tumor prone larger mice in Study #3 that had large background rates of tumors, which make inappropriate the apparent combination of results from Studies #2 and #3 for the multiplicity as percentages of control values; (2) the low and varying number of animals analyzed for PCO values and the variability in PCO control values; (3) the appropriateness of using PCO values from later time points; and (4) the dose-spacing of the experiment.

Similarly, the authors reported a regression analysis that compares "percent of hepatocellular neoplasia," which again, is indicated by tumor multiplicity with TCA dose as represented by mg/kg-day. This regression analysis also is of limited value for the same reasons as that for PCO with added uncertainty, as the exposure concentrations in drinking water have been converted to an internal dose and each study gave different levels of drinking water with one study showing a reduction of drinking water at the 5 g/L level. The authors attempted to identify a NOEL for tumorigenicity using tumor multiplicity and TCA dose. However, it is not an appropriate descriptor for these data, especially given that "statistical significance" of the tumor response is the determinant of the conclusions regarding a dose in which there is no TCA-induced effect. Only the 60-week experiment (i.e., Study #1) is useful for the determination of tumor dose-response due to the issues related to appropriateness of control in Study #3. A power calculation of the 60-week study shows that the type II error, which should be >50% and thus greater than the chances of "flipping a coin," was 41 and 71% for incidence and 7 and 15% for multiplicity of adenomas for the 0.05 and 0.5 g/L TCA exposure groups. For the combination of adenomas and carcinomas, the power was 8 and 92% for incidence and 6 and 56% for multiplicity at 0.05 and 0.5 g/L TCA exposure. Therefore, the designed experiment could accept a false null hypothesis, especially in terms of tumor multiplicity, at the lower exposure doses and erroneously conclude that there is no response due to TCA treatment.

E.2.3.2.14. **DeAngelo et al. (1997)**

The design of this study appears to be similar to that of DeAngelo et al. (2008) but to have been conducted in F344 rats. Rats (28–30 day old rats), reported to be of similar weights, were exposed to 2.0 g/L sodium chloride, 0.05, 0.5, or 5.0 g/L TCA in drinking water for 104 weeks. There were groups of animals sacrificed at 15, 30, 45, and 60 weeks (n = 6) for PCO analysis. There were 23, 24, 19, and 22, animals reported to be examined at terminal sacrifice at 104 weeks and 23, 24, 20, and 22 animals reported to be used in the liver tumor analysis reported by the authors for the control, 0.05, 0.5, and 5.0 g/L treatment groups, respectively. Complete pathological exams were reported to be performed for all tissues from animals in the high-dose TCA group at 104 weeks. No indication was given as to whether a complete necropsy and pathological exam was performed for controls at terminal sacrifice. Tritiated thymidine was reported to be administered at interim sacrifices 5 days prior to sacrifice and to be examined with

autoradiography. The 5 g/L TCA treatment group was reported to have a reduction in growth to 89.3% of controls.

For water consumption, TCA was reported to slightly decrease water consumption at all doses with a 7, 8, and 4% decrease in water consumption reported for 0.05, 0.5, and 5.0 g/L TCA, respectively. Body weight was decreased by 5.0 g/L TCA dose only through 78 weeks of exposure to 89.3% of the control value. All of the percent liver/body weight ratios were reported to be slightly decreased (1–4%) by all of the exposure concentrations of TCA but the data shown do not indicate if the liver weight data were taken at interim sacrifice times and appear to be only for animals at terminal sacrifice of 104 weeks.

No data were shown for hepatocyte proliferation but the authors reported no TCA treatment effects. For PCO, there was a 2.3-fold difference between control values between the 15- and 104-week data. For the 0.05 and 0.5 g/L TCA treatment groups, there was not a statistically significant difference reported between control and treated group PCO levels. At 15 weeks, the PCO activity was reduced by 55%, increased to 1.02-fold, and increased 2.12-fold of control for 0.05, 0.5, and 5.0 g/L TCA exposures, respectively. For the 30-week exposure groups, the 0.05 and 0.5 g/L TCA groups were reported to have PCO levels within 5% of the control level. However, for the 5.0 g/L TCA treatment groups, there was approximately twofold of control PCO activity at the 15, 30, 45, and 60 weeks and at 104 weeks, there was a fourfold of control PCO activity. Of note is that the control PCO value was lowest at 104 weeks, while the TCA treatment group was similar to interim values.

For analysis of liver tumors, there were 20–24 animals examined in each group. Unlike the study of DeAngelo et al. (2008), it appeared that most of the animals that were sacrificed at 104 weeks were used in the tumor analysis without addition of "extra" animals or deletion of animal data. The incidence of adenomas was reported to be 4.4, 4.2, 15, and 4.6% and the incidence of HCCs was reported to be 0, 0, 0, and 4.6% for the control, 0.05, 0.5, and 5.0 g/L TCA exposure groups. The multiplicity or tumors/animal was reported to be 0.04, 0.08, 0.15, and 0.05 for adenomas and 0, 0, 0, and 0.05 for carcinomas for the control, 0.05, 0.5, and 5.0 g/L TCA exposure groups.

Although there was an increase in the incidence of adenomas at 0.5 g/L and an increase in carcinomas at 5.0 g/L TCA, they were not reported to be statistically significant by the authors; neither were the increases in adenoma multiplicity at the 0.05 and 0.5 g/L exposures. However, using such a low number of animals per treatment group (n = 20–24) limits the ability of this study to determine a statistically significant increase in tumor response and to be able to determine that there was no treatment-related effect. A power calculation of the study shows that the type II error, which should be >50% and thus, greater than the chances of "flipping a coin," was <6% for incidence and multiplicity of tumors at all exposure DCA concentrations with the exception of the incidence of adenomas for 0.5 g/L treatment group (58.7%). Therefore, the designed experiment could accept a false null hypothesis, especially in terms of tumor

multiplicity, at the lower exposure doses and erroneously conclude that there is no response due to TCA treatment. Thus, while suggesting a lower response than for mice for TCA-induced liver tumors, the study is inconclusive for determination of whether TCA induces a carcinogenic response in the liver of rats. The experimental design is such that extrahepatic carcinogenicity of TCA in the male rat cannot be determined.

E.2.3.2.15. **DeAngelo et al. (1996)**

In this study, 28-day-old male F344 rats were given drinking water containing DCA at concentrations of 0, 0.05, 0.5, or 5.0 g/L with another group was provided water containing 2.0 g/L sodium chloride for 100 weeks. This experiment modified its exposure protocol due to toxicity (peripheral neuropathy) such that the 5.0 g/L group was lowered to 2.5 g/L at 9 weeks, then 2.0 g/L at 23 weeks, and finally to 1.0 g/L at 52 weeks. When the neuropathy did not reverse or diminish, the animals were sacrificed at 60 weeks and excluded from the results. Based on measured water intake in the 0, 0.05, and 0.5 g/L groups, the TWA doses were reported to be 0, 3.6, and 40.2 mg/kg-day respectively. This experiment was conducted at a U.S. EPA laboratory in Cincinnati and the controls for this group were given 2.0 g/L sodium chloride (Study #1). In a second study, rats were given either deionized water or 2.5 g/L DCA, which was also lowered to 1.5 g/L at 8 weeks and to 1.0 g/L at 26 weeks of exposure (Study #2).

Although 23 animals were reported to be sacrificed at terminal sacrifice that had been given 2 g/L sodium chloride, the number of animals reported to be examined in this group for hepatocellular lesions was 3. The incidence data for this group for adenomas was 4.4%, so this is obviously a typographical error. The number of rats included in the water controls for tumor analysis was reported to be 33, which was the same number as those at final sacrifice. The number of animals at final sacrifice was reported to be 23 for 2 g/L sodium chloride, 21 for 0.05 g/L DCA, 23 for 0.5 g/L DCA in experiment #1, and 33 for deionized water and 28 for the initial dose of 2.5 g/L DCA in experiment #2.

Although these were of the same strain, the initial body weight was 59.1 vs. 76 g for the 2.0 g/L control group vs. deionized water group. The treatment groups in both studies were similar to the deionized water group. The percent liver/body weights were greater (4.4 vs. 3.7% in the sodium chloride vs. deionized water control groups [~20%]). The number of unscheduled deaths was greater in Study #2 (22%) than in Study #1 (12%). Interim sacrifice periods were conducted.

As with the DeAngelo et al. (DeAngelo et al., 2008) study in mice, the number of animals reported at final sacrifice was not the same as the number examined for liver tumors in Study #1 (five more animals examined than sacrificed at the 0.05 g/L DCA and six more animals examined than sacrificed at the 0.5 g/L DCA exposure groups) with n = 23, n = 26, and n = 29 for the 2 g/L sodium chloride, 0.05 g/L DCA, and 0.5 g/L DCA groups utilized in the tumor analysis. For Study #2, the same number of rats was reported to be sacrificed as examined. The source of the

extra animals for tumor analysis in Study #1, whether from interim sacrifice or unscheduled deaths, was not given by the authors and is unknown. Carcinoma prevalence data were not reported for the control group or 0.05 g/L DCA group in Study #1 and multiplicity data were not reported for the control group or 0.05 g/L DCA group. Multiplicity was not reported for adenomas in the 0.05 g/L DCA group in Study #1.

There was a lack of hepatocyte DNA synthesis and necrosis reported at any dose group carried out to final sacrifice at 100 weeks. The authors reported the incidence of adenomas to be 4.4% in 2 g/L sodium chloride control, 0 in 0.05 g/L DCA, and 17.2% in the 0.5 g/L DCA exposure groups. For carcinomas, no data were reported for the control or 0.05 g/L DCA group but an incidence of 10.3% was reported for the 0.5 g/L DCA group. The authors reported increased hepatocellular adenomas and carcinomas in male F344 rats, although no data were reported for carcinomas in the control and 0.05 g/L exposure groups. They reported that for 0.5 g/L DCA, 24.1 vs. 4.4% adenomas and carcinomas combined (Study #1) and 28.6 vs. 3.0% (Study #2) at what was initially 2.5 g/L DCA but continuously reduced. Tumor multiplicity was reported to be significantly increased in the 0.5 g/L DCA group (0.04 adenomas and carcinomas/ animal in control vs. 0.31 in 0.5 g/L DCA in Study #1 and 0.03 in control vs. 0.36 in what was initially 2.5 g/L DCA in Study #2). The issues of use of a small number of animals, additional animals for tumor analysis in Study #1, and most of all, the lack of a consistent dose for the 2.5 g/L animals in Study #2, are obvious limitations for establishment of a dose-response for DCA in rats.

E.2.3.2.16. **Richmond et al. (1995)**

This study was conducted by the same authors as DeAngelo et al. (1996) and appears to report results for the same data set for the 2 g/L sodium chloride control, 0.05 g/L DCA and 0.5 g/L DCA exposed groups. Of note is that while DeAngelo et al. (1996) refer to the 28-day-old rats as ”weanlings,” the same aged rats are referred to as “adults” in this study. Male F344 rats were administered TWA concentrations of 0, 0.05, 0.5, or 2.4 g/L DCA in drinking water. Concentrations were kept constant but due to hind-limb paralysis, all 2.4 g/L DCA rats had been sacrificed by 60 weeks of exposure. In the 104-week sacrifice time, there were 23 rats reported to be analyzed for incidence of hepatocellular adenomas and carcinomas in the control group, 26 rats in the 0.05 g/L DCA group, and 29 rats in the 0.5 g/L DCA exposed group. This is the same number of animals included in the tumor analysis reported in DeAngelo et al. (1996). Tumor multiplicity was not given.

Richmond et al. (1995) reported that there was a 4% incidence of adenomas reported in the 2.0 g/L sodium chloride control animals, 0% at 0.05 g/L DCA, and 21% in the 0.5 DCA group at 104 weeks. These figures are similar to those reported by DeAngelo et al. (1996) for the same data set, with the exception of a 17.2% incidence of adenomas reported for the 0.5 g/L DCA group.

There were no HCCs reported in the control or 0.05 g/L exposure groups, but a 10% incidence reported in the 0.5 g/L DCA exposure group at 104 weeks of exposure. While carcinomas were not reported by DeAngelo et al. (1996) for the control and 0.05 g/L groups, they are assumed to be zero in the summary data for carcinomas and adenomas combined. The 10% incidence at 0.5 g/L DCA is similar to the 10.4% incidence reported for this group by DeAngelo et al. (1996).

At 60 weeks at 2.4 g/L DCA, the incidences of hepatocellular adenomas were reported to be 26% and HCCs to be 4%. This is not similar to the values reported by DeAngelo for 2.5 g/L DCA that was continuously decreased so that the estimated final concentration was 1.6 g/L DCA for 100 weeks. For those animals, the incidence of adenomas was reported by DeAngelo et al. (1996) to be 10.7% and carcinomas 21.4%, probably more a reflection of longer exposure time allowing for adenoma to carcinoma progression. The authors did not report any of the results of DCA-induced increases of adenomas and carcinomas to be statistically significant. As it appears the same data set was used for the 2.0 g/L sodium chloride control, 0.05 g/L DCA, and 0.5 g/L DCA exposure groups as was reported in DeAngelo et al. (1996), the same issues arise as regarding the differences in numbers of animals that were included in tumor analysis than were reported to have been present at final sacrifice. As stated previously for the DeAngelo et al. (1997) study of TCA in rats, the use of small numbers of rats limits the detection of and ability to determine whether there was no treatment-related effects, especially at the low concentrations of DCA exposure.

E.2.4. Summaries and Comparisons Between TCE, DCA, and TCA Studies

There are a number of studies of TCE that have reported effects on the liver. However, the study of this compound is difficult as its concentration does not remain stable in drinking water, some studies have been carried out using TCE with small quantities of a carcinogenic stabilizing agent, some studies have been carried out in whole-body inhalation chambers that resulted in additional oral administration and for which individual animal data were not recorded throughout the experiment, and the results of gavage studies have been limited by gavage-related deaths and vehicle effects. In addition, some studies have been conducted using the i.p. route of administration, which results in route-related toxicity and inflammation. For many studies, liver effects consisted of measured increases in liver weight with little or no description of attendant histological changes induced by TCE treatment. A number of studies were conducted at a few relatively high doses with attendant effects on body weight, indicative of systemic toxicity and affecting TCE-induced liver weight gain. Although many studies have been performed in male mice, the inhalation studies of Kjellstrand et al. (1981b, 1983a, 1983b) indicate that male mice, regardless of strain appear to have a greater variability in response, as measured by TCE-induced liver weight gain, and susceptibility to TCE-induced decreases in body weight than female mice. However, the body of the TCE literature is consistent in identifying the liver as a target of TCE-

induced effects, with the most commonly reported change to be a dose-related TCE-induced increase in liver weight in multiple species, strains, and genders from both inhalation and oral routes of exposure.

The following sections will not only summarize results for studies of TCE reported in Sections E.2.1–E.2.2, but provide comparison of studies of either TCA or DCA that have used similar paradigms or investigated similar parameters described in Sections E.2.3.1 and E.2.3.2. A synopsis of the results from studies of CH and in comparison with TCE results is presented in Section E.2.5. While the study of Bull et al. (2002), described in Section E.2.2.2.2, presents data for combinations of DCA or TCA exposure for comparisons of tumor phenotype with those induced by TCE, the examination of co-exposure studies of TCE metabolites in rodents that are also exposed to a number of other carcinogens, and descriptions of the toxicity data for brominated haloacetates that also occur with TCE in the environment, are presented in Section E.4.3.3.

E.2.4.1. Summary of Results For Short-term Effects of TCE

In regard to early changes in DNA synthesis, the data for TCE are very limited. The study by Mirsalis et al. (1989) used an in vivo-in vitro hepatocyte DNA repair and S-phase DNA synthesis in primary hepatocytes from male F344 rats (180–300 g) and male and female $B6C3F_1$ mice (20–29 g for male mice and 18–25 g female mice) administered TCE by gavage in corn oil. They reported negative results 2–12 hours after treatment from 50 to 1,000 mg/kg TCE in rats and mice (male and female) for UDS and repair using three animals per group. After 24 and 48 hours of 200 or 1,000 mg/kg TCE in male mice (n = 3) and after 48 hours of 200 (n = 3) or 1,000 (n = 4) mg/kg TCE in female mice, similar values of 0.30–0.69% of hepatocytes were reported as undergoing DNA synthesis in those hepatocytes in primary culture with only the 1,000 mg/kg TCE dose in male mice at 48 hours giving a result considered to be positive (~2.2%). No statistical analyses were performed on these measurements, which were obviously limited by both the number of animals examined and the relevance of the paradigm.

TCE-induced increases in liver weight have been reported to occur quickly. The inhalation study of Okino et al. (1991) in male rats demonstrates that liver weight and metabolism were increased with as little as 8 hours of TCE exposure (500 and 2,000 ppm) and as early as 22 hours after cessation of such exposures with little concurrent hepatic necrosis. Laughter et al. (2004) reported increase liver weight in SV129 mice in their 3-day study (see below). Tao et al. (2000) reported a 1.26-fold of control percent liver/body weight in female $B6C3F_1$ mice fed 1,000 mg/kg TCE in corn oil for 5 days. Elcombe et al. (1985) and Dees and Travis (1993) reported gavage results in mice and rats after 10 days exposure to TCE, which showed TCE-induced increases in liver weight (see below for more detail on dose-response). Tucker et al. (1982) reported that 14 days of exposure to 24 and 240 mg/kg TCE via gavage induced a dose-related increase in liver weight in male CD-1 mice but did not show the data.

TCE-induced increases in percent liver/body weight ratios have been studied most extensively in B6C3F$_1$ and Swiss mice. Both strains have been shown to have a TCE-induced increase in liver tumors from long-term exposure as well (see Section E.2.4.3). A number of studies have provided dose-response information for TCE-induced increases in liver weight from 10 days to 13 weeks of exposure in mice. Most studies have reported that the magnitude of increase in TCE exposure concentration is similar to the magnitude increase of percent liver/body weight increase. For example a twofold increase in TCE exposure has often resulted in a twofold increase in the percent change in liver/body weight over control (i.e., 500 mg/kg TCE induces a 20% increase in liver weight and 1,000 mg/kg TCE induces a 50% increase in liver weight as reported by Elcombe et al. (1985). The range in which this relationship is valid has been reported to vary from 100 mg/kg TCE at 10 days (Dees and Travis, 1993) to 1,600 mg/kg (Buben and O'Flaherty, 1985) at 6 weeks and up to 1,500 mg/kg TCE for 13 weeks (NTP, 1990). The consistency in the relationship between magnitude of liver weight increase and TCE exposure concentration has been reported for both genders of mice, across oral and inhalation routes of exposure, and across differing strains of mice tested. For rats, there are fewer studies with fewer exposure levels tested, but both Berman et al. (1995) and Melnick et al. (1987) report that short-term TCE exposures from 150 to ~2,000 mg/kg induced percent liver/body weight that increased proportionally with the magnitude of TCE exposure concentration.

Dependence of PPARα activation for TCE-liver weight gain has been investigated in PPARα null mice by both Nakajima et al. (2000) and Laughter et al. (2004). After 2 weeks of 750 mg/kg TCE exposure to carefully matched SV129 wild-type or PPARα-null male and female mice (n = 6 group), there was a reported 1.50-fold of control in wild-type and 1.26-fold of control percent liver/body weight in PPARα-null male mice by Nakajima et al. (2000). For female mice, there was ~1.25-fold of control percent liver/body weight ratios for both wild-type and PPARα-null mice. Ramdhan et al. (2010) also reported increased liver weight in male PPARα-null mice after high levels of inhalation exposure that were comparable to that in wild type mice after 7 days of exposure (up to 40–50% increases at the highest dose). Thus, TCE-induced liver weight gain was not dependent on a functional PPARα receptor in female mice, and data indicate that a significant portion of it may have also not have been PPARα receptor-dependent in male mice.

Nakajima et al. (2000) report that both wild-type male and female mice have similar increases in the number of peroxisome in the pericentral area of the liver after TCE exposure and, although increased twofold, were still only ~4% of cytoplasmic volume. Female wild-type mice were reported to have less TCE-induced elevation of very long chain acyl-CoA synthetase, D-type peroxisomal bifunctional protein, mitochondrial trifunctional protein α subunits α and β, and CYP 4A1 than males mice, even though peroxisomal volume was similarly elevated in male and female mice. The induction of PPARα protein by TCE treatment was also reported to be slightly less in female than male wild-type mice (2.17- vs. 1.44-fold of control, respectively).

Ramdhan et al. (2010) examined TCE-induced hepatice steatosis and toxicity using male wild type, PPARα-null, and human PPARα-inserted mice (humanized) exposed to high inhalation concentrations of TCE for 7 days. Significant differences were observed among control mice for each genotype with reduced body weight in untreated humanized mice. Liver/body weight ratios were 11% higher in untreated PPARα- null mice than wild type mice. Higher levels of liver triglycerides and hepatic steatosis were reported in the untreated humanized mice and PPARα-null mice than wild type mice. Background expression of a number of genes and protein expression levels were significantly different between the untreated strains. In particular, human PPARα protein levels were >10-fold greater in the humanized mice than mouse PPARα in untreated wild type mice. Insertion of human PPARα in the null mice did not return the mice to a normal state. Both PPARα-null and humanized mice were more susceptible to TCE toxicity as evidenced by serum AST and ALT (liver injury biomarkers), hepatic triglyceride levels, and hepatic steatosis. Hepatomegally was induced in all strains to a similar extent after TCE exposure. However, urinary TCA concentrations were reported to be significantly lower and TCOH levels significantly higher in TCE-treated PPARα-null mice in comparison to treated wild type mice. This difference was not related to changes in expression of metabolic enzymes. Thus, TCE-induced liver toxicity was not dependent on PPARα with dysregulation of the receptor in null or humanized mice, rendering them more susceptible to TCE-induced toxicity.

Laughter et al. (2004) also studied SV129 wild-type and PPARα-null male mice treated with 3 daily doses of TCE in 0.1% methyl cellulose for either 3 days (1,500 mg/kg TCE) or 3 weeks (0, 10, 50, 125, 500, 1,000, or 1,500 mg/kg TCE 5 days/week). However, not only is the paradigm not comparable to other gavage paradigms, but no initial or final body weights of the mice were reported and thus, the influence of differences in initial body weight on percent liver/body weight determinations could not be ascertained. In the 3-day study, while control wild-type and PPARα-null mice were reported to have similar percent liver/body weight ratios (~4.5%), at the end of the 3-week experiment, the percent liver/body weight ratios were reported to be increased in the PPARα-null male mice (5.1%).

TCE treatment for 3 days was reported to increase the percent liver/body weight ratio 1.4-fold of control in the wild-type mice and 1.07-fold of control in the null mice. In the 3-week study, wild-type mice exposed to various concentrations of TCE had percent liver/body weights that were reported to be within ~2% of control values except for the 1,000 and 1,500 mg/kg groups (~1.18- and 1.30-fold of control levels, respectively). For the PPARα-null mice, the variability in percent liver/body weight was reported to be greater than that of the wild-type mice in most of the groups, and the baseline level of percent liver/body weight ratio also 1.16-fold greater. TCE exposure was apparently more toxic in the null mice with death at the 1,500 mg/kg TCE exposure level, resulting in the prevention of recording of percent liver/body weights. At the 1,000 mg/kg TCE exposure level, there was a reported 1.10-fold of control percent liver/body weight in the PPARα-null mice.

None of the increases in percent liver/body weight in the null mice were reported to be statistically significant by Laughter et al. (2004). However, the statistical power of the study was limited due to low numbers of animals and increased variability in the null mice groups. The percent liver/body weight after TCE treatment that was reported in this study was actually greater in the null mice than the wild-type male mice at the 1,000 mg/kg TCE exposure level (5.6 ± 0.4 vs. 5.2 ± 0.5%, for null and wild-type mice, respectively). At 1-week and at 3-weeks, TCE appeared to induce increases in liver weight in PPARα-null mice, although not reaching statistical significance in this study. At a 1,000 mg/kg TCE exposure for 3 weeks, percent liver/body weights were reported to be 1.18-fold of control in wild-type and 1.10-fold of control in null mice. Although the experiments in Laughter et al. (2004) for DCA and TCA were not conducted using the same paradigm, the TCE-induced increase in percent liver/body weight more closely resembled the dose-response pattern for DCA than for DCA wild-type SV129 and PPARα-null mice.

Many studies have used cyanide-insensitive PCO as a surrogate for peroxisome proliferation. Of note is that several studies have shown that this activity is not correlated with the volume or number of peroxisomes that are increased as a result of exposure to TCE or its metabolites (Nakajima et al., 2000; Nelson et al., 1989; Elcombe et al., 1985). This activity appears to be highly variable both as a baseline measure and in response to chemical exposures. Laughter et al. (2004) presented data showing that WY-14,643 induced increases in PCO activity varied up to sixfold between experiments in wild-type mice. They also showed that PCO activity, in some instances, was up to sixfold of wild-type mice values in untreated PPARα-null mice. Parrish et al. (1996) noted that control values between experiments varied as much as a factor of 2-fold for PCO activity and thus, their data were presented as percent of concurrent controls. Goldsworthy and Popp (1987) reported that 1,000 mg/kg TCE induced a 6.25-fold of control PCO activity in B6C3F_1 mice in two 10-day experiments. However, for F344 rats, the increases over control between two experiments conducted at the same dose were reported to vary by >30%. Finally, Melnick et al. (1987) have reported that corn oil administration alone can elevate PCO activity as well as catalase activity.

For TCE there are two key 10-days studies (Dees and Travis, 1993; Elcombe et al., 1985) that examine the effects of short-term exposure in mice and rats via gavage exposure and attempt to determine the nature of the dose- response in a range of exposure concentrations that include levels below which there is concurrent decreased body weights. Although they have limitations, they reported generally consistent results. In regard to liver weight in mice, gavage exposure to TCE at concentrations ranging from 100 to 1,500 mg/kg TCE produced increases in liver/body weight that was dose-related (Dees and Travis, 1993; Elcombe et al., 1985).

Elcombe et al. (1985) reported a small decrease in DNA content with TCE treatment (consistent with hepatocellular hypertrophy) that was not dose-related, increased tritiated thymidine incorporation in whole mouse liver DNA that was that was treatment-related but not

dose-related (i.e., two-, two-, and fivefold of control values in mice treated with 500, 1,000, and 1,500 mg/kg TCE), and slightly increased numbers of mitotic figures that were treatment-related, but not dose-related and not correlated with DNA synthesis as measured by thymidine incorporation. Elcombe et al. (1985) reported an increase in peroxisome volume after TCE exposure that was correlated with the magnitude of increase in peroxisomal-associated enzyme activity at the only dose in which both were tested. Peroxisome increases after TCE treatment in mice livers were identified as being pericentral in location. After TCE treatment, increased peroxisomal volumes in $B6C3F_1$ mice were reported to be not dose-related (i.e., there was little difference between 500 and 1,500 mg/kg TCE exposures). The TCE-induced increases in peroxisomal volumes were also not correlated with the reported increases in thymidine incorporation or mitotic activity in mice.

Neither TCE-induction of peroxisomes nor hepatocellular proliferation, as measured by either mitotic index or thymidine incorporation, was correlated with TCE-induced liver weight increases. Elcombe et al. (1985) only measured PCO activity in a subset of $B6C3F_1$ mice at the 1,000 mg/kg TCE exposure level for 10 days of exposure and reported an 8-fold of control PCO activity and a 1.5-fold of control catalase activity. This result was similar to that of Goldsworthy and Popp (1987), who reported 6.25-fold of control PCO activity in male $B6C3F_1$ mice exposed to 1,000 mg/kg-day TCE for 10 days in two separate experiments.

Similar to Elcombe et al. (1985), who reported no difference in response between 500 and 1,000 mg/kg TCE treatments, (Dees and Travis, 1993) reported that incorporation of tritiated thymidine in DNA from mouse liver was elevated after TCE treatment and the mean peak level of tritiated thymidine incorporation occurred at 250 mg/kg TCE treatment level remaining constant for the 500 and 1,000 mg/kg treated groups. (Dees and Travis, 1993) specifically report that mitotic figures, although very rare, were more frequently observed after TCE treatment, most often in the intermediate zone, and in cells resembling mature hepatocytes. They reported that there was little tritiated thymidine incorporation in areas near the bile duct epithelia or close to the portal triad in liver sections from both male and female mice. They also reported no evidence of increased lipofuscin and that increased apoptosis from TCE exposure "did not appear to be in proportion to the applied TCE dose given to male or female mice" (i.e., the mean number of apoptosis 0, 0, 0, 1, and 8 for control, 100, 250, 500, and 1,000 mg/kg TCE treated groups, respectively). Both Elcombe et al. (1985) and (Dees and Travis, 1993) reported no changes in apoptosis other than increased apoptosis only at a treatment level of 1,000 mg/kg TCE.

Elcombe et al. (1985) reported increases in percent liver/body weight after TCE treatment in both the Osborne-Mendel and Alderley Park rat strain, although to a smaller extent than in mice. For both strains, Elcombe et al. (1985) reported no TCE-induced changes in body weight at doses ranging from 500 to 1,500 mg/kg. For male Osborne-Mendel rats, administration of TCE in corn oil gavage resulted in a 1.18-, 1.26-, and 1.30-fold of control percent liver/body weight at 500, 1,000, and 1,500 mg/kg-day exposures, respectively. For Alderley Park rats, those increases

were 1.14-, 1.17-, and 1.17-fold of control at the same respective exposure levels for 10 days of exposure.

In regard to liver weight increases, Melnick et al. (1987) reported a 1.13- and 1.23-fold of control percent liver/body weight in male F344 rats fed 600 and 1,300 mg/kg-day TCE in capsules, respectively. There was no difference in the extent of TCE-induced liver increase between the two lowest dosed groups administered TCE in corn oil gavage (~20% increase in percent liver/body weight at 600 and 1,300 mg/kg-day TCE) for 14 days. However, the magnitude of increases in percent liver/body weight in these groups was affected by difference between control groups in liver weight although initial and final body weights appeared to be similar. By either type of vehicle, Melnick et al. (1987) reported decreases in body weights in rats treated with concentrations of TCE ≥2,200 mg/kg-day for 14 days. Similarly, Nunes et al. (2001) reported decreased body weight in Sprague-Dawley rats administered 2,000 mg/kg-day for 7 days in corn oil. Melnick et al. (1987) reported that both exposures to either 600 or 1,300 mg/kg-day TCE in capsules did not result in decreased body weight and caused less than minimal focal necrosis randomly distributed in the liver. At 2,200 and 4,800 mg/kg TCE fed via capsule, Melnick et al. (1987) reported that although there was decreased body weight in rats treated at these exposures, there was little TCE-induced necrosis, and no evidence of inflammation, cellular hypertrophy or edema with TCE exposure. Similarly, Berman et al. (1995) reported increases in liver weight gain at doses as low as 50 mg/kg TCE, no necrosis up to doses of 1,500 mg/kg, and hepatocellular hyper trophy only at the 1,500 mg/kg level in female F344 rats.

For rats, Elcombe et al. (1985) reported an increase over untreated rats of 1.13-fold of control PCO activity in Alderley Park rats after 1,000 mg/kg-day TCE exposure for 10 days, while Goldsworthy and Popp (1987) reported a 1.8- and 2.39-fold of control in male F344 rats at the same exposure in two separate experiments. Melnick et al. (1987) reported PCO activity of 1.23- and 1.75-fold of control in male F344 rats fed 600 and 1,300 mg/kg-day TCE for 14 days in capsules. For rats treated by gavage with 600 or 1,200 mg/kg-day TCE corn oil, they reported 1.16- and 1.29-fold of control values. However, control levels of PCO were 16% higher in corn oil controls than in untreated controls. In addition, Melnick et al. (1987) reported little catalase increases in rats fed TCE via capsules in food (<6% increase) but a 1.18- and 1.49-fold of control catalase activity in rats fed 600 or 1,200 mg/kg/TCE via corn oil gavage, indicative of a vehicle effect.

The data from Elcombe et al. (1985) included reports of TCE-induced pericentral hypertrophy and eosinophilia for both rats and mice but with “fewer animals affected at lower doses.” In terms of glycogen deposition, Elcombe et al. (1985) report “somewhat” less glycogen pericentrally in the livers of rats treated with TCE at 1,500 mg/kg than controls with less marked changes at lower doses restricted to fewer animals. They do not comment on changes in glycogen in mice. Dees and Travis (1993) reported TCE-induced changes to “include an increase in

eosinophilic cytoplasmic staining of hepatocytes located near central veins, accompanied by loss of cytoplasmic vacuolization." Since glycogen is removed using conventional tissue processing and staining techniques, an increase in glycogen deposition would be expected to increase vacuolization and thus, the report from Dees and Travis (1993) is consistent with less, not more, glycogen deposition. Neither study produced a quantitative analysis of glycogen deposition changes from TCE exposure. Although not explicitly discussing liver glycogen content or examining it quantitatively in mice, these studies suggest that TCE-induced liver weight increases did not appear to be due to glycogen deposition after 10 days of exposure, and any decreases in glycogen were not necessarily correlated with the magnitude of liver weight gain either.

For both rats and mice, the data from Elcombe et al. (1985) showed that tritiated thymidine incorporation in total liver DNA observed after TCE exposure did not correlate with mitotic index activity in hepatocytes with both Elcombe et al. (1985) and Dees and Travis (1993) reporting a small mitotic indexes and evidence of periportal hepatocellular hypertrophy from TCE exposure. Neither mitotic index or tritiated thymidine incorporation data support a correlation with TCE-induced liver weight increase in the mouse. If higher levels of hepatocyte replication had occurred earlier, such levels were not sustained by 10 days of TCE exposure. Both Elcombe et al. (1985) and Dees and Travis (1993) present data that represent "a snapshot in time," which do not show whether increased cell proliferation may have happened at an earlier time point and then subsided by 10 days. These data suggest that increased tritiated thymidine levels were targeted to mature hepatocytes and in areas of the liver where greater levels of polyploidization occur. Both Elcombe et al. (1985) and Dees and Travis (1993) show that tritiated thymidine incorporation in the liver was approximately twofold of controls between 250 and 1,000 mg/kg TCE, a result consistent with a doubling of DNA. Thus, given the normally quiescent state of the liver, the magnitude of this increase over control levels, even if a result of proliferation rather than polyploidization, would be confined to a very small population of cells in the liver after 10 days of TCE exposure.

Laughter et al. (2004) reported that there was an increase in DNA synthesis after aqueous gavage exposure to 500 and 1,000 mg/kg TCE given as three boluses a day for 3 weeks with BrdU given for the last week of treatment in mice. An examination of DNA synthesis in individual hepatocytes was reported to show that 1 and 4.5% of hepatocytes had undergone DNA synthesis in the last week of treatment for the 500 and 1,000 mg/kg doses, respectively. Both Elcombe et al. (1985) and Dees and Travis (1993) show TCE-induced changes for several parameters at the lowest level tested without toxicity and without evidence of regenerative hyperplasia or sustained hepatocellular proliferation.

In regards to susceptibility to liver cancer induction, the more susceptible ($B6C3F_1$) vs. less susceptible (Alderley Park/Swiss) strains of mice to TCE-induced liver tumors (Maltoni et al., 1988), the "less susceptible" strain was reported by Elcombe et al. (1985) to have a greater baseline level of liver weight/body weight ratio, a greater baseline level of thymidine

incorporation, and greater responses for those endpoints due to TCE exposure. However, both strains showed a hepatocarcinogenic response after TCE exposure, although there are limitations regarding determination of the exact magnitude of response for these experiments as previously discussed.

E.2.4.2. Summary of Results For Short-Term Effects of DCA and TCA: Comparisons With TCE

Short-term exposures from DCA and TCA have been studied either through gavage or in drinking water. Palatability became an issue at the highest level of DCA tested in drinking water experiments (5 g/L), which caused a significant reduction of drinking water intake in mice of 46–64% (Carter et al., 1995). Decreases in drinking water consumption have also been reported for a range of concentrations of DCA and TCA from 0.05 to 5.0 g/L, in both mice and rats, and with generally the higher concentrations producing the highest decrease in drinking water (DeAngelo et al., 1999; DeAngelo et al., 1997; Carter et al., 1995; Mather et al., 1990); (DeAngelo et al., 2008). However, results within studies (e.g., DeAngelo et al., 2008) and between studies have been reported to vary as to the extent of the reduction in drinking water from the presence of TCA or DCA. Some drinking water studies of DCA or TCA have not reported drinking water consumption. Therefore, although in general, DCA and TCA studies have do not include vehicle effects, such as those posed by corn oil, they have been affected by differences in drinking water consumption not only changing the dose received by the rodents and therefore, potentially the shape of the dose-response curve, but also the effects of dehydration are potentially added to any chemically-related reported effects.

Studies have attempted to determine short-term effects on DNA by TCE and its metabolites. Nelson and Bull (1988) administered TCE male to Sprague-Dawley rats and male B6C3F_1 mice and measured the rate of DNA unwinding under alkaline conditions 4 hours later. For rats, there was a significantly increased rate of unwinding at the two highest dose and for mice, there was a significantly increased level of DNA unwinding at a lower dose. In this same study, DCA was reported to be most potent in this assay with TCA being the lowest, while CH closely approximated the dose-response curve of TCE in the rat. In the mouse, the most potent metabolite in the assay was reported to be TCA, followed by DCA with CH considerably less potent. Nelson and Bull (1988) and Nelson et al. (1989) have reported increases in SSBs after DCA and TCA exposure. However, Styles et al. (1991) (for mice) and Chang et al. (1992) (for mice and rats) did not. Austin et al. (1996) report that the alkaline unwinding assay, a variant of the alkaline elution procedure, is noted for its variability and inconsistency depending on the techniques used while performing the procedure. In regard to oxidative damage as measured by TBARS for lipid peroxidation and 8-OHdG levels in DNA, increases appear to be small (<50% greater than control levels) and transient after DCA and TCA treatment in mice (see Section E.3.4.2.3) with TCE results confounded by vehicle or route of administration effects.

Although there is no comparative data for TCE, the study of Styles et al. (1991) is particularly useful for determining effects of TCA from 1 to 4 days of exposure in mice. Styles et al. (1991) reported no change in "hepatic" DNA uptake of tritiated thymidine up to 36 hours, a peak at 72 hours (~6-fold of control), and falling levels by 96 hours (~4-fold of controls) after 500 mg/kg TCA gavage exposure. Incorporation of tritiated thymidine observed for individual hepatocytes decreased between 24 and 36 hours, rose slowly back to control levels at 48 hours, significantly increased by 72 hours, and then decreased by 96 hours. Thus, increases in "hepatic" DNA tritiated thymidine uptake did not capture the decrease observed in individual hepatocytes at 36 hours. By either measure, the population of cells undergoing DNA synthesis was small, with the peak level being <1% of the hepatocyte population. Zonal distribution of labeled hepatocytes were decreased at 36 hours in all zones, appeared to be slightly greater in periportal than midzonal cells with centrilobular cells still below control levels by 48 hours, similarly elevated over controls in all zones by 72 hours, and to have returned to near control levels in the midzonal and centrilobular regions but with periportal areas still elevated by 96 hours. These results are consistent with all hepatocytes showing a decrease in DNA synthesis by 36 hours and then a wave of DNA synthesis to occur, starting at the periportal zone and progressing through the liver acinus that is decreased by 4 days after exposure.

Along with changes in liver weight, DNA synthesis, and glycogen accumulation, several studies of DCA and TCA have focused on the extent of peroxisome proliferation as measured by changes in peroxisome number, cytoplasmic volume and enzyme activity induction as potential "key events" occurring from shorter-term exposures that may be linked to chronic effects such as liver tumorigenicity. As noted above in Section E.2.4.1, TCE-induced liver weight gain has been reported to not be dependent on a functional PPARα receptor in female mice while as well as a significant portion of it not dependent on functional PPARα receptor in male mice. Also as noted, cyanide-insensitive PCO has also been reported to not be correlated with the volume or number of peroxisomes that are increased as a result of exposure to TCE or it metabolites (Nakajima et al., 2000; Nelson et al., 1989; Elcombe et al., 1985) and to be highly variable both as a baseline measure and in response to chemical exposures (e.g., variation of up to 6-fold between after WY-14,643 exposure in mice). Also as noted above, the vehicle used in many TCE gavage experiments, corn oil, has been reported to elevate PCO activity as well as catalase activity.

A number of short-term studies have examined the effects of TCA and DCA on liver weight increases and evidence of peroxisome proliferation and changes in DNA synthesis. In particular, two studies of DCA and TCA used a similar paradigm presented by Elcombe et al. (1985) and Dees and Travis (1993) for TCE effects in mice. Nelson et al. (1989) report findings from gavage doses of unbuffered TCA (500 mg/kg) and DCA (500 mg/kg) in male B6C3F$_1$ mice; Styles et al. (1991) also provide data on peroxisome proliferation using the same paradigm. Nelson et al. (1989) reported levels of PCO activity in mice administered 500 mg/kg DCA or TCA for 10 days with 250 mg/kg Clofibrate administration serving as a positive control. DCA

and TCA exposure were reported to not affect body weight, but both to significantly increase liver weight (1.63-fold of control for DCA and 1.30-fold of control for TCA treatments), and percent liver/body weight ratios (1.53-fold of control for DCA and 1.16-fold of control for DCA treatments). PCO activity was reported to be significantly increased by ~1.63-, 2.7-, and 5-fold of control for DCA, TCA, and Clofibrate treatments, respectively, and indicated that both DCA and TCA were weaker inducers of this activity than Clofibrate.

Results from randomly selected electron photomicrographs showed an increase in peroxisomes per unit area but gave a different pattern than PCO enzyme activity (i.e., 2.5- and 2.4-fold of control peroxisome volume for DCA and TCA, respectively). Evidence of gross hepatotoxicity was reported to not occur in vehicle or TCA-treated mice. Light microscopic sections were reported to show TCA and control hepatocytes to have the same intensity of PAS staining, but with slightly larger hepatocytes occurring in TCA-treated mice throughout the liver section with architecture and tissue pattern of the liver intact. For DCA, the histopathology was reported to be markedly different than control mice or TCA treated mice. DCA was reported to induce a marked increase in the size of hepatocytes throughout the liver with an approximately 1.4-fold of control diameter that was accompanied by increased PAS staining (indicative of glycogen deposition). All DCA-treated mice were reported to have multiple white streaks grossly visible on the surface of the liver corresponding with subcapsular foci of coagulative necrosis that were not encapsulated, varied in size, and accompanied by a slight inflammatory response characterized by neutrophil infiltration.

A quantitative comparison of effects from equivalent exposures of TCE, TCA, and DCA (500 mg/kg for 10 days in mice via corn oil gavage for TCE) shown in Table E-13 can be drawn between the Elcombe et al. (1985), Dees and Travis (1993), Styles et al. (1991), and Nelson et al. (1989) data for relationship to control values for percent liver/body weight, PCO, and qualitatively for glycogen deposition.

Table E-13. Comparison of liver effects from TCE, TCA, and DCA (10-day exposures in mice)

Model	Exposure	% Liver/body weight	Peroxisome volume	Peroxisome enzyme activity	Glycogen deposition
Nelson et al. (1989)[a]					
$B6C3F_1$ male	TCA	1.16-fold	2.4-fold	2.7-fold	No change
	DCA	1.53-fold	2.5-fold	1.63-fold	Increased
Styles et al. (1991)					
$B6C3F_1$ male	TCA	NR	1.9-fold	NR	NR
Elcombe et al. (1985)					
$B6C3F_1$ male	TCE	1.20-fold	8-fold	NR	NR
Alderley Park male (Swiss)	TCE	1.43-fold	4-fold	NR	NR
Dees and Travis (1993)					
$B6C3F_1$ male	TCE	1.05-fold[b]	NR	NR	NR
$B6C3F_1$ female	TCE	1.18-fold	NR	NR	NR

[a]Unbuffered. NR = not reported as no analysis was performed for this dose or the authors did not report this finding (i.e., did not note a change in glycogen in description of exposure-related changes).
[b]Statistically significant although small increase.

Although using a similar species, route of exposure, and dose, the comparison of responses for TCE and its metabolites shown above are in male mice and also are reflective of variability in strain, and variability and uncertainty of initial body weights. As described in more detail in Section E.2.2, initial age and body weight have an impact on TCE-related increases in liver weight. Male mice have been reported to have greater variability in response than female mice within and between studies and most of the comparative data for the 10-day 500 mg/kg doses of TCE or its metabolites were from studies in male mice. Corn oil, used as the vehicle for TCE gavage studies but not those of its metabolites, has been noted to specifically affect peroxisomal enzyme induction, body weight gain, and hepatic necrosis, specifically, in male mice (Merrick et al., 1989). Corn oil alone has also been reported to increase PCO activity in F344 rats and to potentiate the induction of PCO activity of TCA (DeAngelo et al., 1989). Thus, quantitative inferences regarding the magnitude of response in these studies are limited by a number of factors.

The variability in the magnitude of TCE-induced increases in percent liver/body weight across studies is readily apparent, but for TCE, TCA, and DCA, there is an increase in liver weight in mice at this dose after 10 days of exposure. The volume of the peroxisomal compartment in hepatocytes was reported to be more greatly increased from TCE-treatment by Elcombe et al. (1985) than for either TCA or DCA by Nelson et al. (1989) or Styles et al. (1991). However, the control values for the $B6C3F_1$ mice were half that of the other strain reported by Elcombe et al. (1985) and this parameter in general did not match the pattern of PCO activity values reported for TCA and DCA (Nelson et al., 1989). There is no PCO activity data at this

dose for TCE, but Elcombe et al. (1985) reported that the magnitude of TCE-induced increase in peroxisome volume was similar to that of PCO activity at the only dose where both were tested (1,000 mg/kg TCE).

However, Elcombe et al. (1985) reported that increased peroxisomal volumes in $B6C3F_1$ mice after 10 days of TCE treatment were not dose-related (i.e., there was little difference between 500, 1,000, and 1,500 mg/kg TCE exposures in the magnitude of TCE-induced increases in peroxisomal volume). The lack of dose-response for TCE-induced peroxisomal volume increases was not consistent with increases in percent liver/body weight that increased with increasing TCE exposure concentration. Also as noted above, PCO activity appears to be highly variable in untreated and treated rodents and to vary between experiments and between studies.

From the above comparison, it is clear that TCE, DCA, and TCA exposures were associated with increased liver weight in mice but a question arises as to what changes account for the liver weight increases. For TCE and TCA 500 mg/kg treatments, changes in glycogen were not reported in the general descriptions of histopathological changes (Dees and Travis, 1993; Styles et al., 1991; Elcombe et al., 1985) or were specifically described by the authors as being similar to controls (Nelson et al., 1989). However, for DCA, glycogen deposition was specifically noted to be increased with treatment, although no quantitative analyses were presented that could give information as to the nature of the dose-response (Nelson et al., 1989). Issues in regard to not only whether TCE and its metabolites each gives a similar response for a number of parameters, but also potential changes may be associated with carcinogenicity from long-term exposures can be examined by a comparison of the dose-response curves for these parameters from a range of exposure concentrations and durations of exposure. In addition, if glycogen accumulation results from DCA exposure, what proportion of DCA-induced liver weight increases result from such accumulation or other events that may be similar to those occurring with TCE exposure (see Section E.4.2.4)?

As noted in Section E.2.4.1, TCE-induced changes in liver weight appear to be proportional to the exposure concentration across route of administration, gender, and rodent species. As an indication of the potential contribution of TCE metabolites to this effect, a comparison of the shape of the dose-response curves for liver weight induction for TCE and its metabolites is informative. A number of studies of TCA and DCA in drinking water, conducted from 10 days to 4 weeks, have attempted to measure changes in liver weight induction, peroxisomal enzyme activity, and DNA synthesis predominantly in mice to provide insight into the mode(s) of action for liver cancer induction (DeAngelo et al., 2008; Parrish et al., 1996; Carter et al., 1995; Sanchez and Bull, 1990; DeAngelo et al., 1989).

Direct comparisons are harder to make between the drinking water studies of DCA and TCA and the gavage studies of TCE (Tables E-14, E-15, and E-16). Similar to 10-day gavage exposures to TCE, 14-day exposures to TCA or DCA via drinking water were reported to induce dose-related increases in liver weight in male $B6C3F_1$ mice (0.3, 1.0, and 2.0 g/L TCA or DCA)

with a greater increase in liver weight from DCA than TCA at 2 g/L and a difference in the shape of the dose-response curve (Sanchez and Bull, 1990). They reported a 1.08-, 1.31-, and 1.62-fold of control liver weight for DCA and a 1.15-, 1.22-, and 1.38-fold of control values for TCA at 0.3, 1.0, and 2.0 g/L concentrations, respectively (n = 12–14 mice). While the magnitude of difference between the exposures was ~6.7-fold between the lowest and highest dose, the differences between TCA exposure groups for change in percent of liver weight was ~2.5, but for DCA, the slope of the dose-response curve for liver weight increases appeared to be closer to the magnitude of difference in exposure concentrations between the groups (i.e., a difference of 7.7-fold between the highest and lowest dose for liver weight induction).

DeAngelo et al. (1989) reported that after 14 days of exposure to 5 or 2 g/L TCA in male mice, the magnitudes of the difference in the increase in exposure concentration (2.5-fold) was generally higher than the increase percent liver/body weight ratios at these doses (i.e., ~40% for the Swiss-Webster, C3H, and for one of the $B6C3F_1$ mouse experiments, and for the C57BL/6 mouse, there was no difference in liver weight induction between the 2 and 5 g/L TCA exposure groups). There was a range in the magnitude of percent liver/body weight ratio increases between the strains of mice with liver weight induction reported to range between 1.26- and 1.66-fold of control values for the four strains of mice at 5 g/L TCA and to range between 1.16- and 1.63-fold of control values at 2 g/L TCA. One strain, $B6C3F_1$, was chosen to compare responses between DCA and TCA. At 1, 2, and 5 g/L TCA or DCA, DCA was reported to induce a greater increase in liver weight that TCA (i.e., 1.55- vs. 1.39-fold of control percent liver/body weight ratio for 5.0 g/L DCA vs. TCA, respectively). At the 5 g/L exposures, DCA induced ~40% greater percent liver/body weight than TCA. Although as noted above, the majority of the data from this study in mice did not indicate that the magnitude of difference in exposure concentration was the same as that of liver weight induction for TCA, in the particular experiment that examined both DCA and TCA, the increase in percent liver/body weight ratios were similar to the magnitude of difference in dose between the 2 and 5 g/L exposure concentrations for both DCA and TCA (i.e., 2–2.5-fold increase in liver weight change corresponding to a 2.5-fold difference in exposure concentration).

Table E-14. Liver weight induction as percent liver/body weight fold-of-control in male B6C3F$_1$ mice from DCA or TCA drinking water studies

Concentration (g/L)	Duration of exposure				Mean for average of d 14–30
	14 or 15 d	20 or 21 d	25 d	28 or 30 d	
DCA					
0.1		1.02-fold			1.02-fold
0.3	1.08-fold				1.08-fold
0.5	1.12-fold	1.24-fold, 1.05-fold	1.16-fold	1.16-fold	1.15-fold
1.0	1.31-fold				1.31-fold
2.0	1.62-fold	1.46-fold, 2.01-fold	2.04-fold	1.99-fold, 1.42-fold	1.83-fold
5.0	1.67-fold				1.67-fold
TCA					
0.05				1.09-fold	1.09-fold
0.1		0.98-fold			0.98-fold
0.3	1.15-fold				1.15-fold
0.5		1.13-fold		1.16-fold	1.15-fold
1.0	1.23-fold, 1.08-fold				1.16-fold
2.0	1.38-fold, 1.16-fold, 1.26-fold	1.33-fold			1.30-fold
3.0				1.33-fold	1.33-fold
5.0	1.39-fold, 1.35-fold				1.37-fold

Table E-15. Liver weight induction as percent liver/body weight fold-of-control in male B6C3F$_1$ or Swiss mice from TCE gavage studies

Concentration (mg/kg-d)	10 d	28 d	42 d	Mean for average of d 10–42
B6C3F$_1$				
100	1.00-fold			1.00-fold
250	1.00-fold			1.00-fold
500	1.20-fold, 1.06-fold			1.13-fold
600		1.36-fold		1.36-fold
1,000	1.50-fold, 1.17-fold, 1.50-fold			1.39-fold
1,200		1.64-fold		1.64-fold
1,500	1.47-fold			1.47-fold
2,400		1.81-fold		1.81-fold
Swiss				
100			1.12-fold	1.12-fold
200			1.15-fold	1.15-fold
400			1.25-fold	1.25-fold
500	1.43-fold	1.32-fold		1.38-fold
800			1.36-fold	1.36-fold
1,000	1.56-fold	1.41-fold		1.49-fold
1,500	1.75-fold			1.75-fold
1,600			1.63-fold	1.63-fold
2,000		1.38-fold		1.38-fold
2,400		1.69-fold		1.69-fold

Carter et al. (1995) examined 0.5 and 5.0 g/L exposures to DCA in B6C3F$_1$ male mice and reported that percent liver/body weights were increased consistently from 0.5 g/L DCA treatment from 5 to 30 days of treatment (i.e., a range of 1.05–1.16-fold of control). For 5.0 g/L DCA exposure, the range of increase in percent liver/body weight was reported to be 1.37–2.04-fold of control for the same time period. At the 15 days of exposures, the percent liver/body weight ratios were 1.67- and 1.12-fold of control for 5.0 and 0.5 g/L DCA and at 30 days were 1.99- and 1.16-fold, respectively. The difference in magnitude of dose and percent liver/body weight increase is difficult to determine given that the 5 g/L dose of DCA reduced body weight and significantly reduced water consumption by ~50%. The differences in DCA-induced percent liver/body weights were ~6-fold for the 15-, 25-, and 30-day data between the 0.5 and 5 g/L DCA exposures rather than the 10-fold difference in exposure concentration in the drinking water.

Table E-16. B6C3F$_1$ and Swiss (data sets combined)

Concentration (mg/kg-d)	Mean for average of d 10–42
100	1.06-fold
200	1.15-fold
250	1.00-fold
400	1.25-fold
500	1.26-fold
600	1.36-fold
800	1.36-fold
1,000	1.49-fold
1,200	1.64-fold
1,500	1.61-fold
1,600	1.63-fold
2,000	1.38-fold
2,400	1.75-fold

Parrish et al. (1996) reported that for male B6C3F$_1$ mice exposed to TCA or DCA (0, 0.01, 0.5, and 2.0 g/L) for 3 or 10 weeks, the 4–5-fold magnitude of difference in doses resulted in increases in percent liver/body weight for the 21- and 71-day exposures that were greater for DCA than TCA. The percent liver/body weight ratio were 0.98-, 1.13-, and 1.33-fold of control levels at 0.1, 0.5, and 2.0 g/L TCA and for DCA were 1.02-, 1.24-, and 1.46-fold of control levels, respectively, after 21 days of exposure. Both TCA and DCA exposures at 0.1 g/L resulted in difference in percent liver/body weight change of ≤2%. For TCA, although there was a fourfold increase in magnitude between the 0.5 and 2.0 g/L TCA exposure concentrations, the magnitude of increase for percent liver/body weight increase was 2.5-fold between them at both 21 and 71 days of exposure. For DCA, the fourfold difference in dose between the 0.5 and 2.0 g/L DCA

exposure concentrations were reported to result in a ~2-fold increase in percent liver/body weight increase at 21 days and ~4.5-fold increase at 71 days.

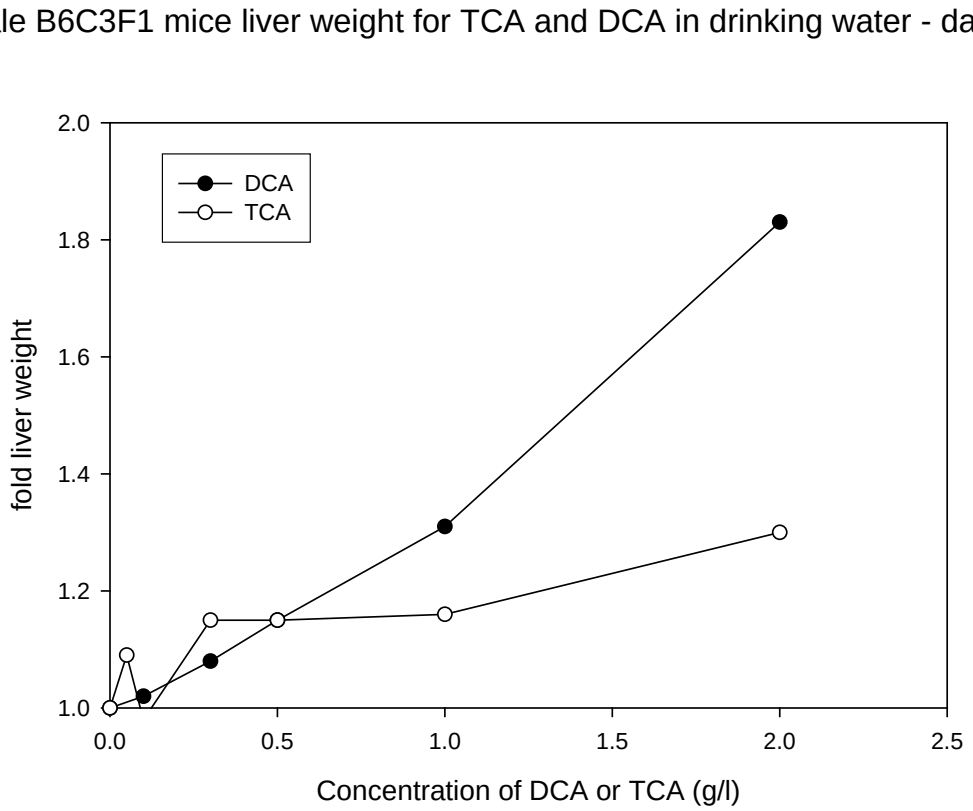
DeAngelo et al. (2008) studied three exposure concentrations of TCA in male $B6C3F_1$ mice, which were an order of magnitude apart, for 4 weeks of exposure. The percent liver/body weight ratios were 1.09-, 1.16-, and 1.35-fold of control levels, for 0.05, 0.5, and 5.0 g/L TCA exposures, respectively. The 10-fold differences in exposure concentration of TCA resulted in ~2-fold differences in percent liver/body weight increases. No dose-response inferences can be drawn from the 4-week study of DCA and TCA in $B6C3F_1$ male mice by Kato-Weinstein et al. (2001), but 2 g/L DCA and 3 g/L TCA in drinking water were reported to induce percent liver/body weights of 1.42- and 1.33-fold of control, respectively (n = 5).

The majority of short-term studies of DCA and TCA in mice have been conducted in the $B6C3F_1$ strain and in males. Studies conducted from 14 to 30 days show a consistent increase in percent liver/body weight induction by TCA or DCA. Analyses of this information regarding inferences for attribution and comparisons of dose-response have been published by Evans et al. (2009), Chiu et al., (2004), and Chiu (2011), and is discussed in Chapter 4 of the TCE assessment document and in Appendix A. A broader discussion of primarily issues and data related to Evans (2009) is contained below.

An examination of all of the data from Parrish et al. (1996), Sanchez and Bull (1990), Carter et al. (1995), Kato-Weinstein et al. (2001), and DeAngelo et al. (2008; 1989) from 14 to 30 days of exposure in male $B6C3F_1$ mice can give an approximation of the dose-response differences between DCA and TCA for liver weight induction as shown in Table E-14 and Figure E-1. Although the data for $B6C3F_1$ mice from Sanchez and Bull (1990) are reported as the fold of liver weight rather that percent liver/body weight increase, they are included in the comparison as both reflect increase in liver weight. Similar data can be assessed for TCE for comparative purposes. Short-duration studies (10–42 days) were selected because: (1) in chronic studies, liver weight increases are confounded by tumor burden; (2) multiple studies are available; (3) in this duration range, Kjellstrand et al. (1981a) reported that TCE-induced increases in liver weight plateau; and (4) TCA studies do not show significant duration-dependent differences in this duration range. These comparisons are presented in Table E-14.

DeAngelo et al. (1989) and Carter et al. (1995) used up to 5 g/L DCA and TCA in their experiments with Carter et al. (1995) noting a dramatic decrease in water consumption in the 5 g/L DCA treatment groups (46–64% reduction), which can affect body weight as well as dose received. DeAngelo et al. (1989) did not report drinking water consumption. The drinking water consumption was reported by DeAngelo et al. (2008) to be reduced by 11, 17, and 30% in the 0.05, 0.5, and 5 g/L TCA treated groups compared to 2 g/L sodium chloride control animals over 60 weeks. DeAngelo et al. (1999) reported mean drinking water consumption to be reduced by 26% in mice exposed to 3.5 g/L DCA over 100 weeks. Carter et al. (1995) reported that DCA at 5 g/L to decrease drinking water consumption by 64 and 46% but 0.5 g/L DCA to not affect

drinking water consumption. Thus, it appears that the 5 g/L concentrations of either DCA or TCA can significantly affect drinking water consumption as well as inducing reductions in body weight. Accordingly, an estimation of the shape of the dose-response curve for comparative purposes between DCA or TCA drinking water studies is best examined at concentrations at ≤2 g/L, especially for DCA.



Reproduced from Section 4.5.

Sources: (2008; Kato-Weinstein et al., 2001; Parrish et al., 1996; Carter et al., 1995; Sanchez and Bull, 1990; DeAngelo et al., 1989)).

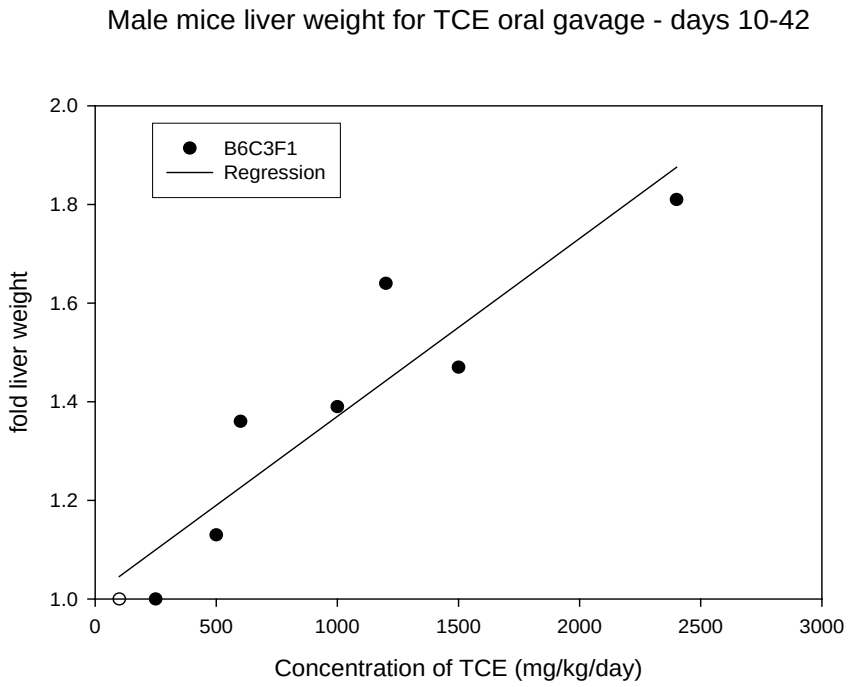
Figure E-1. Comparison of average fold-changes in relative liver weight to control and exposure concentrations of ≤2 g/L in drinking water for TCA and DCA in male B6C3F$_1$ mice for 14–30 days

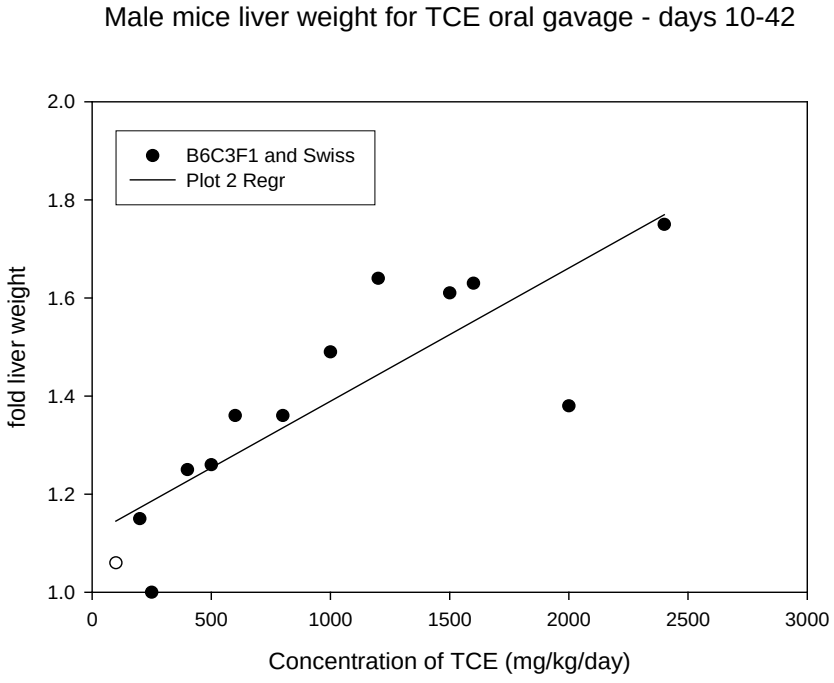
The dose-response curves for similar concentrations of DCA and TCA are presented in Figure E-1 for durations of exposure from 14 to 28 days in the male B6C3F$_1$ mouse, which was the most common sex and strain used. For this comparative analysis, an average is provided between two values for a given concentration and duration of exposure for comparison with other doses and time points. As noted in the discussion of individual experiments, there appears to be a linear correlation between dose in drinking water and liver weight induction up to 2 g/L of DCA. However, the shape of the dose-response curve for TCA appears to be quite different (i.e., lower concentrations of TCA inducing larger increase that does DCA but then the response reaching an

apparent plateau for TCA at higher doses while that of DCA continues to increase). As shown by DeAngelo et al. (2008), 10-fold differences in the magnitude of exposure concentration to TCA corresponded to approximately twofold differences in liver weight induction increases. In addition, TCA studies did not show significant duration-dependent difference in liver weight induction in this duration range as shown in Table E-14.

Of interest is the issue of how the dose-response curves for TCA and DCA compare to that of TCE in a similar model and dose range. Since TCA and DCA have strikingly different dose-response curves, which one, if either, best fits that of TCE and thus, can give insight as to which is causative agent for TCE's effects in the liver? In the case of the TCE database in the mouse two strains have been predominantly studied, Swiss and $B6C3F_1$, and both have been reported to get liver tumors in response to chronic TCE exposure.

Rather than administered in drinking water, oral TCE studies have been conducted via gavage and generally in corn oil for 5 days of exposure per week. The study by Goel et al. (1992) was conducted in ground-nut oil. Vehicle effects, the difference between daily and weekly exposures, the dependence of TCE effects in the liver on its metabolism to a variety of agents capable inducing effects in the liver, differences in response between strains, and the inherent increased variability in use of the male mouse model all add to increased difficulty in establishing the dose-response relationship for TCE across studies and for comparisons to the DCA and TCA database. Despite difference in exposure route, etc., a consistent pattern of dose-response emerges from combining the available TCE data. The effects of oral exposure to TCE from 10 to 42 days on liver weight induction is shown in Figure E-2 using the data of Elcombe et al. (1985), Dees and Travis (1993), Goel et al. (1992), Merrick et al. (1989), Goldsworthy and Popp (1987), and Buben and O'Flaherty (1985). More detailed discussion of the 4–6-week studies is presented in Section E.2.4.3 (e.g., for (Goel et al., 1992; Merrick et al., 1989; Buben and O'Flaherty, 1985)). For this comparative analysis, an average is provided between two values per concentration and duration of exposure for comparison with other doses and time points. As shown by the 10-day data in $B6C3F_1$ mice, there are significant differences in response between studies of male $B6C3F_1$ mice at the same dose of TCE. This variability is similar to findings from inhalation studies of TCE in male mice (Kjellstrand et al., 1983b).





Reproduced from Section 4.5.

Sources: (Dees and Travis, 1993; Merrick et al., 1989; Goldsworthy and Popp, 1987; Elcombe et al., 1985)).

Figure E-2. Comparisons of fold-changes in average relative liver weight and gavage dose of (top panel) male B6C3F$_1$ mice for 10–28 days of exposure and (bottom panel) in male B6C3F$_1$ and Swiss mice.

As shown in Figure E-2, oral TCE administration in male $B6C3F_1$ and Swiss mice appeared to induce a dose-related increase in percent liver/body weight that was generally proportional to the increase in magnitude of dose, though as expected, with more variability than observed for a similar exercise for DCA or TCA in drinking water. Common exposure concentrations between $B6C3F_1$ and Swiss mice were 100, 500, 1,000, 1,500 and 2,400 mg/kg-day TCE, which corresponded to a 5-, 2-, 1.5-, and 1.6-fold difference in the magnitude of dose. For the data from studies in $B6C3F_1$ mice, there was no increase reported at 100 mg/kg-day TCE but between 500 and 1,000, 1,000 and 1,500, and 1,500 and 2,400 mg/kg-day TCE, the magnitude of difference in doses matched that of the magnitude of increase in percent liver/body weight (i.e., a 2.6-, 1.4-, and 1.7-fold increase in liver weight was matched by a 2-, 1.5-, and 1.6-fold increase in TCE exposure concentration at these exposure intervals).

However, only a 10-day interval was available for doses between 100 and 500 mg/kg in $B6C3F_1$ mice and at the lower doses, a 10-day interval may have been too short for the increase in liver weight to have been fully expressed. The database for the Swiss mice, which has more data from 28 and 42 days of exposure, support this conclusion. At 28–42 days of exposure, there was a much greater increase in liver weight from TCE exposure in Swiss mice than the 10-day data in $B6C3F_1$ mice.

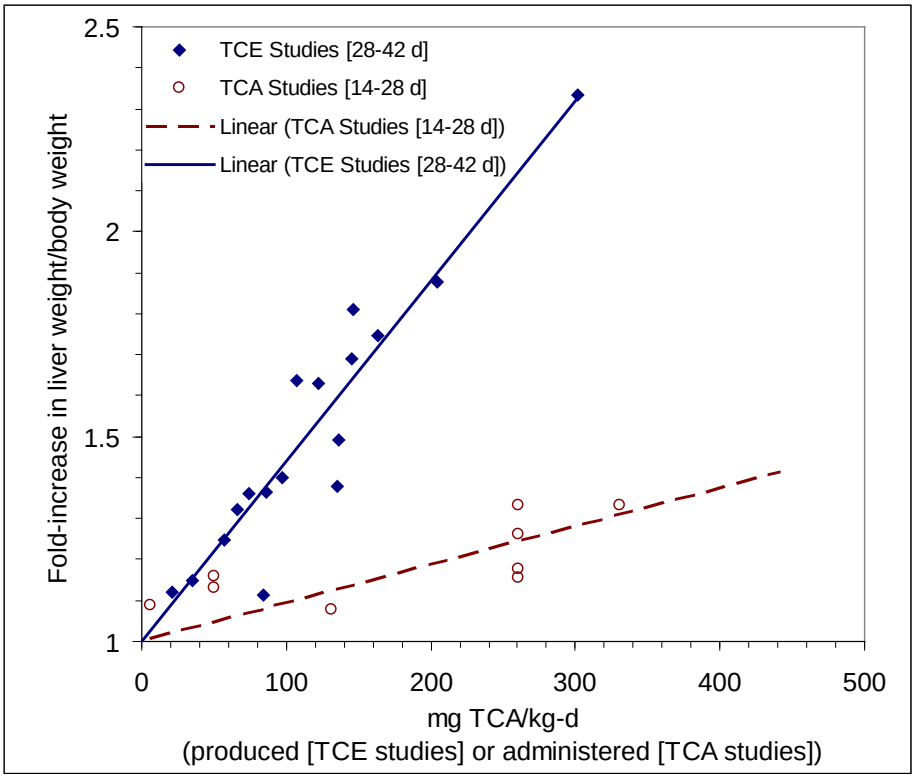
In Figure E-2, the 10-day data are included for comparative purpose for the $B6C3F_1$ data set and the Swiss and $B6C3F_1$ data sets combined. Both the combined TCE data and that for only $B6C3F_1$ mice shows a correlation with the magnitude of dose and magnitude of percent liver/body weight increase. The slope of the dose-response curves are both closer to that of DCA than TCA. The correlation coefficients for the linear regressions presented for the $B6C3F_1$ data are $R^2 = 0.861$ and for the combined data sets is $R^2 = 0.712$. Comparisons of the slopes of the dose-response curves indicate that TCA is not responsible for TCE-induced liver effects. In this regression, all data points were treated equally, although some came from several sets of data and others did not. Of note is that the 2,000 mg/kg TCE data point in the combined data set, which is much lower in liver weight response than the other data, is from one experiment (Goel et al., 1992), from six mice, at one time point (28 days), and one strain (Swiss). Deletion of these data point from the rest of the 23 used in the study results in a better fit to the data of the regression analysis.

A more direct comparison would be on the basis of dose rather than drinking water concentration. The estimations of internal dose of DCA or TCA from drinking water studies have been reported to vary with DeAngelo et al. (1989) calculated DCA drinking water concentrations of 1.0, 2.0, and 5.0 g/L to result in 90, 166, and 346 mg/kg-day, respectively, based on previous analyses in their laboratory. For TCA, 0.05, 0.5, 1.0, 2.0, and 5 g/L drinking water exposures were reported to result in 5.8 (range 3.6–8.0), 50 (range of 32.5–68), 131, 261, and 469 (range 364–602) mg/kg-day doses. The estimations of internal dose of DCA or TCA from drinking water studies, while varying considerably (DeAngelo et al., 2008; 1989), nonetheless suggest that

the doses of TCE used in the gavage experiments were much higher than those of DCA or TCA. However, only a fraction of ingested TCE is metabolized to DCA or TCA, as, in addition to oxidative metabolism, TCE is also cleared by GSH conjugation and by exhalation.

While DCA dosimetry is highly uncertain (see Sections 3.3 and 3.5), the mouse PBPK model, described in Section 3.5 was calibrated using extensive in vivo data on TCA blood, plasma, liver, and urinary excretion data from inhalation and gavage TCE exposures, and makes robust predictions of the rate of TCA production. If TCA were predominantly responsible for TCE-induced liver weight increases, then replacing administered TCE dose (e.g., mg TCE/kg/day) by the rate of TCA produced from TCE (mg TCA/kg/day) should lead to dose-response curves for increased liver weight consistent with those from directly administered TCA.

Figure E-3 shows this comparison using the PBPK model-based estimates of TCA production for four TCE studies from 28 to 42 days in the male NMRI, Swiss, and B6C3F$_1$ mice (Goel et al., 1992; Merrick et al., 1989; Buben and O'Flaherty, 1985; Kjellstrand et al., 1983a) and four oral TCA studies in B6C3F$_1$ male mice at ≤2 g/L drinking water exposures (DeAngelo et al., 2008; Kato-Weinstein et al., 2001; Parrish et al., 1996; DeAngelo et al., 1989) from 14 to 28 days of exposure. The selection of the 28–42 day data for TCE was intended to address the decreased opportunity for full expression of response at 10 days. PBPK modeling predictions of daily internal doses of TCA in terms of mg/kg-day via produced via TCE metabolism would be indeed lower than the TCE concentrations in terms of mg/kg-day given orally by gavage. The predicted internal dose of TCA from TCE exposure studies are of a comparable range to those predicted from TCA drinking water studies at exposure concentrations in which palatability has not been an issue for estimation of internal dose. Thus, although the TCE data are for higher exposure concentrations, they are predicted to produce comparable levels of TCA internal dose estimated from direct TCA administration in drinking water.



(Reproduced from Section 4.5.)

Abscissa for TCE studies consists of the median estimates of the internal dose of TCA predicted from metabolism of TCE using the PBPK model described in Section 3.5 of the TCE risk assessment. Lines show linear regression with intercept fixed at unity. All data were reported fold-change in mean liver weight/body weight ratios, except for Kjellstrand et al. (1983a), with were the fold-change in the ratio of mean liver weight to mean body weight. In addition, in Kjellstrand et al. (1983a), some systemic toxicity as evidence by decreased total body weight was reported in the highest-dose group.

Sources: Kjellstrand et al. (1983a); (Goel et al., 1992; Merrick et al., 1989; Buben and O'Flaherty, 1985); (DeAngelo et al., 2008; Kato-Weinstein et al., 2001; Parrish et al., 1996; DeAngelo et al., 1989).

Figure E-3. Comparison of fold-changes in relative liver weight for data sets in male B6C3F$_1$, Swiss, and NRMI mice between TCE studies [duration 28–42 days]) and studies of direct oral TCA administration to B6C3F$_1$ mice [duration 14–28 days]).

Figure E-3 clearly shows that for a given amount of TCA produced from TCE, but going through intermediate metabolic pathways, the liver weight increases are substantially greater than, and highly inconsistent with, that expected based on direct TCA administration. In particular, the response from direct TCA administration appears to "saturate" with increasing TCA dose at a level of about 1.4-fold, while the response from TCE administration continues to increase with

dose to 1.75-fold at the highest dose administered orally in Buben and O'Flaherty (1985) and over 2-fold in the inhalation study of Kjellstrand et al. (1983a). For this analysis, it is unlikely that strain differences can account for this inconsistency in the dose-response curves.

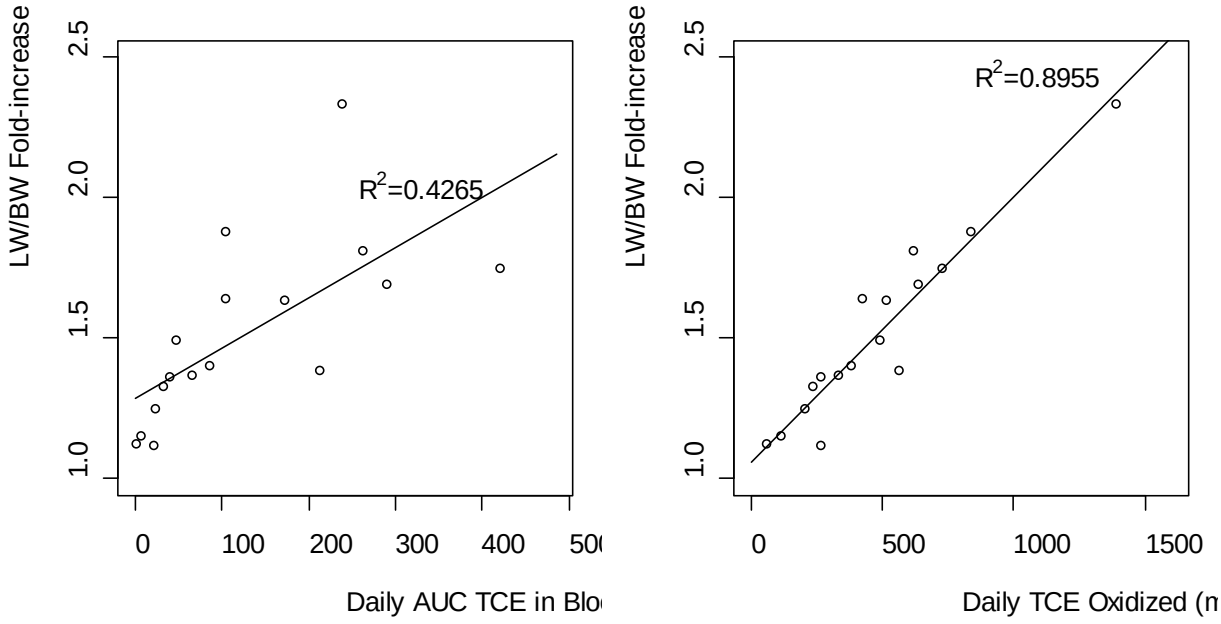
TCE-induced increases in liver weight appear to be generally similar between $B6C3F_1$ and Swiss male mice (see Table E-14) via oral exposure and between NMRI male and female mice after inhalation, although the NMRI strain appeared to be more prone to TCE-induced toxicity in male mice, and females appeared to have a smaller TCE-induced liver weight increase than other strains (Kjellstrand et al., 1983a). As noted previously, the difference in response between strains and between studies in the same strain for TCE liver weight increases can be highly variable. Little data exist to examine this issue for TCA studies, although DeAngelo et al. (1989) report a range of 1.16–1.63-fold of control percent liver/body weight increase after 14 days exposure at 2 g/L TCA in the Swiss-Webster, C3H, C57BL/6, and $B6C3F_1$ strains, with differences also noted between two studies of the $B6C3F_1$ mouse.

Furthermore, while as noted previously, oral studies appear to report a linear relationship between TCE exposure concentration and liver weight induction, the inclusion of inhalation studies on the basis of internal dose led to a highly consistent dose-response curve among TCE studies. Therefore, it is unlikely that differing routes of exposure can explain the inconsistencies in dose-response. The PBPK model predicted that matching average TCA production by TCE with the equivalent average dose from drinking water-administered TCA also led to an equivalent AUC of TCA in the liver.

Moreover, Dees and Travis (1993) administered 100–1,000 mg/kg-day TCA by gavage to male and female $B6C3F_1$ mice for 11 days, and did not observe increases in liver/body weight ratios >1.28-fold, no higher than those observed with drinking water exposures. Finally, the dose-response consistency between TCE inhalation and gavage studies argues against route of exposure significantly impacting liver weight increases. Thus, no level of TCA administration appears able account for the continuing increase in liver weights observed with TCE, quantitatively inconsistent with TCA being the predominant metabolite responsible for TCE-induced liver weight changes. Involvement of other metabolites, besides TCA, is implicated as the causes of TCE-induced liver effects.

Additional analyses do, however, support a role for oxidative metabolism in TCE-induced liver weight increases, and that the parent compound TCE is not the likely active moiety (as suggested previously by Buben and O'Flaherty, 1985). In particular, the same studies are shown in Figure E-4 using PBPK-model based predictions of the AUC of TCE in blood and total oxidative metabolism, which produces chloral, TCOH, DCA, and other metabolites in addition to TCA. The dose-response relationship between TCE blood levels and liver weight increase, while still having a significant trend, shows substantial scatter and a low R^2 of 0.43. On the other hand, using total oxidative metabolism as the dose-metric leads to substantially more consistency dose-response across studies, and a much tighter linear trend with an R^2 of 0.90 (see Figure E-4). A

similar consistency is observed using liver-only oxidative metabolism as the dose-metric, with R^2 of 0.86 (not shown). Thus, while the slope is similar between liver weight increase and TCE concentration in the blood and liver weight increase and rate of total oxidative metabolism, the data are a much better fit for total oxidative metabolism.



(Reproduced from Section 4.5).

Lines show linear regression. Use of liver oxidative metabolism as a dose-metric gives results qualitatively similar to (B), with $R^2 = 0.86$.

Sources: Kjellstrand et al. (1983a); (Goel et al., 1992; Merrick et al., 1989; Buben and O'Flaherty, 1985).

Figure E-4. Fold-changes in relative liver weight for data sets in male B6C3F$_1$, Swiss, and NRMI mice reported by TCE studies of duration 28–42 days using internal dose-metrics predicted by the PBPK model described in Section 3.5: (A) dose-metric is the median estimate of the daily AUC of TCE in blood, (B) dose-metric is the median estimate of the total daily rate of TCE oxidation.

As stated in many of the discussions of individual studies, there is a limited ability to detect a statistically significant change in liver weight change in experiments that use a relatively small number of animals. Many experiments have been conducted with 4–6 mice per dose group. The experiments of Buben and O'Flaherty used 12–14 mice per group, giving it a greater ability to detect a TCE-induced dose response. In some experiments, greater care was taken to document and age and weight match the control and treatment groups before the start of treatment. The approach taken above for the analyses of TCE, TCA, and DCA uses data across several data sets

and gives a more robust description of these dose-response curves, especially at lower exposure levels. For example, the data from DeAngelo et al. (2008) for TCA-induced percent liver/body weight ratio increases in male B6C3F$_1$ mice were only derived from five animals per treatment group after 4 weeks of exposure. The 0.05 and 0.5 g/L exposure concentrations were reported to give a 1.09- and 1.16-fold of control percent liver/body weight ratios, which were consistent with the increases noted in the cross-study database above. However, a power calculation shows that the type II error, which should be >50% and thus, greater than the chances of "flipping a coin," was only a 6 and 7% and therefore, the designed experiment could accept a false null hypothesis.

Although the qualitative similarity to the linear dose-response relationship between DCA and liver weight increases is suggestive of DCA being the predominant metabolite responsible for TCE liver weight increases, due to the highly uncertain dosimetry of DCA derived from TCE, this hypothesis cannot be tested on the basis of internal dose. Similarly, another TCE metabolite, CH, has also been reported to induce liver tumors in mice; however, there are no adequate comparative data to assess the nature of liver weight increases induced by this TCE metabolite (see Section E.2.5). Whether its formation in the liver after TCE exposure correlates with TCE-induced liver weight changes cannot be determined. Of note is the high variability in total oxidative metabolism reported in mice and humans of Section 3.3, which suggests that the correlation of total TCE oxidative metabolism with TCE-induced liver effects should not only lead to a high degree of variability in response in rodent bioassays, which is the case (see Section E.2.4.4), but also make detection of liver effects more difficult in human epidemiological studies.

The bioavailability of TCA has been assumed to be 100% in the analyses in Figure E-3. Further analyses are presented in Appendix A and in Chiu (2011) regarding the assertions by Sweeney, et al. (Sweeney et al., 2009) that previously unpublished kinetic data for mice exposed to TCA in drinking water indicates much lower absorption. The conclusions of Sweeney et al. (2009) were based on the TCE PBPK model of Hack et al. (2006) and not that of Evans et al. (2009) and Chiu et al. (2009). The analyses by Chiu (Chiu, 2011) show that while there is some decreased absorption of TCA at higher doses, it was not as low as that estimated by Sweeney et al. (2009) and as discussed in Appendix A, it may be more accurate to characterize the fractional absorption as an empirical parameter reflecting unaccounted-for biological processes as well as experimental variation. The Chiu (2011) re-analyses the data on TCE- and TCA-induced hepatomegaly, using the central estimates of the fractional absorption of TCA, showed that while reduced fractional absorption inferred from drinking water data reported by Sweeney et al. (2009) accounts for part of the difference in dose-responses between TCE- and TCA-induced hepatomegaly reported by Evans et al. (2009), it does not appear to be able to account for the entire difference. The inability of TCA to account for TCE-induced hepatomegaly was confirmed statistically by ANOVA and even with an assumption of reduced TCA bioavailability,

the available data are inconsistent with the toxicological hypothesis that TCA can fully account for TCE-induced hepatomegaly.

What mechanisms or events are leading to liver weight increases for DCA, TCA, and TCE can be examined by correlations between changes in glycogen content, hepatocyte volume, and evidence of polyploidization noted in short-term assays. Data have been reported regarding the nature of changes the TCE and its metabolites induce in the liver and are responsible for the reported increases in liver weight. Increased liver weight may result from increased size or hypertrophy of hepatocytes through changes in glycogen deposition, but also through increased polyploidization. Increased cell number may also contribute to increased liver weight. As noted in Section E.2.4.1, hepatocellular hypertrophy appeared to be related to TCE-induced liver weight changes after short-term exposures. However, neither glycogen deposition, DNA synthesis, nor increases in mitosis appear to be correlated with liver weight increases. In particular, DNA synthesis increases were similar from 250 to 1,000 mg/kg and peroxisomal volume was similar between 500 and 1,500 mg/kg TCE exposures after 10 days. Autoradiographs identified hepatocytes undergoing DNA synthesis in "mature" hepatocytes that were in areas where polyploidization typically takes place in the liver.

By 14 days of exposure, Sanchez and Bull (1990) reported that both dose-related TCA- and DCA-induced increases in liver weight were generally consistent with changing cell size increases, but were not correlated with patterns of change in hepatic DNA content, incorporation of tritiated thymidine in DNA extracts from whole liver, or incorporation of tritiated thymidine in hepatocytes. There are conflicting reports of DNA synthesis induction in individual hepatocytes for up to 14 days of DCA or TCA exposure and a lack of correlation with patterns observed for this endpoint and those of whole-liver thymidine incorporation. The inconsistency of whole-liver DNA tritiated thymidine incorporation with that reported for hepatocytes was noted by the Sanchez and Bull (1990) to be unexplained. Carter et al. (1995) also report a lack of correlation between hepatic DNA tritiated thymidine incorporation and labeling in individual hepatocytes in male mice. Carter et al. (1995) reported no increase in labeling of hepatocytes in comparison to controls for any DCA treatment group from 5 to 30 days of DCA exposure. Rather than increase hepatocyte labeling, DCA induced a decrease with no change reported from days 5 though 15 but significantly decreased levels between days 20 and 30 for 0.5 g/L that were similar to those observed for the 5 g/L exposures.

The most comparable time periods between TCE, TCA, and DCA results for whole-liver thymidine incorporation are the 10- and 14-day durations of exposure when peak tritiated thymidine incorporation into individual hepatocytes and whole liver for TCA and DCA have been reported to have already passed (Pereira, 1996; Carter et al., 1995; Styles et al., 1991; Sanchez and Bull, 1990). Whole-liver DNA synthesis was elevated over control levels by approximately twofold after from 250 to 1,000 mg/kg TCE exposure after 10 days of exposure but did not correlate with mitosis (Dees and Travis, 1993; Elcombe et al., 1985). After 3 weeks of exposure

to TCE, Laughter et al. (2004) reported that 1 and 4.5% of individual hepatocytes had undergone DNA synthesis in the last week of treatment for the 500 and 1,000 mg/kg TCE levels, respectively. More importantly, these data show that hepatocyte proliferation in TCE-exposed mice at 10 days of exposure or for DCA- or TCA-exposed mice for up to 14 days of exposure is confined to a very small population of cells in the liver.

In regard to cell size, although increased glycogen deposition with DCA exposure was noted by Sanchez and Bull (1990), lack of quantitative analyses of that accumulation in this study precludes comparison with DCA-induced liver weight gain. Although not presenting a quantitative analysis, Sanchez and Bull (1990) reported DCA-treated $B6C3F_1$ mice to have large amounts of PAS staining material and Swiss-Webster mice to have similar increase despite reporting differences of DCA-induced liver weight gain between the two strains. The lack of concordance of the DCA-induced magnitude of increase in liver weight with that of glycogen deposition is consistent with the findings for longer-term exposures to DCA reported by Kato-Weinstein et al. (2001) and Pereira et al. (2004a) in mice (see Section E.2.4.4). Carter et al. (1995) reported that in control mice, there was a large variation in apparent glycogen content and also did not perform a quantitative analysis of glycogen deposition. The variability of this parameter in untreated animals and the extraction of glycogen during normal tissue processing for light microscopy makes quantitative analyses for dose-response difficult unless specific methodologies are employed to quantitatively assess liver glycogen levels as was done by Kato-Weinstein et al. (2001) and Pereira et al. (2004a).

Although suggested by their data, polyploidization was not examined for DCA or TCA exposure in the study of Sanchez and Bull (1990). Carter et al. (1995) reported that hepatocytes from both 0.5 and 5 g/L DCA treatment groups were reported to have enlarged, presumably polyploidy nuclei with some hepatocyte nuclei labeled in the mid-zonal area. There were statistically significant changes in cellularity, nuclear size, and multinucleated cells during 30 days exposure to DCA. The percentage of mononucleated cells hepatocytes was reported to be similar between control and DCA treatment groups at 5- and 10-day exposures.

However, at 15 days and beyond, DCA treatments were reported to induce increases in mononucleated hepatocytes. At later time periods, there were also reports of DCA-induced increases nuclear area, consistent with increased polyploidization without mitosis. The consistent reporting of an increasing number of mononucleated cells between 15 and 30 days could be associated with clearance of mature hepatocytes as suggested by the report of DCA-induced loss of cell nuclei. The reported decrease in the numbers of binucleate cells in favor of mononucleated cells is not typical of any stage of normal liver growth (Brodsky and Uryvaeva, 1977). The linear dose-response in DCA-induced liver weight increase was not consistent with the increased numbers of mononucleate cells and increased nuclear area reported from day 20 onward by Carter et al. (1995). Specifically, the large differences in liver weight induction between the 0.5 g/L treatment group and the 5 g/L treatment groups at all times studied also did

not correlate with changes in nuclear size and percent of mononucleated cells. Thus, DCA-induced increases in liver weight were not a function of cellular proliferation, but probably included hypertrophy associated with polyploidization, increased glycogen deposition, and other factors.

In regard to necrosis, Elcombe et al. (1985) reported only small incidence of focal necrosis in 1,500 mg/kg TCE-exposed mice and no necrosis at exposures up to 1,000 mg/kg for 10 days as did Dees and Travis (1993). Sanchez and Bull (1990) report DCA-induced localized areas of coagulative necrosis both for B6C3F_1 and Swiss-Webster mice at higher exposure levels (1 or 2 g/L) by 14 days but not at the 0.3 g/L level or earlier time points. For TCA treatment, necrosis was reported to not be associated with TCA treatment for up to 2 g/L and up to 14 days of exposure. Carter et al. (1995) reported that mice given 0.5 g/L DCA for 15, 20, and 25 days had midzonal focal cells with less detectable or no cell membranes and loss of the coarse granularity of the cytoplasm, with some cells having apparent karyolysis, but for liver architecture to be normal.

As for apoptosis, both Elcombe et al. (1985) and Dees and Travis (1993) reported no changes in apoptosis other than increased apoptosis only at a treatment level of 1,000 mg/kg TCE. Rather than increases in apoptosis, peroxisome proliferators have been suggested to inhibit apoptosis as part of their carcinogenic mode of action (see Section E.3.4.1). However, the age and species studied appear to greatly affect background rates of apoptosis. Snyder et al. (1995) report that control mice were reported to exhibit apoptotic frequencies ranging from ~0.04 to 0.085%, that over the 30-day period of their study, the frequency rate of apoptosis declined, and suggest that this pattern is consistent with reports of the livers of young animals undergoing rapid changes in cell death and proliferation. They reported rat liver to have a greater the estimated frequency of spontaneous apoptosis (~0.1%) and therefore, greater than that of the mouse.

Carter et al. (1995) reported that after 25 days of 0.5 g/L DCA treatment apoptotic bodies were reported as well as fewer nuclei in the pericentral zone and larger nuclei in central and midzonal areas. This would indicate an increase in the apoptosis associated potential increases in polyploidization and cell maturation. However, Snyder et al. (1995) report that mice treated with 0.5 g/L DCA over a 30-day period had a similar trend as control mice of decreasing apoptosis with age. The percentage of apoptotic hepatocytes decreased in DCA-treated mice at the earliest time point studied and remained statistically significantly decreased from controls from 5 to 30 days of exposure. Although the rate of apoptosis was very low in controls, treatment with 0.5 g/L DCA reduced it further (~30–40% reduction) during the 30-day study period. The results of this study not only provide a baseline of apoptosis in the mouse liver, which is very low, but also to show the importance of taking into account the effects of age on such determinations. The significance of the DCA-induced reduction in

apoptosis reported in this study, from a level that is already inherently low in the mouse, to account for the mode of action for induction of DCA-induced liver cancer is difficult to discern.

Finally, short-term inhalation studies by Ramdhan et al. (2010) indicate that in wild type, PPARα-null, and humanized null mice, relatively high exposures to TCE induced increased liver size after 7 days of inhalation exposure. At the same highest concentration of TCE, although urinary TCA concentrations were lower in PPARα-null mice than wild type mice, the sum of urinary TCOH and TCA concentrations were the same, increases in percent liver/body weight were the same, and liver triglyceride content was much greater in the PPARα-null mice than wild type mice after TCE exposure. Hepatic steatosis was also greater as a baseline condition along with hepatic triglyceride content in the PPARα-null mice than wild type mice. These parameters were more elevated in humanized mice as a background dysregulation and even more elevated after treatment with TCE. Therefore, the nature of hepatomegally induced by TCE is complex and dependent on baseline lipid dysregulation states.

E.2.4.3. Summary of TCE Subchronic and Chronic Studies

The results of longer-term (Toraason et al., 1999; Channel et al., 1998; Parrish et al., 1996) studies of "oxidative stress" for TCE and its metabolites are discussed in Section E.3.4.2.3. Of note are the findings that the extent of increased enzyme activities associated with peroxisome proliferation do not appear to correlate with measures of oxidative stress after longer-term exposures (Parrish et al., 1996) and SSBs (Chang et al., 1992).

Similar to the reports of Melnick et al. (1987) in rats, Merrick et al. (1989) report that vehicle (aqueous or gavage) affects TCE-induced toxicity in mice. Vehicle type made a large difference in mortality, extent of liver necrosis, and liver weight gain in male and female $B6C3F_1$ mice after 4 weeks of exposure. The lowest dose used in this experiment was 600 mg/kg-day in males and 450 mg/kg-day in females. Administration of TCE via gavage using Emulphor resulted in mortality of all of the male mice and most of the female mice at a dose in corn oil that resulted in few deaths. However, use of Emulphor vehicle induced little, if any, focal necrosis in males at concentrations of TCE in corn oil gavage that caused significant focal necrosis, indicating vehicle effects.

As discussed in Section E.2.4.2, the extent of TCE-induced liver weight increases was consistent between 4 and 6 weeks of exposure and between 10-day and 4-week exposures at higher dose levels. In general, the reported elevations of enzymatic markers of liver toxicity and results for focal hepatocellular necrosis were not consistent and did not reflect TCE dose-responses observed for induction of liver weight increases (Merrick et al., 1989). Female mice given corn oil and male and female mice given TCE in Emulphor were reported to have "no to negligible necrosis," although they had increased liver weight from TCE exposure.

Using a different type of oil vehicle, Goel et al. (1992) exposed male Swiss mice to TCE in groundnut oil at concentrations ranging from 500 to 2,000 mg/kg for 4 weeks and reported no

changes in body weight up to 2,000 mg/kg. There was a 15% decrease at the highest dose and increased TCE-induced percent liver/body weight ratio. At a dose of 1,000 and 2,000 mg/kg, liver swelling, vacuolization, and widespread degenerative necrosis of hepatocytes was reported along with marked proliferation of "endothelial cells" but no quantitation regarding the extent or location of hepatocellular necrosis was reported, nor whether there was a dose-response relationship in these events. They reported a TCE-related dose-response in catalase and liver protein, but a decreased induction at the 2,000 mg/kg level where body weight had decreased.

Three studies were published by Kjellstrand and colleagues that examined effects of TCE inhalation primarily in mice using whole-body inhalation chambers (Kjellstrand et al., 1983a; Kjellstrand et al., 1983b; Kjellstrand et al., 1981a). Liver weight changes were used as the indication of TCE-induced effects. The quantitative results from these experiments had many limitations due to their experimental design including failure to determine body weight changes for individual animals and inability to determine the exact magnitude of TCE due to concurrent oral TCE ingestion from food and grooming behavior. An advantage of this route of exposure was that there were not confounding vehicle effects. The results from Kjellstrand et al. (1981a) were particularly limited by experimental design errors showed similar increases in liver weight gain in gerbils and rats exposed at 150 ppm TCE. For rats, Kjellstrand et al. (1981a) reported increases in liver/body weight ratios of 1.26- and 1.21-fold of control in male and female rat 30 days of continuous TCE inhalation exposure.

The unpublished report of Woolhiser et al. (2006) reports 1.05-, 1.07-, and 1.13-fold of control percent liver/body weight changes in 100, 300, and 1,000 ppm exposure groups that are exposed for 6 hours/day, 5 days/week for 4 weeks in groups of eight female Sprague-Dawley rats. At the two highest exposure levels, body weight was reduced by TCE exposure. The 150 ppm continuous exposure concentrations of Kjellstrand were analogous to 750 ppm exposures using the paradigm of Woolhiser et al. (2006) in terms of total daily dose. Therefore, the very limited inhalation database for rats does indicate TCE-related increases in liver weight.

The study of Kjellstrand et al. (1983b) employed a more successful experimental design that recorded liver weight changes in carefully matched control and treatment groups to determine TCE-treatment related effects on liver weight in seven strains of mice after 30 days of continuous inhalation exposure at 150 ppm TCE. Individual animal body weight changes were not recorded so that such an approach cannot take into account the effects of body weight changes and determine a relative percent liver/body weight ratio. The data presented in this report were for absolute liver weight changes between treated and nontreated groups with carefully matched average body weights at the initiation of exposure. A strength of the experimental design is its presentation of results between duplicate experiments and thus, its ability to show the differences in results between similar exposed groups that were conducted at different times. This information gives a measure of variability in response with time. Mouse strain groups that did not experience TCE-induced decreased body weight gain in comparison

to untreated groups (i.e., DBA and wild-type mice) represented the most accurate determination of TCE-induced liver weight changes given that systemic toxicity that affects body weight can also affect liver weight.

The C57BL, B6CBA, and NZB groups all had at least one group out of two of male mice with changes in final body weight due to TCE exposure. Only one group of NMRI mice were reported in this study and that group had TCE-induced decreases in final body weight. The A/sn group not only had both male groups with decreased final body weight after TCE exposure (along with differences between exposed and control groups at the initiation of exposure), but also a decrease in body weight in one of the female groups and thus, appears to be the strain with the greatest susceptibility to TCE-induced systemic toxicity. In strains of male mice in which there were no TCE-induced affects on final body weight (wild-type and DBA), the influence of gender on liver weight induction and variability of the response could be more readily assessed. In wild-type mice, there was a 1.76- and 1.80-fold of control liver weight in groups 1 and 2 for female mice, and for males, a 1.84- and 1.62-fold of control liver weight for groups 1 and 2, respectively. For DBA mice, there was a 1.87- and 1.88-fold of control liver weight in groups 1 and 2 for female mice, and for males, a 1.45- and 2.00-fold of control liver weight for groups 1 and 2, respectively. Of note, as described previously, the size of the liver is under strict control in relation to body size. An essential doubling of the size of the liver is a profound effect with the magnitude of liver weight size increase physiologically limited.

Overall, the consistency between groups of female mice of the same strain for TCE-induced liver weight gain, regardless of strain examined, was striking, as was the lack of body weight changes at TCE exposure levels that induced body weight changes in male mice. In the absence of body weight changes, the difference in TCE-response in female mice appeared to be reflective of strain and initial weight differences. Groups of female mice with higher body weights, regardless of strain, generally had higher increases in TCE-induced liver weight increases. For the C57BL and As/n strains, female mice starting weights were averaged 17.5 and 15.5 g, while the average liver weights were 1.63- and 1.64-fold of control after TCE exposure, respectively. For the B6CBA, wild-type, DBA, and NZB female groups, the starting body weights averaged 22.5, 21.0, 23.0, and 21.0 g, while the average liver weights were 1.70-, 1.78-, 1.88-, and 2.09-fold of control after TCE exposure, respectively. The NMRI group of female mice, did not follow this general pattern and had the highest initial body weight for the single group of 10 mice reported (i.e., 27 g) associated with 1.66-fold of control liver weight.

The results of Kjellstrand et al. (1983b) suggested that there was more variability between male mice than female mice in relation to TCE-induced liver weight gain. More strains exhibited TCE-induced body weight changes in male mice than female mice, suggesting increased susceptibility of male mice to TCE toxicity as well as more variability in response. Initial body weight also appeared to be a factor in the magnitude of TCE-induced liver weight induction rather than just strain. In general, the strains and groups within strain that had

TCE-induced body weight decreases had smaller TCE-induced increase in liver weight. Therefore, only examining liver weight in males as an indication of TCE treatment effects would not be an accurate predictor of strain sensitivity nor the magnitude or response at doses that also affect body weight. The results from this study show that comparison of the magnitude of TCE response, as measured by liver weight increases, should take into account strain, gender, initial body weight, and systemic toxicity. It shows a consistent pattern of increased liver weight in both male and female mice after TCE exposure of 150 ppm for 30 days.

Kjellstrand et al. (1983a) presented data in the NMRI strain of mice (a strain that appeared to be more prone to TCE-induced toxicity in male mice and a smaller TCE-induced increase in liver weight in female mice) after inhalation exposure of 37–300 ppm TCE. They used the same experimental paradigm as that reported in Kjellstrand et al. (1983b) except for exposure concentration.

For female mice exposed to concentrations of TCE ranging from 37 to 300 ppm TCE continuously for 30 days, only the 300 ppm group experienced a 16% decrease in body weight between control and exposed animals. Therefore, changes in TCE-induced liver weight increases were affected by changes in body weight only for that group. Initial body weights in the TCE-exposed female mice were similar in each of these groups (i.e., range of 29.2–31.6 g, or 8%), with the exception of the females exposed to 150 ppm TCE for 30 days (i.e., initial body weight of 27.3 g), reducing the effects of differences in initial body weight on TCE-induced liver weight induction. Exposure to TCE continuously for 30 days was reported to result in a linear dose-dependent increase in liver weight in female mice with 1.06-, 1.27-, 1.66-, and 2.14-fold of control liver weights reported at 37, 75, 150, and 300 ppm TCE, respectively.

In male mice, there were more factors affecting reported liver weight increases from TCE exposure. For male mice, both the 150 and 300 ppm exposed groups experienced a 10 and 18% decrease in final body weight after TCE exposure, respectively. The 37 and 75 ppm groups did not have decreased final body weight due to TCE exposure but varied by 12% in initial body weight. TCE-induced increases in liver weight were reported to be 1.15-, 1.50-, 1.69-, and 1.90-fold of control for 37, 75, 150, and 300 ppm TCE exposure in male mice, respectively. The flattening of the dose-response curve at the two highest doses is consistent with the effects of toxicity on final body weight.

Kjellstrand et al. (1983a) noted that liver mass increased and the changes in liver cell morphology were similar in TCE-exposed male and female mice. They report that after 150 ppm exposure for 30 days, liver cells were generally larger and often displayed a fine vacuolization of the cytoplasm, changes in nucleoli appearance. Kupffer cells of the sinusoid were reported to be increased in cellular and nuclear size. The intralobular connective tissue was infiltrated by inflammatory cells. Exposure to TCE in higher or lower concentrations during the 30 days was reported to produce a similar morphologic picture.

For mice that were exposed to 150 ppm TCE for 30 days and then examined 120 days after the cessation of exposure, liver weights were 1.09-fold of control for TCE-exposed female mice and the same as controls for TCE-exposed male mice. However, the livers were not the same as untreated liver in terms of histopathology. The authors reported that "after exposure to 150 ppm for 30 days, followed by 120 days of rehabilitation, the morphological picture was similar to that of the air-exposure controls except for changes in cellular and nuclear sizes." The authors did not present any quantitative data on the lesions they describe, especially in terms of dose-response, and most of the qualitative description is for the 150 ppm exposure level in which there are consistent reports of TCE induced body weight decreases in male mice.

Although stating that Kupffer cells were increased in cellular and nuclear size, no differential staining was applied to light microscopy sections and used to distinguish Kupffer from endothelial cells lining the hepatic sinusoid in this study. Without differential staining, such a determination is difficult at the light microscopic level and a question remains as to whether these are the same cells as described by Goel et al. (1992) as a proliferation of sinusoidal endothelial cells after exposures of 1,000 and 2,000 mg/kg-day TCE exposure for 28 days in male Swiss mice. As noted in Section E.2.4.2, the discrepancy in DNA synthesis measures between hepatocyte examinations of individual hepatocytes and whole liver measures in several reports of TCE metabolite exposure, is suggestive of increased DNA synthesis in the nonparenchymal cell compartment of the liver. Thus, nonparenchymal cell proliferation is suggested as an effect of subchronic TCE exposures in mice without concurrent focal necrosis via inhalation studies (Kjellstrand et al., 1983a) and with focal necrosis in the presence of TCE in a groundnut oil vehicle (Goel et al., 1992).

Although Kjellstrand et al. (1983a) did not discuss polyploidization, the changes in cell size and especially the continued change in cell size and nuclear staining characteristics after 120 days of cessation of exposure are consistent with changes in polyploidization induced by TCE that were suggested in studies from shorter durations of exposure (Dees and Travis, 1993; Elcombe et al., 1985) and of longer durations (e.g., Buben and O'Flaherty, 1985). Of note is that in the histological descriptions provided by Kjellstrand et al. (1983a), there was no mention of focal necrosis or apoptosis resulting from these exposures to TCE to mice. Vacuolization is reported and consistent with hepatotoxicity or lipid accumulation, which is lost during routine histological slide preparation. The lack of reported focal necrosis in mice exposed through inhalation is consistent with reports of gavage experiments of TCE in mice that do not use corn oil as the vehicle (Merrick et al., 1989).

Buben and O'Flaherty (1985) reported the effects of TCE via corn oil gavage after 6 weeks of exposure at concentrations ranging from 100 to 3,200 mg/kg-day. This study was conducted with older mice than those generally used in chronic exposure assays (male Swiss-Cox outbred mice between 3 and 5 months of age). Liver weight increases, decreases in liver G6P activity, increases in liver triglycerides, and increases in SGPT activity were examined as

parameters of liver toxicity. Few deaths were reported during the 6-week exposure period except at the highest dose and related to CNS depression. TCE exposure caused dose-related increases in percent liver/body weight with a dose as low as 100 mg/kg-day reported to cause a statistically significant increase (i.e., 112% of control).

The increases in liver size were attributed to hepatocyte hypertrophy, as revealed by histological examination and by a decrease in the liver DNA concentration, and although enlarged, were reported to appear normal. A dose-related trend toward triglyceride concentration was also noted. A dose-related decrease in glucose-6-phophatase activity was reported with similar small decreases (~10%) observed in the TCE exposed groups that did not reach statistical significance until the dose reached 800 mg/kg TCE exposure. SGPT activity was not observed to be increased in TCE-treated mice except at the two highest doses and even at the 2,400 mg/kg dose, half of the mice had normal values. The large variability in SGPT activity was indicative of heterogeneity of this response between mice at the higher exposure levels for this indicator of liver toxicity. Such variability of response in male mice is consistent with the work of Kjellstrand and colleagues. Thus, the results from Buben and O'Flaherty (1985) suggest that hepatomegaly is a robust response that was reported to be observed at the lowest dose tested, dose-related, and not accompanied by overt toxicity.

In terms of histopathology, Buben and O'Flaherty (1985) reported swollen hepatocytes with indistinct borders; their cytoplasm was clumped and a vesicular pattern was apparent and not simply due to edema in TCE-treated male mice. Karyorrhexis (the disintegration of the nucleus) was reported to be present in nearly all specimens from TCE-treated animals and suggestive of impending cell death. It was not present in controls, appeared at a low level at 400 mg/kg TCE exposure level, and appeared to be slightly higher at 1,600 mg/kg TCE exposure level. Central lobular necrosis was present only at the 1,600 mg/kg TCE exposure level and at a very low level. Buben and O'Flaherty (1985) report increased polyploidy in the central lobular region for both 400 and 1,600 mg/kg TCE and described it as hepatic cells having two or more nuclei or enlarged nuclei containing increased amounts of chromatin, but at the lowest level of severity or occurrence. Thus, the results of this study are consistent with those of shorter-term studies via gavage, which report hepatocellular hypertrophy in the centralobular region, increased liver weight induced at the lowest exposure level tested and at a level much lower than those inducing overt toxicity, and that TCE exposure is associated with changes in ploidy.

The NTP 13-week study of TCE gavage exposure in 10 F344/N rats (125–2,000 mg/kg [males] and 62.5–1,000 mg/kg [females]) and in B6C3F_1mice (375–6,000 mg/kg) reported that all rats survived the 13-week study. However, male rat receiving 2,000 mg/kg exhibited a 24% difference in final body weight. The study descriptions of pathology in rats and mice were not very detailed and included only mean liver weights. The rats had increased pulmonary

vasculitis at the highest concentration of TCE and viral titers were positive for Sendai virus. No liver effects were noted for them in the study.

For mice, liver weights (both absolute and percent liver/body weight) were reported to increase in a dose-related fashion with TCE exposure and to be increased by >10% in 750 mg/kg TCE-exposed males and ≥1,500 mg/kg TCE-exposed females. Hepatotoxicity was reported as centrilobular necrosis in 6/10 males and 1/10 females exposed to 6,000 mg/kg TCE and multifocal areas of calcifications scattered throughout 3,000 mg/kg TCE exposed male mice and only a single female 6,000 mg/kg dose, considered to be evidence of earlier hepatocellular necrosis. One female mouse exposed to 3,000 mg/kg TCE also had a hepatocellular adenoma, an extremely rare lesion in female mice of this age (20 weeks). At the lowest dose of exposure, there was a consistent decrease in liver weight in female and male mice after 13 weeks of TCE exposure.

Kawamoto et al. (1988b) exposed rats to 2 g/kg TCE subcutaneously for 15 weeks and reported TCE-induced increases in liver weight. They also reported increase in CYP, cytochrome b-5, and NADPH cytochrome c reductase. The difficulties in relating this route of exposure to more environmentally relevant ones is discussed in Section E.2.2.11.

For 2-year or lifetime studies of TCE exposure, a consistent hepatocarcinogenic response has been observed in mice of differing strains and genders and from differing routes of exposure. However, for rats, some studies have been confounded by mortality from gavage error or the toxicity of the dose of TCE administered. In some studies, a relative insensitive strain of rat has been used. However, in general, it appears that the mouse is more sensitive than the rat to TCE-induced liver cancer. Three studies give results the authors consider to be negative for TCE-induced liver cancer in mice, but have either design and/or reporting limitations, or are in strains and paradigms with apparent low ability for liver cancer induction or detection.

Fukuda et al. (1983) reported a 104-week inhalation bioassay in female Crj:CD-1 (ICR) mice and female Crj:CD (Sprague-Dawley) rats exposed to 0, 50, 150, and 450 ppm TCE (n = 50). There were no reported incidences of mice or rats with liver tumors for controls indicative of relatively insensitive strains used in the study for liver effects. While TCE was reported to induce a number of other tumors in mice and rats in this study, the incidence of liver tumors was <2% after TCE exposure. Of note is the report of cystic cholangioma reported in one group of rats.

Henschler et al. (1980) exposed NMRI mice and WIST random bred rats to 0, 100, and 500 ppm TCE for 18 months (n = 30). This study is limited by short duration of exposure, low number of animals, and low survival in rats. Control male mice were reported to have one HCC and one hepatocellular adenoma with the incidence rate unknown. In the 100 ppm TCE exposed group, two hepatocellular adenomas, and one mesenchymal liver tumor were reported. No liver tumors were reported at any dose of TCE in female mice or controls. For male rats, only one

hepatocellular adenomas at 100 ppm was reported. For female rats no liver tumors were reported in controls, but one adenoma and one cholangiocarcinoma was reported at 100 ppm TCE and at 500 ppm TCE, two cholangioadenomas, a relatively rare biliary tumor, was reported. The difference in survival in mice, did not affect the power to detect a response, as was the case for rats. However, the low number of animals studied, abbreviated exposure duration, and apparently low sensitivity of this paradigm (i.e., no background response in controls) suggests a study of limited ability to detect a TCE carcinogenic liver response. Of note is that both Fukuda et al. (1983) and Henschler et al. (1980) report rare biliary cell derived tumors in rats in relatively insensitive assays.

Van Duuren et al. (1979) exposed mice to 0.5 mg/mouse to TCE via gavage once a week in 0.1 mL trioctanion (n = 30). Inadequate design and reporting of this study limit that ability to use the results as an indicator of TCE carcinogenicity.

The NCI (1976) study of TCE was initiated in 1972 and involved the exposure of Osborne-Mendel rats and B6C3F_1 mice to varying concentrations of TCE. The animals were co-exposed to a number of other carcinogens as exhalation as multiples studies and control animals all shared the same laboratory space. Treatment duration was 78 weeks and animals received TCE via gavage in corn oil at two doses (n = 20 for controls, but n = 50 for treatment groups). For rats, the high dose was reported to result in significant mortality (i.e., 47/50 high-dose rats died before scheduled termination of the study). A low incidence of liver tumors was reported for controls and carbon tetrachloride positive controls in rats from this study. In B6C3F_1 mice, TCE was reported to increase incidence of HCCs in both doses and both genders of mice (~1,170 and 2,340 mg/kg for males and 870 and 1,740 mg/kg for female mice). HCC diagnosis was based on histologic appearance and metastasis to the lung. The tumors were described in detail and to be heterogeneous “as described in the literature” and similar in appearance to tumors generated by carbon tetrachloride. The description of liver tumors in this study and tendency to metastasize to the lung are similar to descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation exposure.

For male rats, noncancer pathology in the NCI (1976) study was reported to include increased fatty metamorphosis after TCE exposure and angiectasis or abnormally enlarged blood vessels. Angiectasis can be manifested by hyperproliferation of endothelial cells and dilatation of sinusoidal spaces. The authors conclude that due to mortality, “the test is inconclusive in rats.” They note the insensitivity of the rat strain used from their data on the positive control of carbon tetrachloride exposure.

The NTP (1990) study of TCE exposure in male and female F344/N rats, and B6C3F_1 mice (500 and 1,000 mg/kg for rats and 1,000 mg/kg for mice) was limited in the ability to demonstrate a dose-response for hepatocarcinogenicity. There was also little reporting of non-neoplastic pathology or toxicity and no report of liver weight at termination of the study. However, by the end of a 2-year cancer bioassay, liver tumor induction can be a significant

factor in any changes in liver weight. No treatment-related increases in necrosis in the liver were observed in mice. A slight increase in the incidence of focal necrosis was noted for TCE-exposed male mice (8 vs. 2% in control) with a slight reduction in fatty metamorphosis in treated male mice (0 treated vs. 2 control animals). In female mice, there was a slight increase in focal inflammation (29 vs. 19% of animals) and no other changes. Therefore, this study did not show concurrent evidence of liver toxicity but did show TCE-induced neoplasia after 2 years of TCE exposure in mice. The administration of TCE was reported to cause earlier expression of tumors as the first animals with carcinomas were reported to have them 57 weeks for TCE-exposed animals and 75 weeks for control male mice.

The NTP (1990) study reported that TCE exposure was associated with increased incidence of HCC (tumors with markedly abnormal cytology and architecture) in male and female mice. Hepatocellular adenomas were described as circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent but the tumors lacked typical lobular organization. HCCs had markedly abnormal cytology and architecture with abnormalities in cytology cited as including increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies, and variations in nuclear appearance. Furthermore, in many instances, several or all of the abnormalities were present in different areas of the tumor and variations in architecture with some of the HCCs having areas of trabecular organization. Mitosis was variable in amount and location. Therefore, the phenotype of tumors reported from TCE exposure was heterogeneous in appearance between and within tumors.

For rats, the NTP (1990) study reported no treatment-related non-neoplastic liver lesions in males and a decrease in basophilic cytological change reported from TCE-exposure in females. The results for detecting a carcinogenic response in rats were considered to be equivocal because both groups receiving TCE showed significantly reduced survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the high-dose group) of death by gavage error.

The NTP (1988) study of TCE exposure in four strains of rats to “diisopropylamine-stabilized TCE” was also considered inadequate for either comparing or assessing TCE-induced carcinogenesis in these strains of rats because of chemically induced toxicity, reduced survival, and incomplete documentation of experimental data. TCE gavage exposures of 0, 500, or 1,000 mg/kg-day (5 days/week, for 103 weeks) male and female rats was also marked by a large number of accidental deaths (e.g., for high-dose male Marshal rats, 25 animals were accidentally killed).

Results from a 13-week study were briefly mentioned in the report and indicated that exposure levels of 62.5–2,000 mg/kg TCE were not associated with decreased survival (with the

exception of three male August rats receiving 2,000 mg/kg TCE). Administration of the chemical for 13 weeks was not associated with histopathological changes.

In regard to evidence of liver toxicity, the 2-year study of TCE exposure reported no evidence of TCE-induced liver toxicity described as non-neoplastic changes ACI, August, Marshal, and Osborne-Mendel rats. Interestingly, for the control animals of these four strains, there was, in general, a low background level of focal necrosis in the liver of both genders. In summary, the negative results in this bioassay are confounded by the killing of a large portion of the animals accidently by experimental error but TCE-induced overt liver toxicity was not reported.

Maltoni et al. (1986) reported the results of several studies of TCE via inhalation and gavage in mice and rats. A large number of animals were used in the treatment groups but the focus of the study was detection of a neoplastic response with only a generalized description of tumor pathology phenotype given and limited reporting of non-neoplastic changes in the liver. Accidental death by gavage error was reported not to occur in this study. In regards to effects of TCE exposure on survival, "a nonsignificant excess in mortality" correlated to TCE treatment was observed only in female rats (treated by ingestion with the compound) and in male $B6C3F_1$ mice.

TCE-induced effects on body weight were reported to be absent in mice except for one experiment (BT 306 bis) in which a slight nondose correlated decrease was found in exposed animals. "Hepatoma" was the term used to describe all malignant tumors of hepatic cells, of different subhistotypes, and of various degrees of malignancy, and were reported to be unique or multiple and have different sizes (usually detected grossly at necropsy) from TCE exposure. In regard to phenotype, tumors were described as usual type observed in Swiss and $B6C3F_1$ mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens and to frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. Swiss mice from this laboratory were reported to have a low incidence of hepatomas without treatment (1%). The relatively larger number of animals used in this bioassay (n = 90–100), in comparison to NTP standard assays, allows for a greater power to detect a response.

TCE exposure for 8 weeks via inhalation at 100 or 600 ppm may have been associated with a small increase in liver tumors in male mice in comparison to concurrent controls during the life span of the animals. In Swiss mice exposed to TCE via inhalation for 78 weeks, there a reported increase in hepatomas associated with TCE treatment that was dose-related in male, but not female, Swiss mice. In $B6C3F_1$ mice exposed via inhalation to TCE for 78 weeks, the results from one experiment indicated a greater increase in liver cancer in females than male mice, but in a second experiment in males, there was a TCE-exposure associated increase in hepatomas. Although the mice were supposed to be of the same strain, the background level of liver cancer was significantly different in male mice. The finding of differences in response in animals of the same strain but from differing sources has also been reported in other studies for

other endpoints (see Section E.3.1.2). However, for both groups of male B6C3F$_1$ mice, the background rate of liver tumors over the lifetime of the mice was <20%.

For rats, there were four liver angiosarcomas reported (one in a control male rat, one each in a TCE-exposed male and female at 600 ppm TCE for 8 weeks, and one in a female rat exposed to 600 ppm TCE for 104 weeks), but the specific results for incidences of hepatocellular "hepatomas" in treated and control rats were not given. Although Maltoni et al. (1986) concluded that the small number of these tumors was not treatment-related, the findings were brought forward because of the extreme rarity of this tumor in control Sprague-Dawley rats, untreated or treated with vehicle materials. In rats treated for 104 weeks, there was no report of a TCE treatment-related increase in liver cancer in rats. This study only presented data for positive findings, so it did not give the background or treatment-related findings in rats for liver tumors in this study. Thus, the extent of background tumors and sensitivity for this endpoint cannot be determined.

Of note is that the Sprague-Dawley strain used in this study was also noted in the Fukuda et al. (1983) study to be relatively insensitive for spontaneous liver cancer and to also be negative for TCE-induced hepatocellular liver cancer induction in rats. However, like the Fukuda et al. (1983) and Henschler et al. (1980) studies, which reported rare biliary tumors in insensitive strains of rat for hepatocellular tumors, Maltoni et al. (1986) reported a relatively rare tumor type, angiosarcoma, after TCE exposure in a relatively insensitive strain for "hepatomas." As noted above, many of the rat studies were limited by premature mortality due to gavage error or premature mortality (NTP, 1990, 1988; Henschler et al., 1980; NCI, 1976), which was reported not occur in Maltoni et al. (1986).

There were other reports of TCE carcinogenicity in mice from chronic exposures that were focused primarily on detection of liver tumors with limited reporting of tumor phenotype or non-neoplastic pathology. Herren-Freund et al. (1987) reported that male B6C3F$_1$ mice given 40 mg/L TCE in drinking water had increased tumor response after 61 weeks of exposure. However, concentrations of TCE fell by about half at this dose of TCE during the twice a week change in drinking water solution, so the actual dose of TCE the animals received was <40 mg/L. The percent liver/body weight was reported to be similar for control and TCE-exposed mice at the end of treatment. Despite difficulties in accurately establishing the dose received, an increase in adenomas per animal and an increase in the number of animals with HCCs were reported to be associated with TCE exposure after 61 weeks of exposure and without apparent hepatomegaly.

Anna et al. (1994) reported tumor incidences for male B6C3F$_1$ mice receiving 800 mg/kg-day TCE via gavage (5 days/week for 76 weeks). All TCE-treated mice were reported to be alive after 76 weeks of treatment. Although the control group contained a mixture of exposure durations (76–134 weeks) and concurrent controls had a very small number

of animals, TCE-treatment appeared to increase the number of animals with adenomas and the mean number of adenomas and carcinomas, but with no concurrent TCE-induced cytotoxicity.

E.2.4.4. Summary of Results for Subchronic and Chronic Effects of DCA and TCA: Comparisons With TCE

There are no similar studies for TCA and DCA conduced at 6 weeks and with the range of concentrations examined in Buben and O'Flaherty (1985) for TCE. In general, many studies of DCA and TCA have been conducted at few and high concentrations, with shortened durations of exposure, and varying and low numbers of animals to examine primarily a liver tumor response in mice. However, the analyses presented in Section E.2.4.2 gives comparisons of administered TCA and DCA dose-responses for liver weight increases for a number of studies in combination as well as comparing such dose-responses to that of TCE and its oxidative metabolism. As stated above, many subchronic studies of DCA and TCA have focused on elucidating a relationship between dose and hypothesized events that may be indicators of carcinogenic potential that have been described in chronic studies with a focus on indicators of peroxisome proliferation and DNA synthesis. Many chronic studies have focused on the nature of the DCA and TCA carcinogenic response in mouse liver through examination of the tumors induced.

Almost all of the chronic studies for DCA and TCA have been carried out in mice. As the database for examination of the ability of TCE to induce liver tumors in rats includes several studies that have been limited in ability determine a carcinogenic response in the liver, the database for DCA and TCA in rats is even more limited. For TCA, the only available study in rats (DeAngelo et al., 1997) has been frequently cited in the literature to indicate a lack of response in this species for TCA-induced liver tumors. Although reporting an apparent dose-related increase in multiplicity of adenomas and an increase in carcinomas over control at the highest dose, DeAngelo et al. (1997) use such a low number of animals per treatment group (n = 20–24) that the abilities of this study to determine a statistically significant increase in tumor response and to be able to determine that there was no treatment-related effect were limited. A power calculation of the study shows that the type II error, which should be >50%, was <8% probability for incidence and multiplicity of all tumors at all exposure TCA concentrations with the exception of the incidence of adenomas and adenomas and carcinomas for 0.5 g/L treatment group (58%) in which there was an increase in adenomas reported over control (15 vs. 4%) that was the same for adenomas and carcinomas combined. Therefore, the designed experiment could accept a false null hypothesis and erroneously conclude that there is no response due to TCA treatment. While suggesting a lower response than for mice for liver tumor induction, it is inconclusive for determination of whether TCA induces a carcinogenic response in the liver of rats.

For DCA, there are two reported long-term studies in rats (DeAngelo et al., 1996; Richmond et al., 1995) that appear to have reported the majority of their results from the same data set and which consequently were subject to similar design limitations and DCA-induced neurotoxicity in this species. DeAngelo et al. (1996) reported increased hepatocellular adenomas and carcinomas in male F344 rats exposed for 2 years. However, the data from exposure concentrations at a 5 g/L dose had to be discarded and the 2.5 g/L DCA dose had to be continuously lowered during the study due to neurotoxicity. There was a DCA-induced increased in adenomas and carcinomas combined reported for the 0.5 g/L DCA (24.1 vs. 4.4% adenomas and carcinomas combined in treated vs. controls) and an increase at a variable dose started at 2.5 g/L DCA and continuously lowered (28.6 vs. 3.0% adenomas and carcinomas combined in treated vs. controls). Only combined incidences of adenomas and carcinomas for the 0.5 g/L DCA exposure group were reported to be statistically significant by the authors, although the incidence of adenomas was 17.2 vs. 4% in treated vs. control rats.

Hepatocellular tumor multiplicity was reported to be increased in the 0.5 g/L DCA group (0.31 adenomas and carcinomas/animal in treated vs. 0.04 in control rats) but was reported by the authors to not be statistically significant. At the starting dose of 2.5 g/L, continuously lowered due to neurotoxicity, the increased multiplicity of HCCs was reported by the authors to be to be statistically significant (0.25 carcinomas/animals vs. 0.03 in control) as well as the multiplicity of combined adenomas and carcinomas (0.36 adenomas and carcinomas/animals vs. 0.03 in control rats).

Issues that affected the ability to determine the nature of the dose-response for this study include: (1) the use of a small number of animals (n = 23, n =21, and n = 23 at final sacrifice for the 2.0 g/L sodium chloride control, 0.05, and 0.5 g/L treatment groups) that limit the power of the study both to determine statistically significant responses and to determine that there are not treatment-related effects (i.e., power); (2) apparent addition of animals for tumor analysis not present at final sacrifice (i.e., 0.05 and 0.5 g/L treatment groups); and (3) most of all, the lack of a consistent dose for the 2.5 g/L DCA exposed animals.

Similar issues were present for the study of Richmond et al. (1995) that was conducted by the same authors as DeAngelo et al. (1996) and appeared to be from the same data set. The Richmond et al. (1995) data for the 2 g/L sodium chloride, 0.05 g/L DCA, and 0.5 g/L DCA exposure groups were the same data set reported by DeAngelo et al. (1996) for these groups. Additional data was reported for F344 rats administered and 2.5 g/L DCA that, due to hind-limb paralysis, were sacrificed 60 weeks (DeAngelo et al., 1996). Tumor multiplicity was not reported by the authors. There was a small difference in reports of the results between the two studies for the same data for the 0.5 g/L DCA group in which Richmond et al. (1995) reported a 21% incidence of adenomas and DeAngelo et al. (1996) reported a 17.2% incidence. The authors did not report any of the results of DCA-induced increases of adenomas and carcinomas to be statistically significant. The same issues discussed above for DeAngelo et al. (1996) apply

to this study. Similar to the DeAngelo study of TCA in rats (DeAngelo et al., 1997) the study of DCA exposure in rats reported by DeAngelo et al. (1996) and Richmond et al. (1995), the use of small numbers of rats limits the detection of treatment-related effects and the ability to determine whether there was no treatment related effects (Type II error), especially at the low concentrations of DCA exposure.

For mice, the data for both DCA and TCA is much more extensive and has shown that both DCA and TCA induced liver tumors in mice. Many of the studies are for relatively high concentrations of DCA or TCA, have been conducted for ≤1 year, and have focused on the nature of tumors induced to ascertain potential modes of action and to make inferences as to whether TCE-induced tumors in mice are similar. As shown previously in Section E.2.4.2, the dose-response curves for increased liver weight for TCE administration in male mice are more similar to those for DCA administration and TCE oxidative metabolism than for direct TCA administration. There are two studies in male $B6C3F_1$ mice that attempt to examine multiple concentrations of DCA and TCA for 2-year studies (DeAngelo et al., 2008; DeAngelo et al., 1999) at doses that do not induce cytotoxicity and attempt to relate them to subchronic changes and peroxisomal enzyme induction. However, the DeAngelo et al. (2008) study was carried out in $B6C3F_1$ mice that were of large size and prone to liver cancer and premature mortality, limiting its use for the determination of TCA-dose response in a 2-year bioassay. One study in female $B6C3F_1$ mice describes the dose-response for liver tumor induction at a range of DCA and TCA concentrations after 51 or 82 weeks (Pereira, 1996) with a focus on the type of tumor each compound produced.

DeAngelo et al. (1999) conducted a study of DCA exposure to determine a dose response for the hepatocarcinogenicity of DCA in male $B6C3F_1$ mice over a lifetime exposure and especially at concentrations that did not illicit cytotoxicity or were for abbreviated exposure durations. DeAngelo et al. (1999) used 0.05, 0.5, 1.0, 2.0, and 3.5 g/L exposure concentrations of DCA in their 100-week drinking water study. The number of animals at final sacrifice was generally low in the DCA treatment groups and variable (i.e., n = 50, n = 33, n = 24, n = 32, n = 14, and n = 8 for control, 0.05, 0.5, 1, 2.0, and 3.5 g/L DCA exposure groups). It was apparent that animals that died unscheduled deaths between weeks 79 and 100 were included in data reported for 100 weeks. Although the authors did not report how many animals were included in the 100-week results, it appeared that the number was no greater than 1 for the control, 0.05, and 0.5 exposure groups and varied between 3 and 7 for the higher DCA exposure groups.

The multiplicity or number of HCCs/animals was reported to be significantly increased over controls in a dose-related manner at all DCA treatments including 0.05 g/L DCA, and a NOEL reported not to be observed by the authors (i.e., 0.28, 0.58, 0.68, 1.29, 2.47, and 2.90 HCCs/animal for control, 0.05, 0.5, 1.0, 2.0, and 3.5 g/L DCA). Between the 0.5 and 3.5 g/L exposure concentrations of DCA, the magnitude of increase in multiplicity was similar

to the increases in magnitude in dose. The incidence of HCCs was reported to be increased at all doses as well, but not reported to be statistically significant at the 0.05 g/L exposure concentration. However, given that the number of mice examined for this response (n = 33), the power of the experiment at this dose was only 16.9% to be able to determine that there was not a treatment-related effect. The authors did not report the incidence or multiplicity of adenomas for the 0.05 g/L exposure group in the study and neither did they report the incidence or multiplicity of adenomas and carcinomas in combination. For the animals surviving from 79 to 100 weeks of exposure, the incidence and multiplicity of adenomas peaked at 1 g/L, while HCCs continued to increase at the higher doses. This would be expected where some portion of the adenomas would either regress or progress to carcinomas at the higher doses.

DeAngelo et al. (1999) reported that peroxisome proliferation was significantly increased at 3.5 g/L DCA only at 26 weeks, not correlated with tumor response, and not increased at either 0.05 or 0.5 g/L treatments. The authors concluded that DCA-induced carcinogenesis was not dependent on peroxisome proliferation or chemically sustained proliferation, as measured by DNA synthesis. DeAngelo et al. (1999) reported not only a dose-related increase in DCA-induced liver tumors, but also a decrease in time-to-tumor associated with DCA exposure at the lowest levels examined. In regards to cytotoxicity, there appeared to be a treatment-related, but not dose-related, increase in hepatocellular necrosis that did not involve most of the liver from 1 to 3.5 g/L DCA exposures for 26 weeks of exposure. By 52 weeks, this effect was diminished with no necrosis observed at the 0.5 g/L DCA treatment for any exposure period.

Hepatomegaly was reported to be absent by 100 weeks of exposure at the 0.05 and 0.5 g/L exposures, while there was an increase in tumor burden reported. However, slight hepatomegaly was present by 26 weeks in the 0.5 g/L group and decreased with time. Not only did the increase in multiplicity of HCCs increase proportionally with DCA exposure concentration after 79–100 weeks of exposure, but so did the increases in percent liver/body weight.

DeAngelo et al. (1999) presented a figure comparing the number of HCCs/animal at 100 weeks compared with the percent liver/body weight at 26 weeks that showed a linear correlation (r^2 = 0.9977), while peroxisome proliferation and DNA synthesis did not correlate with tumor induction profiles. The proportional increase in liver weight with DCA exposure was also reported for shorter durations of exposure as noted in Section E.2.4.2. The findings of the study illustrate the importance of examining multiple exposure levels at lower concentrations, at longer durations of exposure, and with an adequate number of animals to determine the nature of a carcinogenic response. Although Carter et al. (1995) suggested that there is evidence of DCA-induced cytotoxicity (e.g., loss of cell membranes and apparent apoptosis) at higher levels, the 0.5 g/L exposure concentration was shown by DeAngelo et al.

(1999) to increase hepatocellular tumors after 100 weeks of treatment without concurrent peroxisome proliferation or cytotoxicity in mice.

As noted in detail in Section E.2.3.2.13, DeAngelo et al. (2008) exposed male B6C3F$_1$ mice to neutralized TCA in drinking water to male B6C3F$_1$ mice in three studies. Rather than using five exposure levels that were generally twofold apart, as was done in DeAngelo et al. (1999) for DCA, DeAngelo et al. (2008) studied only three doses of TCA that were an order of magnitude apart, which limits the elucidation of the shape of the dose-response curve. In addition, DeAngelo et al. (2008) contained two studies, each conducted in a separate laboratories, for the 104-week data so that the two lower doses were studied in one study and the highest dose in another. The first study was conducted using 2 g/L sodium chloride, or 0.05, 0.5, or 5 g/L TCA in drinking water for 60 weeks (Study #1), while the other two studies were conducted for a period of 104 weeks (Study #2 with 2.5 g/L neutralized acetic acid or 4.5 g/L TCA exposure groups and Study #3 with deionized water, 0.05 and 0.5 g/L TCA exposure groups). In the studies reported in DeAngelo et al. (2008), a small number of animals has been used for the determination of a tumor response (~n = 30 at final necropsy), but for the data for liver weight or PCO activity at interim sacrifices, the number was even smaller (n = 5).

The percent liver/body weight changes at 4 weeks in Study #1 have been included in the analysis for all TCA data in Section E.2.4.2, and are consistent with that data. Although there was a 10-fold difference in TCA exposure concentration, there was a 9, 16, and 35% increase in liver weight over control for the 0.05, 0.5, and 5 g/L TCA exposures. PCO activity varied 2.7-fold as baseline controls, but the increase in PCO activity at 4 weeks was 1.3-, 2.4-, and 5.3-fold of control for the 0.05, 0.5, and 5 g/L TCA exposure groups in Study #1. The incidence data for adenomas observed at 60 weeks was 2.1-, 3.0-, and 5.4-fold of control values and the fold increases in multiplicity were similar after 0.05, 0.5, and 5.0 g/L TCA. Thus, in general, the dose-response for TCA-induced liver weight increases at 4 weeks was similar to the magnitude of induction of adenomas at 60 weeks. Such a result is more consistent with the ability of TCA to induce tumors and increases in liver weight at low doses with little change with increasing dose as shown by this study and the combined data for TCA liver weight induction by administered TCA presented in Section E.2.4.2.

While the 104-week data from Studies #2 and #3 could have been more valuable for determination of the dose-response, as it would have allowed enough time for full tumor expression, serious issues were apparent for Study #3, which was reported to have a 64% incidence rate of adenomas and carcinomas for controls, while that of Study #2 was 12%. As stated in Section E.2.3.2.13, the mice in Study #3 were of larger size than those of either Study #1 or #2 and the large background rate of tumors reported is consistent with mice of these size (Leakey et al., 2003a). However, the large background rate and increased mortality for these mice limit their use for determining the nature of the dose-response for TCA liver carcinogenicity.

Examination of the data for treatment groups shows that there was no difference in any of the results between the 0.5 g/L (Study #3) and 5 g/L (Study #2) TCA exposure groups (i.e., adenoma, carcinoma, and combinations of adenoma and carcinoma incidence and multiplicity) for 104 weeks of exposure. For these same exposure groups, but at 60 weeks of exposure (Study #1), there was a twofold increase in multiplicity for adenomas, and for adenomas and carcinomas combined between the 0.5 and 5.0 g/L TCA exposure groups. At the two lowest doses of 0.05 and 0.5 g/L TCA from Study #3 in the large-tumor prone mice, the differences in the incidences and multiplicities for all tumors were twofold at 104 weeks. These results are consistent with: the two highest exposure levels reaching a plateau of response after a long enough duration of exposure for full expression of the tumors (i.e., ~90% of animals having liver tumors at the 0.5 and 5 g/L exposures) with the additional tumors observed in a tumor-prone paradigm. Thus, without use of the 0.05 and 0.5 g/L TCA data from Study #3, only the 4.5 g/L TCA data from Study #2 can be used for determination of the TCA cancer response in a 2-year bioassay.

To put the 64% incidence data for carcinomas and adenomas reported in DeAngelo et al. (2008) for the control group of Study #3 in context, other studies cited in this review for male $B6C3F_1$ mice show a much lower incidence in liver tumors with: (1) NCI (1976) reporting a colony control level of 6.5% for vehicle and 7.1% incidence of HCCs for untreated male $B6C3F_1$ mice (n = 70–77) at 78 weeks; (2) Herren-Freund et al. (1987) reporting a 9% incidence of adenomas in control male $B6C3F_1$ mice with a multiplicity of 0.09 ± 0.06 and no carcinomas (n = 22) at 61 weeks; (3) NTP (1990) reporting an incidence of 14.6% adenomas and 16.6% carcinomas in male $B6C3F_1$ mice after 103 weeks (n = 48); and (4) Maltoni et al. (1986) reporting that $B6C3F_1$ male mice from the "NCI source" had a 1.1% incidence of "hepatoma" (carcinomas and adenomas) and those from "Charles River Co." had a 18.9% incidence of "hepatoma" during the entire lifetime of the mice (n = 90 per group).

The importance of examining an adequate number of control or treated animals before confidence can be placed in those results in illustrated by Anna et al. (1994), in which at 76 weeks, 3/10 control male $B6C3F_1$ mice that were untreated and 2/10 control animals given corn oil were reported to have adenomas, but from 76 to 134 weeks, 4/32 mice were reported to have adenomas (multiplicity of 0.13 ± 0.06) and 4/32 mice were reported to have carcinomas (multiplicity of 0.12 ± 0.06). Thus, the reported combined incidence of carcinomas and adenomas of 64% reported by DeAngelo et al. (2008) for the control mice of Study #3, is not only inconsistent and much higher than those reported in Studies #1 and #2, but also much higher than reported in a number of other studies of TCE.

Trying to determine a correspondence with either liver weight increases or increases in PCO activity after shorter periods of exposure will be depend on whether data reported in Study #3 in the 104-week studies can be used. DeAngelo et al. (2008) reported a regression analyses that compared "percent of hepatocellular neoplasia," indicated by tumor multiplicity, with TCA

dose, represented by estimations of the TCA dose in mg/kg-day, and with PCO activity for the 60- and 104-week data. Whether adenomas and carcinomas combined or individual tumor type were used in these analysis was not reported by the authors. Concerns arise also from comparing PCO activity at the end of the experiments, when there was already a significant tumor response, rather than at earlier time points. Such PCO data may not be useful as an indicator key event in tumorigenesis when tumors are already present.

In addition, regression analyses of these data are difficult to interpret because of the dose spacing of these experiments as the control and 5 g/L exposure levels will basically determine the shape of the dose-response curve. The 0.05 and 0.5 g/L exposure levels are close to the control value in comparison to the 5 g/L exposure level, the dose response appears to be linear between control and the 5.0 g/L value with the two lowest doses not affect changing the slope of the line (i.e., "leveraging" the regression). Thus, the value of these analyses is limited by: (1) use of data from Study #3 in a tumor-prone mouse that is not comparable to those used in Studies #1 and #2; (2) the appropriateness of using PCO values from later time points and the variability in PCO control values; (3) the uncertainty of the effects of palatability on the 5 g/L TCA results, which were reported in one study to reduce drinking water consumption; and (4) the dose-spacing of the experiment.

DeAngelo et al. (2008) attempted to identify a NOEL for tumorigenicity using tumor multiplicity data and estimated TCA dose. However, it is not an appropriate descriptor for these data, especially given that "statistical significance" of the tumor response is the determinant used by the authors to support the conclusions regarding a dose in which there is no TCA-induced effect. Due to issues related to the appropriateness of use of the concurrent control in Study #3, only the 60-week experiment (i.e., Study #1) is useful for the determination of tumor dose-response. However, there is no allowance for full expression of a tumor response at the 60-week time point. In addition, a power calculation of the 60-week study shows that the type II error, which should be >50% and thus, greater than the chances of "flipping a coin," was 41 and 71% for incidence and 7 and 15% for multiplicity of adenomas for the 0.05 and 0.5 g/L TCA exposure groups. For the combination of adenomas and carcinomas, the power calculation was 8 and 92% for incidence and 6 and 56% for multiplicity at 0.05 and 0.5 g/L TCA exposure. Therefore, the designed experiment could accept a false null hypothesis, especially in terms of tumor multiplicity, at the lower exposure doses and erroneously conclude that there is no response due to TCA treatment.

Pereira (1996) examined the tumor induction in female B6C3F_1 mice and demonstrated that foci, adenoma, and carcinoma development in mice are dependent on duration of exposure, (or period of observation in the case of controls) for full expression of a carcinogenic response. In control female mice, a 360- vs. 576-day observation period showed that at 360 days, no foci or carcinomas and only 2.5% of animals had adenomas, whereas by 576 days of observation, 11% had foci, 2% adenomas, and 2% had carcinomas. For DCA and TCA treatments, foci,

adenomas, and carcinoma incidence and multiplicity did not reach full expression until 82 weeks at the three doses employed (2.58 g/L DCA, 0.86 g/L DCA, 0.26 g/L DCA, 3.27 g/L TCA, 1.1.0 g/L TCA, and 0.33 g/L TCA). Although the numbers of animals were relatively low and variable at the two highest doses (18–28 mice), there were 50–53 mice studied at the lowest dose level and 90 animals studied in the control group.

The results of Pereira (1996) showed that not only were the incidences of mice with foci, adenoma, and carcinomas greatly increased with duration of exposure, but concentration also affected the nature and magnitude of the response in female mice. At 2.86, 0.86, and 0.26 g/L DCA exposures and controls, after 82 weeks, the incidence of adenomas in female B6C3F_1 mice was reported to be 84.2, 25.0, 6.0, and 2.2%, respectively, and carcinomas to be 26.3, 3.6, 0, and 2.2%, respectively. For the multiplicity or number of tumors/animal at these same exposure levels of DCA, the multiplicity was reported to be 5.58, 0.32, 0.06, and 0.02 adenomas/animal, and 0.37, 0.04, 0, and 0.02 carcinomas/animal. Thus, for DCA exposure in female mice, for ~3-fold increases in DCA exposure concentration, after 82 weeks of exposure, there was a similar magnitude of increase in adenomas incidence with much greater increases in multiplicity. For HCC induction, there was no increase in the incidence or multiplicity or carcinomas between the control and 0.33 g/L DCA dose.

At 3.27, 1.10, and 0.33 g/L TCA and controls, after 82 weeks, the incidence of adenomas in female B6C3F_1 mice was reported to be 38.9, 11.1, 7.6, and 2.2%, respectively, and carcinomas to be 27.8, 18.5, 0, and 2.2%, respectively. At these same exposure levels of TCA, the multiplicity was reported to be 0.61, 0.11, 0.08, and 0.02 adenomas/animal, and 0.39, 0.22, 0, and 0.02 carcinomas/animal, respectively. Thus, for TCA, the incidences of adenomas were lower at the two highest doses than DCA and the ~3-fold differences in dose between the two lowest doses only resulted in ~50% increase in incidences of adenomas. For incidence of carcinomas, the ~3-fold difference in dose between the two highest doses only resulted in ~50% increase in carcinoma incidence. A similar pattern was reported for multiplicity after TCA exposure. Foci were also examined and, in general, were similar to adenomas regarding incidence and multiplicity. Thus, the dose-response curve for tumor induction in female mice differed between DCA and TCA after 82 weeks of exposure with TCA having a much less steep dose-response curve than DCA. This is consistent with the pattern of liver weight increases reported for male B6C3F_1 mice in Section E.2.4.2.

DeAngelo et al. (1999) reported a linear increase in incidence and multiplicity of HCCs that was proportional to dose and as well as proportional to the magnitude of liver weight increase from subchronic exposure to DCA. However, the studies of DeAngelo et al. (2008) and Pereira (1996) are suggestive that TCA induced increase in tumor incidence are less proportional to increases in dose as are liver weight increases from subchronic exposure.

Given that TCE subchronic exposure also induced an increase in liver weight that was proportional to dose (i.e., similar to DCA but not TCA), it is of interest as to whether the dose-

response for TCE induced liver cancer in mice was similar. The database for TCE, while consistently showing a induction of liver tumors in mice, is very limited for making inferences regarding the shape of the dose-response curve. For many of these experiments, multiplicity was not given, only liver tumor incidence. NTP (1990), Bull et al. (2002), and Anna et al. (1994) conducted gavage experiments in which they only tested one dose of ~1,000 mg/kg-day TCE. NCI (1976) tested two doses that were adjusted during exposure to an average of 1,169 and 2,339 mg/kg-day in male mice with only twofold dose spacing in only two doses tested. Maltoni et al. (1988) conducted inhalation experiments in two sets of $B6C3F_1$ mice and one set of Swiss mice at three exposure concentrations that were threefold apart in magnitude between the low and mid-dose and twofold apart in magnitude between the mid- and high-dose. However, for one experiment in male $B6C3F_1$ mice, the mice fought and suffered premature mortality and for two the experiments in $B6C3F_1$ mice, although using the same strain, the mice were obtained from differing sources with very different background liver tumor levels.

For the Maltoni et al. (1988) study, a general descriptor of "hepatoma" was used for liver neoplasia rather than describing hepatocellular adenomas and carcinomas so that comparison of that data with those from other experiments is difficult. More importantly, while the number of adenomas and carcinomas may be the same between treatments or durations of exposure, the number of adenomas may decrease as the number of carcinomas increase during the course of tumor progression. Such information is lost by using only a hepatoma descriptor.

Maltoni et al. (1988) did not report an increase over control for 100 ppm TCE for the Swiss group and one of the $B6C3F_1$ groups and only a slight increase (1.12-fold) in the second $B6C3F_1$ group. At 300 ppm TCE exposure, the incidences of hepatoma were 2-fold of control values for the Swiss, 4-fold of control for group of $B6C3F_1$ mice, and 1.6-fold of control for the other group of $B6C3F_1$ mice. At 600 ppm TCE, the incidences of hepatoma were 3.3-fold of control for the Swiss group, 6.1-fold of control for one group of $B6C3F_1$ mice, and 1.2-fold for the other group of $B6C3F_1$ mice. Thus, for each group of TCE exposed mice in the Maltoni et al. (1988) inhalation study, the background levels of hepatomas and the shape of the dose-response curve for TCE-hepatoma induction were variable. However, an average of the increases, in terms of fold of control, between the three experiments gives a ~2.9-fold increase between the low- and mid-dose (100 and 300 ppm) and ~1.4-fold increase between the mid- and high-dose (300 and 600 ppm) groups.

Although such a comparison obviously has a high degree of uncertainty associated with it, it suggests that the magnitude of TCE-induced hepatoma increases over control is similar to the three- and twofold difference in the magnitude of exposure concentrations between these doses. Therefore, the increase in TCE-induced liver tumors would roughly be proportional to the magnitude of exposure dose. This result would be similar to the result for the concordance of the increases in liver weight and exposure concentration observed at 28–42-day exposures to

TCE (see Section E.2.4.2) using oral data from $B6C3F_1$ and Swiss mice, and inhalation data from NMRI mice.

The available inhalation data for TCE-induced liver weight dose-response is from one study in a strain derived from Swiss mice (Kjellstrand et al., 1983a) and was conducted in male and female mice with comparable doses of 75 and 300 ppm TCE. However, male mice of this strain exhibited decreased body weight at the 300 ppm level, which can affect percent liver/body weight increases. The magnitude of TCE-induced increases in liver weight between the 75 and 300 ppm exposures were ~1.80-fold for males (1.50 vs. 1.90-fold of control liver weights) and 4.2-fold for females (1.27- vs. 2.14-fold of control liver weight) in this strain.

Female mice were examined in one study each of Swiss and $B6C3F_1$ mice by Maltoni et al. (1988). Both the Swiss and $B6C3F_1$ mice studies reported increases in incidences of hepatomas over controls only at the 600 ppm TCE level in female mice, indicating less of a response than males. Similarly, the Kjellstrand et al. (1983a) data also showed less of a response in females compared to males in terms TCE induction of liver weight at the 37–150 ppm range of exposure in NMRI strain. While the data for TCE dose-response of liver tumor induction is very limited, it is suggestive of a correlation of TCE-induced increases in liver weight correlating liver tumor induction with a pattern that is dissimilar to that of TCA.

Of those experiments conducted at ~1,000 mg/kg-day gavage dose of TCE in male $B6C3F_1$ mice for at least 79 weeks (Bull et al., 2002; Anna et al., 1994; NTP, 1990; NCI, 1976), the control values were conducted in varying numbers of animals (some as low as n = 15, i.e., (Bull et al., 2002) and with varying results). The incidence of HCCs ranged from 1.2 to 16.7% (Anna et al., 1994; NTP, 1990; NCI, 1976) and the incidence of adenomas ranged from 1.2 to 14.6% (Anna et al., 1994; NTP, 1990) in control $B6C3F_1$ mice. After ~1,000 mg/kg-day TCE treatment, the incidence of carcinomas ranged from 19.4 to 62% (Bull et al., 2002; Anna et al., 1994; NTP, 1990; NCI, 1976) with three of the studies (Anna et al., 1994; NTP, 1990; NCI, 1976) reporting a range of incidences between 42.8 and 62.0%). The incidence of adenomas ranged from 28 to 66.7% (Bull et al., 2002; Anna et al., 1994; NTP, 1990). These data are illustrative of the variability between experiments to determine the magnitude and nature of the TCE response in the same gender (male), strain ($B6C3F_1$), time of exposure (3/4 studies were for 76–79 weeks and 1 was for 2 years duration), and roughly the same dose (800–1,163 mg/kg-day TCE).

Given that the TCE-induced liver response, as measured by liver weight increase, is highly correlated with total oxidative metabolism to a number of agents that are hepatoactive agents and hepatocarcinogens, the variability in response from TCE exposure would be expected to be greater than studies of exposure to a single metabolite such as TCA or DCA.

Caldwell et al. (2008b) and Caldwell and Keshava (2006) have commented on the limitations of experimental paradigms used to study liver tumor induction by TCE metabolites and show that 51-week exposure duration has consistently produced a tumor response for these

chemicals, but with greater lesion incidence and multiplicity at 82 weeks. As reported by DeAngelo et al. (1999) and Pereira (1996), full expression of tumor induction in the mouse does not occur until 78–100 weeks of DCA or TCA exposure, especially at lower concentrations. Thus, use of abbreviated exposure durations and concurrently high exposure concentrations limits the ability of such experiments to detect a treatment-related effect with the occurrence of additional toxicity not necessarily associated with tumor induction. Caldwell et al. (2008b) present a table that shows that the differences in the ability of the studies to detect treatment-related effects could also be attributed to a varying and low number of animals in some exposure groups and that because of the low numbers of animals tested at higher exposures, the power to detect a statistically significant change is very low and, in fact for many of the endpoints, is considerably less than "50% chance." Table E-17 from Caldwell et al. (2008b) illustrates the importance of experimental design and the limitations in many of the studies in the TCE metabolite database.

Table E-17. Power calculations[a] for experimental design described in text, using Pereira and colleagues (1996) as an example

Exposure concentration[b] in female B6C3F$_1$ mice	Number of animals	Power calculation for foci	Power calculation for adenomas	Power calculation for carcinomas
20.0 mmol/L NaCl (control) (82 wks)	90	Null hypothesis	Null hypothesis	Null hypothesis
2.58 g/L DCA (82 wks)	19	0.03	0.03	0.13
0.86 g/L DCA (82 wks)	28	0.74	0.20	0.91
0.26 g/L DCA (82 wks)	50	0.99	0.98	–
3.27 g/L TCA (82 wks)	18	0.15	0.09	0.14
1.10 g/L TCA (82 wks)	27	0.60	0.64	0.3
0.33 g/L TCA (82 wks)	53	0.93	0.91	–

[a]The power calculations represent the probability of rejecting the null hypothesis when, in fact, the alternate hypothesis is true for tumor multiplicity (i.e., the total number of lesions/number of animals). The higher the power number calculated, the more confidence we have in the null hypothesis. Assumptions made included: normal distribution for the fraction of tumors reported, null hypothesis represents what we expected the control tumor fraction to be, the probability of a Type I error was set to 0.05, and the alternate hypothesis was set to 4 times the null hypothesis value.
[b]Conversion of mmol/L to g/L from the original reports of Pereira (1996) and Pereira and Phelps (1996) is as follows: 20.0 mmol/L DCA = 2.58 g/L, 6.67 mmol/L DCA = 0.86 g/L, 2.0 mmol/L = 0.26 g/L, 20.0 mmol/L TCA = 3.27 g/L, 6.67 mmol/L TCA = 1.10 g/L, and 2.0 mmol/L TCA = 0.33 g/L.

Bull et al. (1990) examined male and female B6C3F$_1$ mice (age 37 days) exposed from 15 to 52 weeks to neutralized DCA and TCA (1 or 2 g/L) but tumor data were not suitable for dose response. They reported effects of DCA and TCA exposure on liver weight and percent liver/body changes that gave a pattern of hepatomegaly generally consistent with short-term exposure studies. Only 10 female mice were examined at 52 weeks, but the female mice were

reported to be as responsive as males at the exposure concentration tested. After 37 weeks of treatment and then a cessation of exposure for 15 weeks, liver weights and percent liver/body weight were reported to be elevated over controls, which Bull et al. (1990) partially attribute the remaining increases in liver weight to the continued presence of hyperplastic nodules in the liver.

Macroscopically, livers treated with DCA were reported to have multifocal areas of necrosis and frequent infiltration of lymphocytes on the surface and interior of the liver. For TCA-treated mice, similar necrotic lesions were reported but at such a low frequency that they were similar to controls. Marked cytomegaly was reported from exposure to either 1 or 2 g/L DCA throughout the liver. Cell size was reported to be increased from TCA and DCA treatment with DCA producing the greatest change. The 2 g/L TCA exposures were observed to have increased accumulations of lipofuscin but no quantitative analysis was done. Photographs of light microscopic sections, that were supposed to be representative of DCA- and TCA-treated livers at 2 g/L, showed such great hepatocellular hypertrophy from DCA treatment that sinusoids were obscured. Such a degree of cytomegaly could have resulted in reduction of blood flow and contributed to focal necrosis observed at this level of exposure.

As discussed in Sections E.3.2 and E.3.4.2.1, glycogen accumulation has been described to be present in foci in both humans and animals as a result from exposure to a wide variety of carcinogenic agents and predisposing conditions in animals and humans. Bull et al. (1990) reported that glycogen deposition was uniformly increased from 2 g/L DCA exposure with photographs of TCA exposure showing slightly less glycogen staining than controls. However, the abstract and statements in the paper suggest that there was increased PAS positive material from TCA treatment that has caused confusion in the literature in this regard. Kato-Weinstein et al. (2001) reported that in male B6C3F$_1$ mice exposed to DCA and TCA, the DCA treatment increased glycogen, and TCA decreased glycogen content of the liver by using both chemical measurement of glycogen in liver homogenates and by using ethanol-fixed sections stained with PAS, a procedure designed to minimize glycogen loss. Kato-Weinstein et al. (2001) reported that glycogen rich and poor cells were scattered without zonal distribution in male B6C3F$_1$ mice exposed to 2 g/L DCA for 8 weeks. For TCA treatments, they reported centrilobular decreases in glycogen and ~25% decreases in whole liver by 3 g/L TCA.

Kato-Weinstein et al. (2001) reported whole-liver glycogen to be increased ~1.50-fold of control (90 vs. 60 mg glycogen/g liver) by 2 g/L DCA after 8 weeks exposure in male B6C3F$_1$ mice with a maximal level of glycogen accumulation occurring after 4 weeks of DCA exposure. Pereira et al. (2004a) reported that after 8 weeks of exposure to 3.2 g/L DCA, liver glycogen content was 2.20-fold of control levels (155.7 vs. 52.4 mg glycogen/g liver) in female B6C3F$_1$ mice. Thus, the baseline level of glycogen content reported by (~60 mg/g) and the increase in glycogen after DCA exposure was consistent between Kato-Weinstein et al. (2001) and Pereira et al. (2004a). However, the increase in liver weight reported by Kato-Weinstein et al. (2001) of 1.60-fold of control percent liver/body weight cannot be accounted for by the 1.50-fold of

control glycogen content. Glycogen content only accounts for 5% of liver mass so that 50% increase in glycogen cannot account for the 60% increase liver mass induced by 2 g/L DCA exposure for 8 weeks reported by Kato-Weinstein (2001). Thus, DCA-induced increases in liver weight are occurring from other processes as well.

Carter et al. (2003) and DeAngelo et al. (1999) reported increased glycogen after DCA treatment at much lower doses after longer periods of exposure (100 weeks). Carter et al. (2003) reported increased glycogen at 0.5 g/L DCA and DeAngelo et al. (1999) reported increased glycogen at 0.03 g/L DCA in mice. However, there was no quantitation of that increase.

The issues involving identification of a mode of action through tumor phenotype analysis are discussed in detail below for the more general case of liver cancer as well as for specific hypothesized modes of action (see Sections E.3.1.4, E.3.1.8, E.3.2.1, and E.3.4.1.5). For TCE and its metabolites, c-Jun staining, H-rats mutation, tincture, and heterogeneity in dysplacity have been used to describe and differentiate liver tumors in the mouse.

Bull et al. (2002) reported 1,000 mg/kg TCE administered via gavage daily for 79 weeks in male B6C3F_1 mice to produce liver tumors and also reported deaths by gavage error (6/40 animals). The limitations of the experiment are discussed in Caldwell et al. (2008b). Specifically, for the DCA and TCA exposed animals, the experiment was limited by low statistical power, a relatively short duration of exposure, and uncertainty in reports of lesion prevalence and multiplicity due to inappropriate lesions grouping (i.e., grouping of hyperplastic nodules, adenomas, and carcinomas together as "tumors"), and incomplete histopathology determinations (i.e., random selection of gross lesions for histopathology examination).

For the TCE results, a high prevalence (23/36 B6C3F_1 male mice) of adenomas and HCC (7/36) was reported. For determinations of immunoreactivity to c-Jun, as a marker of differences in "tumor" phenotype, Bull et al. (2002) included all lesions in most of their treatment groups, decreasing the uncertainty of his findings. However, for immunoreactivity results hyperplastic nodules, adenomas, and carcinomas were grouped and thus, changes in c-Jun expression between the differing types of lesions were not determined.

Bull et al. (2002) reported lesion reactivity to c-Jun antibody to be dependent on the proportion of the DCA and TCA administered after 52 weeks of exposure. Given alone, DCA was reported to produce lesions in mouse liver for which approximately half displayed a diffuse immunoreactivity to a c-Jun antibody, half did not, and none exhibited a mixture of the two. After TCA exposure alone, no lesions were reported to be stained with this antibody. When given in various combinations, DCA and TCA co-exposure induced a few lesions that were only c-Jun+, many that were only c-Jun-, and a number with a mixed phenotype whose frequency increased with the dose of DCA. For TCE exposure of 79 weeks, TCE-induced lesions were reported to also have a mixture of phenotypes (42% c-Jun+, 34% c-Jun-, and 24% mixed) and to be most consistent with those resulting from DCA and TCA co-exposure but not either metabolite alone.

Stauber and Bull (1997) exposed male B6C3F$_1$ mice (7 weeks old at the start of treatment) to 2.0 g/L neutralized DCA or TCA in drinking water for 38 or 50 weeks, respectively, and then exposed (n = 12) to 0, 0.02, 0.1, 0.5, 1.0, and 2.0 g/L DCA or TCA for an additional 2 weeks. Foci and tumors were combined in reported results as "lesions" and prevalence rates were not reported. The DCA-induced larger "lesions" were reported to be more "uniformly reactive to c-Jun and c-Fos" but many nuclei within the lesions displaying little reactivity to c-Jun. Stauber and Bull (1997) stated that while most DCA-induced "lesions" were homogeneously immunoreactive to c-Jun and C-Fos (28/41 lesions), the rest were stained heterogeneously. For TCA-induced lesions, the authors reported no difference in staining between "lesions" and normal hepatocytes in TCA-treated animals. These results are slightly different that those reported by Bull et al. (2002) for DCA, who report c-Jun positive and negative foci in DCA-induced liver tumors but no mixed lesions. Because "lesions" comprised of foci and tumors, different stages of progression reported in these results. The duration of exposures also differed between DCA and TCA treatment groups that can affect phenotype. The shorter duration of exposure can also prevent full expression of the tumor response.

Stauber et al. (1998) presented a comparison of in vitro results with "tumors" from Stauber and Bull (1997) and note that 97.5% of DCA-induced "tumors" were c-Jun+, while none of the TCA-induced "tumors" were c-Jun+. However, the concentrations used to give tumors in vivo for comparison with in vitro results were not reported. This appears to differ from the heterogeneity of result for c-Jun staining reported by Bull et al. (2002) and Stauber and Bull (1997). There was no comparison of c-Jun phenotype for spontaneous tumors with the authors stating that because of such short time, no control tumors results were given. However, the results of Bull et al. (2002) and Stauber and Bull (1997), do show TCA-induced lesions to be uniformly c-Jun negative and thus, the phenotypic marker was able to show that TCE-induced tumors were more like those induced by DCA than TCA.

The premise that DCA induced c-Jun positive lesions and TCA induced c-Jun negative lesions in mouse liver was used as the rationale to study induction of "transformed" hepatocytes by DCA and TCE treatment in vitro. Stauber et al. (1998) isolated primary hepatocytes from 5–8-week-old male B6C3F$_1$ mice (n = 3) and subsequently cultured them in the presence of DCA or TCA. In a separate experiment, 0.5 g/L DCA was given to mice as pretreatment for 2 weeks prior to isolation. The authors assumed that the anchorage-independent growth of these hepatocytes was an indication of an "initiated cell." DCA and TCA solutions were neutralized before use.

After 10 days in culture with DCA or TCA (0, 0.2, 0.5, and 2.0 mM), concentrations of ≥0.5 mM DCA and TCA both induced an increase in the number of colonies that was statistically significant, increased with dose with DCA, and slightly greater for DCA. In a time-course experiment, the number of colonies from DCA treatment in vitro peaked by 10 days and did not change through days 15–25 at the highest dose and, at lower concentrations of DCA,

increased time in culture induced similar peak levels of colony formation by days 20–25 as that reached by 10 days at the higher dose. Therefore, the number of colonies formed was independent of dose if the cells were treated long enough in vitro.

However, not only did treatment with DCA or TCA induce anchorage-independent growth, but untreated hepatocytes also formed larger numbers of colonies with time, although at a lower rate than those treated with DCA. The level reached by untreated cells in tissue culture at 20 days was similar to the level induced by 10 days of exposure to 0.5 mM DCA. The time course of TCA exposure was not tested to see if it had a similar effect with time as did DCA. The colonies observed at 10 days were tested for c-Jun expression with the authors noting that "colonies promoted by DCA were primarily c-Jun positive in contrast to TCA promoted colonies that were predominantly c-Jun negative." Of the colonies that arose spontaneously from tissue culture conditions, 10/13 (76.9%) were reported to be c-Jun+, those treated with DCA 28/34 (82.3%) were c-Jun+, and those treated with TCA 5/22 (22.7%) were c-Jun+. Thus, these data show heterogeneity in cell in colonies, but with more c-Jun+ colonies occurring by tissue culture conditions alone and in the presence of DCA, rather than in the presence of TCA.

The authors reported that with time (24, 48, 72, and 96 hours) of culture conditioning the number of c-Jun+ colonies was increased in untreated controls. The authors reported that DCA treatment delayed the increase in c-Jun+ expression induced by tissue culture conditions alone in untreated controls, while TCA treatment was reported to not affect the increasing c-Jun+ expression that increased with time in tissue culture. These results seems paradoxical given that DCA induced a higher number of colonies at 10 days of tissue culture than TCA and that most of the colonies were c-Jun positive. The number of colonies was greater for pretreatment with DCA, but the magnitude of difference over the control level was the same after DCA treatment in vitro with and without pretreatment. As to the relationship of c-Jun staining and peroxisome proliferators as a class, as pointed out by Caldwell and Keshava (2006), although Bull et al. (2004) have suggested that the negative expression of c-Jun in TCA-induced tumors may be consistent with a characteristic phenotype shown in general by peroxisome proliferators as a class, there is no supporting evidence of this.

An approach to determine the potential modes of action of DCA and TCA through examination of the types of tumors each "induced" or "selected" was to examine H-ras activation (Bull et al., 2002; Ferreira-Gonzalez et al., 1995; Anna et al., 1994; Nelson et al., 1990). This approach has also been used to try to establish an H-ras activation pattern for "genotoxic" and "nongenotoxic" liver carcinogens compounds and to make inferences concerning peroxisome proliferator-induced liver tumors.

However, as noted by Stanley et al. (1994), the genetic background of the mice used and the dose of carcinogen may affect the number of activated H-ras containing tumors that develop. In addition, the stage of progression of "lesions" (i.e., foci vs. adenomas vs. carcinomas) also has been linked the observance of H-ras mutations.

Fox et al. (1990) note that tumors induced by phenobarbital (0.05% drinking water (H_2O), 1 year), chloroform (200 mg/kg corn oil gavage, 2 times weekly for 1 year), or Ciprofibrate (0.0125% diet, 2 years) had a much lower frequency of H-ras gene activation than those that arose spontaneously (2-year bioassays of control animals) or induced with the "genotoxic" carcinogen benzidine-2 hydrochloric acid (HCl; 120 ppm, drinking H_2O, 1 year) in mice. In that study, the term "tumor" was not specifically defined, but a correlation between the incidence of H-ras gene activation and development of either a hepatocellular adenoma or HCC was reported to be made with no statistically significant difference between the frequency of H-ras gene activation in the hepatocellular adenomas and carcinomas. Histopathological examination of the spontaneous tumors, tumors induced with benzidine-2HCL, phenobarbital, and chloroform was not reported to reveal any significant changes in morphology or staining characteristics.

Spontaneous tumors were reported to have 64% point mutation in codon 61 (n = 50 tumors examined) with a similar response for benzidine of 59% (n = 22 tumors examined), whereas for phenobarbital, the mutation rate was 7% (n = 15 tumors examined), chloroform 21% (n = 24 tumors examined), and Ciprofibrate 21% (n = 39 tumors examined). The Ciprofibrate-induced tumors were reported to be more eosinophilic as were the surrounding normal hepatocytes.

Hegi et al. (1993) tested Ciprofibrate-induced tumors in the NIH3T3 cotransfection-nude mouse tumorigenicity assay, which the authors stated is capable of detecting a variety of activated proto-oncogenes. The tumors examined (Ciprofibrate-induced or spontaneously arising) were taken from the Fox et al. (1990) study, screened previously, and found to be negative for H-ras activation. With the limited number of samples examined, Hegi et al. (1993) concluded that ras proto-oncogene activation or activation of other proto-oncogenes using the nude mouse assay were not frequent events in Ciprofibrate-induced tumors and that spontaneous tumors were not promoted with it. Using the more sensitive methods, the H-ras activation rate was reported to be raised from 21 to 31% for Ciprofibrate-induced tumors and from 64 to 66% for spontaneous tumors.

Stanley et al. (1994) studied the effect of methylclofenapate (MCP) (25 mg/kg for up to 2 years), a peroxisome proliferator, in $B6C3F_1$ (relatively sensitive) and C57BL/10J (relatively resistant) mice for H-ras codon 61 point mutations in MCP-induced liver tumors (hepatocellular adenomas and carcinomas). In the $B6C3F_1$ mice, the number of tumors with codon 61 mutations was 11/46 and for C57BL/10J mice 4/31. Unlike the findings of Fox et al. (1990), Stanley et al. (1994) reported an increase in the frequency of mutation in carcinomas, which was reported to be twice that of adenomas in both strains of mice, indicating that stage of progression was related to the number of mutations in those tumors, although most tumors induced by MCP did not have this mutation.

In terms of liver tumor phenotype, Anna et al. (1994) reported that the H-ras codon 61 mutation frequency was not statistically different in liver tumors from DCA and TCE-treated

mice from a highly variable number of tumors examined. In regard to mutation spectra in H-ras oncogenes in control or spontaneous tumors, the patterns were slightly different but mostly similar to that of DCA-induced tumors (0.5% in drinking water). From their concurrent controls they reported that H-ras codon 61 mutations in 17% (n = 6) of adenomas and 100% (n = 5) of carcinomas. For historical controls (published and unpublished), they reported mutations in 73% (n = 33) of adenomas and mutations in 70% (n = 30) of carcinomas. For tumors from TCE treated animals, they reported mutations in 35% (n = 40) of adenomas and 69% (n = 36) of carcinomas, while for DCA-treated animals, they reported mutations in 54% (n = 24) of adenomas and in 68% (n = 40) of carcinomas. Anna et al. (1994) reported more mutations in TCE-induced carcinomas than adenomas.

The study of Ferreira-Gonzalez et al. (1995) in male B6C3F_1 mice has the advantage of comparison of tumor phenotype at the same stage of progression (HCC), for allowance of the full expression of a tumor response (i.e., 104 weeks), and an adequate number of spontaneous control lesions for comparison with DCA or TCA treatments. However, tumor phenotype at an endstage of tumor progression reflects of tumor progression and not earlier stages of the disease process. In spontaneous liver carcinomas, 58% were reported to show mutations in H-61 as compared with 50% of tumor from 3.5 g/L DCA-treated mice and 45% of tumors from 4.5.g/L TCA-treated mice. Thus, there was a heterogeneous response for this phenotypic marker for the spontaneous, DCA-, and TCA-treatment induced HCCs and not a pattern of reduced H-ras mutation reported for a number of peroxisome proliferators.

A number of peroxisome proliferators have been reported to have a much smaller mutation frequency than spontaneous tumors (e.g., 13–24% H-ras codon 61 mutations after Methylclofenopate depending on mouse strain, Stanley et al. (1994)): 21–31% for Ciprofibrate-induced tumors and 64–66% for spontaneous tumors, Fox et al. (1990) and Hegi et al. (1993).

Bull (2000) suggested that "the report by Anna et al. (1994) indicated that TCE-induced tumors possessed a different mutation spectra in codon 61 of the H-ras oncogene than those observed in spontaneous tumors of control mice." Bull (2000) stated that "results of this type have been interpreted as suggesting that a chemical is acting by a mutagenic mechanism" but went on to suggest that it is not possible to a priori rule out a role for selection in this process and that differences in mutation frequency and spectra in this gene provide some insight into the relative contribution of different metabolites to TCE-induced liver tumors. Bull (2000) noted that data from Anna et al. (1994), Ferreira-Gonzalez et al. (1995), and Maronpot et al. (1995a) indicated that mutation frequency in DCA-induced tumors did not differ significantly from that observed in spontaneous tumors. Bull (2000) also noted that the mutation spectra found in DCA-induced tumors has a striking similarity to that observed in TCE-induced tumors, and DCA-induced tumors were significantly different than that of TCA-induced liver tumors.

Bull et al. (2002) reported that mutation frequency spectra for the H-ras codon 61 in mouse liver "tumors" induced by TCE (n = 37 tumors examined) to be significantly different

than that for TCA (n = 41 tumors examined), with DCA-treated mice tumors giving an intermediate result (n = 64 tumors examined). In this experiment, TCA-induced "tumors" were reported to have more mutations in codon 61 (44%) than those from TCE (21%) and DCA (33%). This frequency of mutation in the H-ras codon 61 for TCA is the opposite pattern as that observed for a number of peroxisome proliferators in which the number of mutations at H-ras 61 in tumors has been reported to be much lower than spontaneously arising tumors (see Section E.3.4.1.5). Bull et al. (2002) noted that the mutation frequency for all TCE, TCA, or DCA tumors was lower in this experiment than for spontaneous tumors reported in other studies (they had too few spontaneous tumors to analyze in this study), but that this study utilized lower doses and was of shorter duration than that of Ferreira-Gonzalez et al. (1995). These are concerns in addition to the effects of lesion grouping in which a lower stage of progression is grouped with more advanced stages. In a limited subset of tumors that were both sequenced and characterized histologically, only 8 of 34 (24%) TCE-induced adenomas but 9/15 (60%) of TCE-induced carcinomas were reported to have mutated H-ras at codon 61, which the authors suggest is evidence that this mutation is a late event.

Thus, in terms of H-ras mutation, the phenotype of TCE-induced tumors appears to be more like DCA-induced tumors (which are consistent with spontaneous tumors), or those resulting from a co-exposure to both DCA and TCA (Bull et al., 2002), than from those induced by TCA. As noted above, Bull et al. (2002) reported the mutation frequency spectra for the H-ras codon 61 in mouse liver tumors induced by TCE to be significantly different than that for TCA, with DCA-treated mice tumors giving an intermediate result and for TCA-induced tumors to have a H-ras profile that is the opposite than those of a number of other peroxisome proliferators. More importantly, these data suggest that using measures, other than dysplasticity and tincture, mouse liver tumors induced by TCE are heterogeneous in phenotype.

With regard to tincture, Stauber and Bull (1997) reported the for male $B6C3F_1$ mice, DCA-induced "lesions" contained a number of smaller lesions that were heterogeneous and more eosinophilic with larger "lesions" tending to less numerous and more basophilic. For TCA results using this paradigm, the "lesions" were reported to be less numerous, more basophilic, and larger than those induced by DCA.

Carter et al. (2003) used tissues from the DeAngelo et al. (1999) study and examined the heterogeneity of the DCA-induced lesions and the type and phenotype of preneoplastic and neoplastic lesions pooled across all time points. Carter et al. (2003) examined the phenotype of liver tumors induced by DCA in male $B6C3F_1$ mice and the shape of the dose-response curve for insight into its mode of action. They reported a dose-response of histopathologic changes (all classes of premalignant lesions and carcinomas) occurring in the livers of mice from 0.05 to 3.5 g/L DCA for 26–100 weeks and suggest that foci and adenomas demonstrated neoplastic progression with time at lower doses than observed DCA genotoxicity. Preneoplastic lesions

were identified as eosinophilic, basophilic, and/or clear cell (grouped with clear cell and mixed cell) and dysplastic.

Altered foci were 50% eosinophilic with about 30% basophilic. As foci became larger and evolved into carcinomas, they became increasingly basophilic. The pattern held true throughout the exposure range. There was also a dose and length of exposure related increase in atypical nuclei in "noninvolved" liver. Glycogen deposition was also reported to be dose-dependent with periportal accumulation at the 0.5 g/L exposure level. Carter et al. (2003) suggested that size and evolution into a more malignant state are associated with increasing basophilia, a conclusion consistent with those of Bannasch (1996) and that there is a greater periportal location of lesions suggestive as the location from which they arose.

Consistent with the results of DeAngelo et al. (1999), Carter et al. (2003) reported that DCA (0.05–3.5 g/L) increased the number of lesions per animal relative to animals receiving distilled water, shortened the time to development of all classes of hepatic lesions, and that the phenotype of the lesions was similar to those spontaneously arising in controls. Along with basophilic and eosinophilic lesions or foci, Carter et al. (2003) concluded that DCA-induced tumors also arose from isolated, highly dysplastic hepatocytes in male B6C3F_1 mice chronically exposed to DCA, suggesting another direct neoplastic conversion pathway other than through eosinophilic or basophilic foci.

Rather than male B6C3F_1 mice, Pereira (1996) studied the dose-response relationship for the carcinogenic activity of DCA and TCA and characterized their lesions (foci, adenomas, and carcinomas) by tincture in females (the generally less sensitive gender). Like the studies of TCE by Maltoni et al. (1986), female mice were also reported to have increased liver tumors after TCA and DCA exposures. Pereira (1996) pooled lesions for phenotype analyses so the effect of duration of exposure could not be determined, nor could adenomas be separated from carcinomas for "tumors."

However, as the concentration of DCA was decreased, the number of foci was reported by Pereira (1996) to be decreased but the phenotype of the foci to go from primarily eosinophilic foci (i.e., ~95% eosinophilic at 2.58 g/L DCA) to basophilic foci (~57% eosinophilic at 0.26 g/L). For TCA, the number of foci was reported to ~40 basophilic and ~60 eosinophilic regardless of dose. Spontaneously occurring foci were more basophilic by a ratio of 7/3. Pereira (1996) described the foci of altered hepatocytes and tumors induced by DCA in female B6C3F_1 mice to be eosinophilic at higher exposure levels but at lower or intermittent exposures to be half eosinophilic and half basophilic. Regardless of exposure level, half of the TCA-induced foci were reported to be half eosinophilic and half basophilic with tumors 75% basophilic. In control female mice, the limited numbers of lesions were mostly basophilic, with most of the rest being eosinophilic with the exception of a few mixed tumors. The limitations of descriptions tincture and especially for inferences regarding peroxisome proliferator from the description of "basophilia" is discussed in Section E.3.4.1.5.

The results appear to differ between male and female $B6C3F_1$ mice in regard to tincture for DCA and TCA at differing doses. What is apparent is that the tincture of the lesions is dependent on the stage of tumor progression, agent (DCA or TCA), gender, and dose. Also what is apparent from these studies is the both DCA and TCA are heterogeneous in their tinctoral characteristics as well as phenotypic markers such as mutation spectra or expression of c-Jun.

The descriptions of TCE-induced tumors in mice reported by the NCI, NTP, and Maltoni et al. studies are also consistent with phenotypic heterogeneity as well as consistency with spontaneous tumor morphology (see Section E.3.4.1.5). As noted in Section E.3.1, HCCs observed in humans are also heterogeneous. For mice, Maltoni et al. (1986) described malignant tumors of hepatic cells to be of different subhistotypes, and of various degrees of malignancy and were reported to be unique or multiple, and have different sizes (usually detected grossly at necropsy) from TCE exposure. In regard to phenotype, tumors were described as usual type observed in Swiss and $B6C3F_1$ mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens and to frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns.

For the NCI (1976) study, the mouse liver tumors were described in detail and to be heterogeneous "as described in the literature" and similar in appearance to tumors generated by carbon tetrachloride. The description of liver tumors in this study and tendency to metastasize to the lung are similar to descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation exposure.

The NTP (1990) study reported TCE exposure to be associated with increased incidence of HCC (tumors with markedly abnormal cytology and architecture) in male and female mice. Hepatocellular adenomas were described as circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent but the tumors lacked typical lobular organization. HCCs were reported to have markedly abnormal cytology and architecture with abnormalities in cytology cited as including increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies, and variations in nuclear appearance. Furthermore, in many instances, several or all of the abnormalities were reported to be present in different areas of the tumor and variations in architecture with some of the HCCs having areas of trabecular organization. Mitosis was variable in amount and location. Therefore, the phenotype of tumors reported from TCE exposure was heterogeneous in appearance between and within tumors from all three of these studies.

Caldwell and Keshava (2006) reported:

> that Bannasch (2001) and Bannasch et al. (2001) describe the early phenotypes of preneoplastic foci induced by many oncogenic agents (DNA-reactive chemicals,

radiation, viruses, transgenic oncogenes and local hyperinsulinism) as insulinomimetic. These foci and tumors have been described by tincture as eosinophilic and basophilic and to be heterogeneous.

The tumors derived from them after TCE exposure are consistent with the description for the main tumor lines of development described by Bannasch et al. (2001) (see Section E.3.4.1.5). Thus, the response of liver to DCA (glycogenosis with emergence of glycogen poor tumors) is similar to the progression of preneoplastic foci to tumors induced from a variety of agents and conditions associated with increased cancer risk.

Furthermore, Caldwell and Keshava (2006) noted that Bull et al. (2002) reported expression of insulin receptor (IR) to be elevated in tumors of control mice or mice treated with TCE, TCA, and DCA but not in nontumor areas, suggesting that this effect is not specific to DCA.

There is a body of literature that has focused on the effects of TCE and its metabolites after rats or mice have been exposed to "mutagenic" agents to "initiate" hepatocarcinogenesis and this is discussed in Section E.4.2, below. TCE and its metabolites were reported to affect tumor incidence, multiplicity, and phenotype when given to mice as a co-exposure with a variety of "initiating" agents and with other carcinogens. Pereira and Phelps (1996) reported that MNU alone induced basophilic foci and adenomas. MNU and low concentrations of DCA or TCA in female mice were reported to induce heterogeneous for foci and tumor with a higher concentration of DCA inducing more eosinophilic and a higher concentration of TCA inducing more tumors that were basophilic. Pereira et al. (2001) reported that not only dose, but also gender affected phenotype in mice that had already been exposed to MNU and were then exposed to DCA. As for other phenotypic markers, Lantendresse and Pereira (1997) reported that exposure to MNU and TCA or DCA induced tumors that had some commonalities and were heterogeneous, but for female mice, were overall different between DCA and TCA as co-exposures with MNU.

Stop experiments, which attempt to ascertain whether progression differences exist between TCA and DCA, have used higher concentrations at much lower durations of exposure. A question arises as to whether the differences in results occurred because animals in which treatment was suspended were not allowed to have full expression of response rather than "progression" as well as the effects of using large doses.

After 37 weeks of treatment and then a cessation of exposure for 15 weeks, Bull et al. (1990) reported that liver weight and percent liver/body weight still was statistically significantly elevated after DCA or TCA treatment. The authors partially attribute the remaining increases in liver weight to the continued presence of hyperplastic nodules in the liver. In terms of liver tumor induction, the authors stated that "statistical analysis of tumor incidence employed a general linear model ANOVA with contrasts for linearity and deviations from linearity to

determine if results from groups in which treatments were discontinued after 37 weeks were lower than would have been predicted by the total dose consumed."

The multiplicity of tumors observed in male mice exposed to DCA or TCA at 37 weeks and then sacrificed at 52 weeks were reported by the authors to have a response in animals that received DCA very close to that which would be predicted from the total dose consumed by these animals. The response to TCA was reported by the authors to deviate significantly ($p = 0.022$) from the linear model predicted by the total dose consumed. Multiplicity of lesions per mouse and not incidence was used as the measure. Most importantly, the data used to predict the dose response for "lesions" used a different methodology at 52 weeks than those at 37 weeks. Not only were not all animal's lesions examined, but foci, adenomas, and carcinomas were combined into one measure. Therefore, foci, of which a certain percentage have been commonly shown to spontaneously regress with time, were included in the calculation of total "lesions."

Pereira and Phelps (1996) note that in MNU-treated mice that were then treated with DCA, the yield of altered hepatocytes decreases as the tumor yields increase between 31 and 51 weeks of exposure, suggesting progression of foci to adenomas. Initiated and noninitiated control mice were reported to also have fewer foci/mouse with time. Because of differences in methodology and the lack of discernment between foci, adenomas, and carcinomas for many of the mice exposed for 52 weeks, it is difficult to compare differences in composition of the "lesions" after cessation of exposure in the Bull et al. (1990) study.

For TCA treatment, the number of animals examined for determination of which "lesions" were foci, adenomas, and carcinomas was 11/19 mice with "lesions" at 52 weeks, while all 4 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. For DCA treatment, the number of animals examined was only 10/23 mice with "lesions" at 52 weeks, while all 7 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. Most importantly, when lesions were examined microscopically, they did not all turn out to be preneoplastic or neoplastic. Two lesions appeared "to be histologically normal" and one necrotic. Not only were a smaller number of animals examined for the cessation exposure than continuous exposure, but only the 2 g/L exposure levels of DCA and TCA were studied for cessation. The number of animals bearing "lesions" after 37 weeks and then 15 cessation weeks was 7/11 (64%), while the number of animals bearing lesions at 52 weeks was 23/24 (96%) after 2 g/L DCA exposure. For TCA, the number of animals bearing lesions at 37 weeks and then 15 weeks cessation was 4/11 (35%), while the number of animals bearing lesions at 52 weeks was 19/24 (80%). While suggesting that cessation of exposure diminished the number of "lesions," conclusions regarding the identity and progression of those lesion with continuous vs. noncontinuous DCA and TCA treatment are tenuous.

E.2.5. Studies of CH

Given that total oxidative metabolism appears to be highly correlated with TCE-induced increases in liver weight in the mouse rather than merely the presence of TCA, other metabolites are of interest as potential agents mediating the effects observed for TCE. Recently, Caldwell and Keshava (2006) provided a synopsis of the results of more recent studies involving CH. A large fraction of TCE oxidative metabolism appears to go through CH, with subsequent metabolism to TCA and TCOH (Chiu et al., 2006b). Merdink et al. (2008) demonstrated that CH administered to humans can be extremely variable and complex in its pharmacokinetic behavior with a peak plasma concentration of CH in plasma 40–50 times higher than observed at the same time interval for other subjects. Studies of CH toxicity in rodents are consistent, with the general presumption that oxidative metabolites are important for TCE-induced liver tumors, but whether CH and its metabolites are sufficient to explain all of the TCE liver tumorigenesis remains unclear, particularly because of uncertainties regarding how DCA may be formed (Chiu et al., 2006b). Studies of CH may enable a comparison between toxicity of TCE and CH and may help elucidate its role in TCE effects. As with other TCE metabolites, the majority of the studies have focused on the mouse liver tumor response. For rats, while the limited data suggest that there is less of a response than mice to CH, those studies are limited in power or reporting.

Daniel et al. (1992) exposed adult male B6C3F_1 (C57B1/6jC male mice bred to C3Heb/Fej female mice) 28-day-old mice to CH, 2-chloroacetaldehyde, or DCA in two different phases (I and II) with initial weights ranging from 9.4 to 13.6 g. The test compounds were buffered and administered in drinking water for 30 and 60 weeks (n = 5 for interim sacrifice), and for 104 weeks (n = 40). The concentration of CH was 1 g/L and the concentration of DCA was 0.5 g/L; the estimated doses of DCA were 85, 93, and 166 mg/kg-day for the DCA group I, DCA group II, and CH exposed group, respectively. Microscopic examination of tissues was conducted for all tissues for five animals of the CH groups with liver, kidneys, testes, and spleen, in addition to all gross lesions, reported to be examined microscopically in all of the 104-week survivors.

The initial body weight for drinking water controls was reported to be 12.99 ± 3.04 g for group I (n = 23) and 10.48 ± 1.70 for group II (n = 10). For DCA-treated animals, initial body weights were 13.44 ± 2.57 g for group I (n = 23) and 9.65 ± 2.72 g for group II (n = 10). For the CH-treated group, the initial body weights were reported to be 10.42 ± 2.49 g (n = 40). It is not clear from the report what control group best matched, if any, the CH group. Thus, the mean initial body weights of the groups as well as the number of animals varied considerably in each group (i.e., ~40% difference in mean body weights at the beginning of the study).

The number of animals surviving until the termination of the experiment was 10, 10, 16, 8, and 24 for the control group I, control group II, DCA group I, DCA group II, and CH groups, respectively. An increase in absolute and relative liver weight was reported to be observed at 30 weeks for DCA and CH groups and at 60 weeks for CH but data were not shown in the study.

At 104 weeks, the data for the surviving control groups were combined as was the data for the two DCA treatment groups. Of note was that for CH treated survivors (n = 24), water consumption was significantly reduced in comparison to controls. Absolute liver weight was reported to be 2.09 ± 0.6, 3.17 ± 1.3, and 2.87 ± 1.1 g for control, DCA, and CH treatment groups, respectively. The % liver to body weight was reported to be similarly elevated (1.57-fold of control for DCA and 1.41-fold of control for CH) at 104 weeks.

At 104 weeks, the treatment-related liver lesions in histological sections were reported to be most prominently hepatocytomegaly and vacuolization in DCA-treated animals. Cytomegaly was also reported to be in 5, 92, and 79% of control, DCA, and CH treatment groups, respectively. Cytomegaly in CH-treated mice was described as minimal and associated with an increased number of basophilic granules (rough endoplasmic reticulum). Hepatocellular necrosis and chronic active inflammation were reported to be mildly increased in both prevalence and severity in all treated groups. The histological findings, from interim sacrifices (n = 5), were considered by the authors to be unremarkable and were not reported.

Liver tumors were increased by DCA and CH treatment. The percent incidence of liver carcinomas and adenomas combined in the surviving animals was 15, 75, and 71% in control, DCA, and CH treated mice, respectively. In the CH-treated group, the incidence of HCC was 46%. The number of tumors/animals was also significantly increased with CH treatment. Most importantly, morphologically, the authors noted that there did not appear to be any discernable differences in the visual appearance of the DCA- and CH-induced tumors.

George et al. (2000) exposed male B6C3F$_1$ mice and male F344/N rats to CH in drinking water for 2 years (up to 162.6 mg/kg-day). Target drinking water concentrations were 0, 0.05, 0.5, and 2 g/L CH in rats and 0, 0.05, 0.5, and 1.0 g/L CH in mice. Groups of animals (n = 6/group) were sacrificed at 13 (rats only), 26, 52, and 78 weeks following the initiation of dosing with terminal sacrifices at week 104. A complete pathological examination was performed on five rats and mice from the high-dose group, with examination primarily of gross lesions except for liver, kidney, spleen, and testes. BrdU incorporation was measured in the interim sacrifice groups in rats and mice with PCO examined at 26 weeks in mice. In rats, the number of animals surviving >78 weeks and examined for hepatocellular proliferative lesions was 42, 44, 44, and 42 for the control, 7.4, 37.4, and 163.6 mg/kg-day CH treatment groups, respectively. Only 32, 36, 35, and 32 animals were examined at the final sacrifice time.

Only the lowest treatment group had increased liver tumors, which were marginally significantly increased by treatment. The percent of animals with hepatocellular adenomas and carcinomas was reported to be 2.4, 14.3, 2.3 and 6.8% in male rats. In mice, preneoplastic foci and adenomas were reported to be increased in the livers of all CH treatment groups (13.5–146.6 mg/kg-day) at 104 weeks. The incidences of adenomas were reported to be statistically increased at all dose levels, the incidences of carcinomas significantly increased at the highest dose, and time-to-tumor decreased in all CH-treatment groups. The percent incidence of

hepatocellular adenomas was reported to be 21.4, 43.5, 51.3, and 50% in control, 13.5, 65.0, and 146.6 mg/kg-day treatment groups, respectively. The percent incidence of HCCs was reported to be 54.8, 54.3, 59.0, and 84.4% in these same groups. The resulting percent incidence of hepatocellular adenomas and carcinomas was reported to be 64.3, 78.3, 79.5, and 90.6%.

The number of mice surviving >78 weeks was reported to be 42, 46, 39, and 32 and the number surviving to final sacrifice was 34, 42, 31, and 25 for control, 13.5, 65.0, and 146.56 mg/kg-day, respectively. CH exposure was reported to not alter serum chemistry, hepatocyte proliferation (i.e., DNA synthesis), or hepatic PCO activity (an enzyme associated with PPARα agonism) in rats and mice at any of the time periods monitored (all interim sacrifice periods for BrdU incorporation, 52 or 78 weeks for serum enzymes, and 26 weeks for PCO) with the exception of 0.58 g/L CH at 26 weeks slightly increasing hepatocyte labeling (~2–3-fold increase over controls) in rats and mice, but the percent labeling still represented ≤3% of hepatocytes.

With regard to other carcinogenic endpoints, only five animals were examined at the high dose, thereby limiting the study's power to determine an effect. Control mice were reported to have a high spontaneous carcinoma rate (54%), thereby limiting the ability to detect a treatment-related response. No descriptions of the foci or tumor phenotype were given. However, of note is the lack of induction of PCO response with CH at 26 weeks of administration in either rats or mice.

Leakey et al. (2003b) studied the effects of CH exposure (0, 25, 50, and 100 mg/kg, 5 days/week for 104–105 weeks via gavage) in male B6C3F_1 mice with dietary control used to manipulate body growth (n = 48 for 2-year study and n = 12 for the 15-month interim study). Dietary control was reported to decrease background liver tumor rates (incidence of 15–20%) and was reported to be associated with decreased variation in liver-to-body weight ratios, thereby potentially increasing assay sensitivity. In dietary-controlled groups and groups fed ad libitum, liver adenomas and carcinomas (combined) were reported to be increased with CH treatment. With dietary restriction, there was a more discernable CH tumor-response with overall tumor incidence reduced, and time-to-tumor increased by dietary control in comparison to ad-libitum-fed mice. Incidences of hepatocellular adenoma and carcinoma overall rates were reported to be 33, 52, 49, and 46% for control, 25, 50, and 100 mg/kg ad-libitum-fed mice, respectively. For dietary-controlled mice, the incidence rates were reported to be 22.9, 22.9, 29.2, and 37.5% for controls, 25, 50, and 100 mg/kg CH, respectively. Body weights were matched and carefully controlled in this study.

After 2 years of CH treatment, the heart weights of ad-libitum-fed male mice administered 100 mg/kg CH were reported to be significantly less and kidney weights of the 50 and 100 mg/kg were less than vehicle controls. No other significant organ weight changes due to CH treatment were reported to be observed in either diet group except for liver. The liver weights of CH treated groups for by dietary groups were reported to be increased at 2 years and

the absolute liver weights of dosed groups to be generally increased at 15 months, with percent liver/body weight ratios increased in CH treated dietary-controlled mice at 15 months. There was 1.0-, 0.87-, and 1.08-fold of control percent liver/body weight for ad-libitum-fed mice exposed to 25, 50, and 100 mg/kg CH, respectively. For dietary-controlled mice, there was 1.05-, 1.08-, and 1.11-fold of control percent liver/body weight for the same dose groups at 15 months. Thus, there was no corresponding dose-response for percent liver/body weight in the ad-libitum-fed mice, which were reported to show a much larger variation in liver-to-body-weight ratios (i.e., the SD and SEs were 2–17-fold lower in dietary-controlled groups than for ad-libitum-fed groups).

Liver weight increases at 15 months did not correlate with 2-year tumor incidences with this group. However, for dietary-controlled groups, the increase in percent liver/body weights at 15 months were generally correlated with increases in liver tumors at 2 years.

The incidences of peripheral or focal fatty change were reported to be increased in all CH-treated groups of ad-libitum-fed mice at 15 months (approximately half the animals showed these changes for all dose groups, with no apparent dose-response). Of the enzymes associated with PPARα agonism (total CYP, CYP2B isoform, CYP4A, or lauric acid β-hydroxylase activity), only CYP4A and lauric acid β-hydroxylase activity were significantly increased at 15 months of exposure in the dietary-restricted group administered 100 mg/kg CH, with no other groups reported showing a statistically significant increased response (n = 12/group). Although not statistically significant, the 100 mg/kg CH exposure group of ad-libitum-fed mice also had an increase in CYP4A and lauric acid β-hydroxylase activity.

The authors reported that the increase in magnitude of CYP4A and lauric acid β-hydroxylase activity at 100 mg/kg CH at 15 months in dietary controlled mice correlated with the increase incidence of mice with tumors. However, there was no correlation of tumor incidence and the increased enzyme activity associated with peroxisome proliferation in the ad-libitum-fed mice. No descriptions of liver pathology were given other than incidence of mice with fatty liver changes. Hepatic malondialdehyde concentration in ad-libitum-fed and dietary controlled mice did not change with CH exposure at 15 months, but the dietary-controlled groups were all approximately half that of the ad-libitum-fed mice. Thus, while overall increased tumors observed in the ad libitum diet correlated with increased malondialdehyde concentration, there was no association between CH dose and malondialdehyde induction for either diet.

Induction of peroxisome-associated enzyme activities was also reported for shorter times of CH exposure. Seng et al. (2003) described CH toxicokinetics in mice at doses up to 1,000 mg/kg-day for 2 weeks with dietary control and caloric restriction slightly reducing acute toxicity. Lauric acid β-hydroxylase and PCO activities were reported to be induced only at doses >100 mg/kg in all groups, with dietary-restricted mice showing the greatest induction. Differences in serum levels of TCA, the major metabolite remaining 24 hours after dosing, were reported not to correlate with hepatic lauric acid β-hydroxylase activities across groups.

Leuschner and Beuscher (1998) examined the carcinogenic effects of CH in male and female Sprague-Dawley rats (69–79 g, 25–29 days old at initiation of the experiment) administered 0, 15, 45, and 135 mg/kg CH in unbuffered drinking water 7 days/week (n = 50/group) for 124 weeks in males and 128 weeks in females. Two control groups were noted in the methods section without explanation as to why they were conducted as two groups.

The mean survival for males was similar in treated and control groups, with 20, 24, 20, 24, and 20% of Control I, Control II, 15, 45, and 135 mg/kg CH-treated groups, respectively, surviving until the end of the study. For female rats, the percent survival was 12, 30, 24, 28, and 16% for of Control I, Control II, 15, 45, and 135 mg/kg CH-treated groups, respectively. The authors reported no substance-related influence on organ weights and no macroscopic evidence of tumors or lesions in male or female rats treated with CH for 124 or 128 weeks. However, no data were presented on the incidence of tumors using this paradigm, especially background rates.

The authors reported a statistically significant increase in the incidence of hepatocellular hypertrophy in male rats at the 135 mg/kg dose (14/50 animals vs. 4/50 and 7/50 in Controls I and II). For female rats, the incidence of hepatocellular hypertrophy was reported to be 10/50 rats (Control I) and 16/50 (Control II) rats with 18/50, 13/50, and 12/50 female rats having hepatocellular hypertrophy after 15, 45, and 135 mg/kg CH, respectively. The lack or reporting in regard to final body weights, histology, and especially background and treatment group data for tumor incidences, limit the interpretation of this study. Whether this paradigm was sensitive for induction of liver cancer cannot be determined.

From the CH studies in mice, there is an apparent increase in liver adenomas and carcinomas induced by CH treatment by either drinking water or gavage with all available studies performed in male $B6C3F_1$ mice. However, the background levels of hepatocellular adenomas and carcinomas in the mice in George et al. (2000) and body weight data from this study show that it is from a tumor-prone mouse model.

Comparisons with concurrent studies of mice exposed to DCA revealed that while both CH and DCA induced hepatomegaly and cytomegaly, DCA-induced cytomegaly was accompanied by vacuolization, while that of CH was associated with increased number of basophilic granules (rough endoplasmic reticulum), which would suggest separate effects. However, the morphology of the CH-induced tumors was reported to be similar between DCA- and CH-induced tumors (Daniel et al., 1992).

Using a similar paradigm (2-year study of $B6C3F_1$ male mice), DeAngelo et al. (1999) and Carter et al. (2003) described DCA-induced tumors to be heterogeneous. This is the same description given for TCE-induced tumors in the studies by NTP, NCI, and Maltoni et al. and to be a common description for tumors caused by a variety of carcinogenic agents. Similar to the studies cited above for CH, DeAngelo et al. (1999) reported that PCO levels were only elevated at 26 weeks at 3.5 g/L DCA and had returned to control levels by 52 weeks. Similar to CH, no

increased tritiated thymidine was reported for DCA at 26 and 52 weeks, with only twofold of control values reported at 0.05 g/L at 4 weeks.

Leakey et al. (2003b) reported that ad-libitum-fed male mice exhibited a similar degree of increase in the incidence of peripheral or focal fatty change at 15 months for all CH doses; however, enzymes associated with peroxisome proliferation were not similarly altered at all CH doses. While dietary restriction seemed to have decreased background levels of tumors and increased time-to-tumor, CH-gave a clear dose-response in dietary restricted animals. However, while the overall level of tumor induction was reduced, there was a greater induction of PPARα enzymes by CH. Induction of liver tumors by CH observed in ad-libitum-fed mice were not correlated with PPARα induction, with dietary restriction alone appearing to have greater levels of lauric acid ω-hydrolase activity in control mice at 15 months. Seng et al. (2003) report that lauric acid β-hydroxylase and PCO were induced only at exposure levels >100 mg/kg CH, again with dietary restricted groups showing the greatest induction. Such data argues against the role of peroxisome proliferation in CH-liver tumor induction in mice.

E.2.6. Serum Bile Acid Assays

Serum bile acids (SBA) have been suggested as a sensitive indicator of hepatotoxicity to a variety of halogenated solvents with an advantage of increased sensitivity and specificity over conventional liver enzyme tests that primarily reflect the acute perturbation of hepatocyte membrane integrity and "cell leakage" rather than liver functional capacity (i.e., uptake, metabolism, storage, and excretion functions of the liver) (Neghab et al., 1997; Bai et al., 1992b). While some studies have reported negative results, a number of studies have reported elevated SBA in organic solvent-exposed workers in the absence of any alterations in normal liver function tests. These variations in results have been suggested to arise from failure of some methods to detect some of the more significantly elevated SBA and the short-lived and reversible nature of the effect (Neghab et al., 1997).

Neghab et al. (1997) have reported that occupational exposure to 1,1,2-trichloro-1,2,2-trifluoroethane and TCE has resulted in elevated SBA and that several studies have reported elevated SBA in experimental animals to chlorinated solvents such as carbon tetrachloride, chloroform, hexachlorobutadiene, tetrachloroethylene, 1,1,1-trichloroethane, and TCE at levels that do not induce hepatotoxicity (Hamdan and Stacey, 1993; Bai et al., 1992b; Wang and Stacey, 1990). Toluene, a nonhalogenated solvent, has also been reported to increase SBA in the absence of changes in other hepatobiliary functions (Neghab and Stacey, 1997). Thus, disturbance in SBA appears to be a generalized effect of exposure to chlorinated solvents and nonchlorinated solvents and not specific to TCE exposure.

Neghab et al. (1997) reported that 8-hour TWA exposures to TCE of 8.9 ppm, measured in the breathing zone using a charcoal tube personal sampler for the whole mean duration of exposure of 3.4 years, do not result in significant changes in albumin, bilirubin, ALP, ALT,

5'-nucleosidase, γ-glutamyltransferase, but do have significantly increased total serum bile acids. Not only were total bile acids significantly increased in these TCE-exposed workers compared to controls (approximately twofold of control), but specifically, deoxycholic acid and subtotal of free bile acids were increased. Neghab et al. (1997) did not show the data, but also reported that "despite the apparent overall low level of exposure, there was a very good correlations (r = 0.94) between the degree of increase in serum concentration of total bile acids and level of TCE." Neghab et al. (1997) noted that while a sensitive indicator or exposure to such solvents in asymptomatic workers, there is no indication that actual liver injury occurs in conjunction with SAB increases.

Wang and Stacey (1990) administered TCE in corn oil via i.p. injection to male Sprague-Dawley rats (300–500 g) at concentrations of 0.01, 0.1, 1, 5, and 10 mmol/kg on 3 consecutive days (n = 4, 5, or 6) with liver enzymes and SBA examined 4 hours after the last TCE treatment. At these doses, there were no differences between treated and control animals in regard to ALP and SDH concentrations, and an elevation of ALT was noted only at the highest dose. However, there was generally a reported dose-related increase in cholic acid, chenodeoxycholic acid, deoxycholic acid, taurocholic acid, and tauroursodeoxycholic acid, with cholic acid and taurochlolic acid increased at the lowest dose. The authors reported that "examination of liver sections under light microscopy yielded no consistent effects that could be ascribed to trichloroethylene."

In the same study, rats were also exposed to TCE via inhalation (n = 4) at 200 ppm for 28 days, and 1,000 ppm for 6 hours/day. Using this paradigm, cholic acid and taurocholic acid were significantly elevated at the 200 ppm level, (~10- and ~5-fold of control, respectively) with very large SEs. At the 1,000 ppm level (6 hours/day), cholic acid and taurocholic acid were elevated to approximately twofold of control but neither was statistically significant. The large variability in responses between rats and the low number of rats tested in this paradigm limit its ability to determine quantitative differences between groups. Nevertheless, without the complications associated with i.p. exposure (see Section E.2.2.1), inhalation exposure of TCE at a relative low exposure level was also associated with increased SBA levels. The authors stated that "no increases in alanine amino transferase levels were observed in the rats exposed to trichloroethylene via inhalation." No histopathology results were reported for rats exposed via inhalation.

As stated by Wang and Stacey (1990), "intraperitoneal injection is not particularly relevant to humans," which was the rationale given for the inhalation exposure experiments in the study. They point out that intestinal interactions require consideration because a major determinant of SBA is that their absorption from the gut and intestinal flora may play a role in bile acid metabolism. They also noted that grooming done by the experimental rats would probably result in low exposure via ingestion of TCE as well. However, Wang and Stacey (1990) reported consistent results in terms of TCE-induced changes in SBA at relatively low

concentrations by either inhalation or i.p. routes of exposure that were not associated with other measures of toxicity.

Hamdan and Stacey (1993) administered TCE in corn oil (1 mmol/kg) in male Sprague-Dawley rats (300–400 g) and followed the time-course of SBA elevation, TCE concentration, and TCOH in the blood at 2, 4, 8, and 16 hours after dosing (n = 4, 5, or 6 per group). Liver and blood concentration of TCE were reported to peak at 4 hours, while those of TCOH peaked at 8 hours after dosing. TCE levels were not detectable by 16 hours in either blood or liver, while those of TCOH were still elevated. Elevations of SBA were reported to parallel those of TCE, with cholic acid and taurochloate acid reported to show the highest levels of bile acids. The dose given was based on that reported by Wang and Stacey (1990) to give no hepatotoxicity but an increase in SBA. The authors stated that liver injury parameters were checked and found unaffected by TCE exposure but do not show the data. Thus, it was TCE concentration and not that of its metabolite that was most closely related to changes in SBA and after a single exposure, the effect was reversible.

In an in vitro study by Bai and Stacey (1993), TCE was studied in isolated rat hepatocytes with TCE reported to cause a dose-related suppression of initial rates of cholic acid and taurocholic acid but with no significant effects on enzyme leakage or intracellular calcium contents, further supporting a role for the parent compound in this effect. The authors noted that the changes in SBA result from interference with a physiological process rather "than an event associated with significant pathological consequences."

E.3. STATE OF SCIENCE OF LIVER CANCER MODES OF ACTION

The experimental evidence in mice shows that TCE and its metabolites induce foci, hepatocellular adenomas, and carcinomas that are heterogeneous in nature as indicated by phenotypic differences in tincture, mutational markers, or gene expression markers. The tumors induced by TCE are reflective of phenotypes that are either similar to those induced by mixtures of DCA and TCA exposure, or more like those induced by DCA. These tumors have been described to be similar also to those arising spontaneously in mice or from chemically induced hepatocarcinogenesis and to arise from preneoplastic foci, and in the case of DCA, single dysplastic hepatocytes as well as foci. HCC observed in humans also has been described to be heterogeneous and to be associated with formation of preneoplastic nodules. Although several conditions have been associated with increased risk of liver cancer in humans, the mechanism of HCC is unknown at this time. A great deal of attention has been focused on predicting which cellular targets (e.g., "stem-cell" or mature hepatocyte) are associated with HCC as well as on phenotypic markers in HCC that can provide insight not only into mode of action and origin of tumor, but also for prediction of clinical course. Examination of pathways and epigenetic changes associated with cancer and the relationship of these changes to liver cancer are also discussed below.

The field of cancer research has been transformed by the recent discoveries of epigenetic changes and their role in cancer and chronic disease states. The following discussion describes not only these advances, but also the issues involved with the technologies that have emerged to describe them (see Section E.3.1.2). Exposure to TCE and its metabolites, like many others, induces a heterogeneous response, even in a relatively homogeneous genetic paradigm as the experimental laboratory rodent model. The importance of phenotypic anchoring is a major issue in the study of any modes of action using these new technologies of gene expression pattern. Although a large amount of information is now available using microarray technologies and transgenic mouse models, specifically for TCE and in study of suggested modes of action for TCE and its metabolites, use of these approaches has limitations that need to be considered in the interpretation of data and conclusions derived from such data, especially quantitative conclusions.

For TCE and its metabolites, the extent of acute to subchronic induction of hepatomegaly correlated with hepatocellular carcinogenicity, although each had differing factors contributing to that hepatomegaly from periportal glycogen deposition to hepatocellular hypertrophy and increased polyploidy. The extent of transient DNA synthesis, peroxisome proliferation, or cytotoxicity was not correlated with carcinogenicity. Hepatomegaly is also a predictor of carcinogenicity for a number of other compounds in mice and rats. Allen et al. (2004) examined the NTP database (87 compounds for rat and 83 for mice) and tried to correlate specific hepatocellular pathology in prechronic studies with carcinogenic endpoints in the chronic 2-year assays. The best single predictor of liver cancer in mice was hepatocellular hypertrophy. Hepatocellular cytomegaly and hepatocyte necrosis also contributed, although the numbers of positive findings were less than hypertrophy.

With regard to genotoxicity studies, there was no evidence of a correlation between mouse liver tumor chemicals and *Salmonella* or micronucleus assay outcome. None of the prechronic liver lesions examined were correlated with either *Salmonella* or Micronucleus assays. In rats, no single prechronic liver lesions (when considered individually) was a strong predictor of liver cancer in rats. The most predictive lesions was hepatocellular hypertrophy. There was not a significant correlation between liver tumors/toxicity and the two mutagenicity measures.

Although the lack of correlation with the mutagenicity assays could be interpreted as rodent assays predominantly identifying nongenotoxic liver carcinogens, this conclusion could be questioned because it is solely dependent on *Salmonella* mutagenicity and additional genotoxic endpoints could conceivably shift the association between liver cancer and genotoxicity towards a more positive correlation. As to questions of the usefulness of the mouse bioassay, the two mutagenicity assays did not correlate with rat results either and an important indicator for carcinogenicity would be lost.

Examination of tumor phenotype from TCE, DCA, and TCA exposures in mice shows a large heterogeneity, which is also consistent with the heterogeneity observed in human HCC (see Section E.3.1.8). The heterogeneity of tumor phenotype has been correlated with survival outcome and tumor aggressiveness in humans and in transgenic mouse models that share some of the same perturbations in gene pathway expression (see Sections E.3.1.8 and E.3.2.1, below). An examination of common pathway disturbances that may be common to all cancers and those of liver tumors shows that there are pathways in common, but that there is greater heterogeneity in disturbance of hepatic pathways in cancer that may make is useful as a marker of disturbances indicative of different targets of carcinogenicity depending on the cellular context and target. Thus, although primate and human liver may not be as susceptible to HCC as the rodent liver, the pathways leading to HCC in rodents and humans appear to be similar and heterogeneous, with some indicative of other susceptible cellular targets for neoplasia in a differing context.

E.3.1. State of Science for Cancer and Specifically Human Liver Cancer

E.3.1.1. Epigenetics and Disease States (Transgenerational Effects, Effects of Aging, and Background Changes)

Wood et al. (2007) published their work on "genomic landscapes" of human breast and colorectal cancers that significantly forwards the understanding of "key events" involved with induction of cancer. They state that there are ~80 DNA mutations that alter amino acid in a typical cancer, but that examination of the overall distribution of these mutations in different cancers of the same type leads to a new view of cancer genome landscapes: they are composed of a handful of commonly mutated genes "mountains" but are dominated by a much larger number of infrequently mutated gene "hills."

> Statistical analyses suggested that most of the ~ 80 mutation in an individual tumor were harmless and that <15 were likely to be responsible for driving the initiation, progression, or maintenance of the tumor…Historically the focus of cancer research has been on the gene mountains, in part because they were the only alterations that could be identified with available technologies. However, our data show that vast majority of mutations in cancers do not occur in such mountains. This new view of cancer is consistent with the idea that a large number of mutations, each associated with a small fitness advantage, drive tumor progression. It is the "hills" and not the "mountains" that dominate the cancer genomic landscape.

The large number of "hills" actually reflects alterations in a much smaller number of cell signaling pathways. Indeed, pathways rather than individual genes appear to govern the course of tumorigenesis.

> It is becoming increasingly clear that pathways rather than individual genes govern the course of tumorigenesis. Mutations in any of several genes of a single pathway can thereby cause equivalent increases in net cell proliferation.…This new view of cancer is consistent with the idea that a large number of mutations, each associated with a small fitness advantage, drive tumor progression.

Thus, when pathways are altered, the same phenotype can arise from alterations in any of several genes.

Consistent with the arguments put forth by Wood et al. (2007) for mutations in cancer is the additional insight into pathway alterations by epigenomic mechanisms, which can act similarly as mutation. Weidman et al. (2007) report that:

> cell phenotype is not only dependent on its genotype but also on its unique epigenotype, which is shaped by developmental history and environmental exposures. The human and mouse genome projects identified approximately 15,500 and 29,000 CpG islands, respectively. Hypermethylation of CpG-rich regions of gene promoters inhibit expression by blocking the initiation of transcription. DNA methylation is also involved in the allelic inactivation of imprinted genes, the silencing of genes on the inactive X chromosome, and the reduction of expression of transposable elements. Because epigenomic modifications are copied after DNA synthesis by DNMT1, they are inherited during somatic cell replication…Inherited and spontaneous or environmentally induced epigenetic alterations are increasingly being recognized as early molecular events in cancer formation. Furthermore, such epigenetic alterations are potentially more adverse than nucleotide mutations because their effects on regional chromatin structure can spread, thereby affecting multiple genetic loci. Although tumor suppressor gene silencing by DNA methylation occurs frequently in cancer, genome-wide hypomethylation is one of the earliest events to occur in the genesis of cancer. Demethylation of the genome can lead to the reactivation of transposable elements, thereby altering the transcription of adjacent genes, the activation of oncogenes such as H-Ras, and biallelic expression of imprinted loci (e.g., loss of IGF2 imprinting).

Thus, epigenetic modification may be worse than mutation in terms of cancer induction.

Dolinoy et al. (2007) report on the role of environmental exposures on the epigenome, especially during critical periods of development and their role in adult disease susceptibility. They report that:

> aberrant epigenetic gene regulation has been proposed as a mechanism of action for nongenotoxic carcinogenesis, imprinting disorders, and complex disorders including Alzheimer's disease, schizophrenia, asthma, and autism. Epigenetic modifications are inherited not only during mitosis but also can be transmitted transgenerationally (Anway et al., 2005; Rakyan et al., 2003; Rakyan et al., 2002)). The influence on environmental factors on epigenetic gene regulation may also persist transgenerationally despite lack of continued exposure in second,

> third, and fourth generations (Anway et al., 2005). Therefore if the genome is compared to the hardware in a computer, the epigenome is the software that directs the computer's operation…The epigenome is particularly susceptible to deregulation during gestation, neonatal development, puberty and old age. Nevertheless, it is most vulnerable to environmental factors during embryogenesis because DNA synthetic rate is high, and the elaborate DNA methylation pattern and chromatin structure required for normal tissue development is established during early development…83 imprinted genes have been identified in mice and humans with 29 or about one third being imprinted in both species. Since imprinted genes are functionally haploid, they are denied the protection from recessive mutations that diploidy would normally afford. Imprinted genes that have been linked to carcinogenesis include IGF2 (bladder, lung, ovarian and others), IGF2R (breast, colon, lung, and others), and Neuronatin (pediatric leukemia).

Bjornsson et al. (2008) recently reported that not only were there time-dependent changes in global DNA methylation within the same individuals in two separate populations in widely separated geographic locations, but also these changes showed familial clustering in both increased and decreased methylation. These results were suggested not only to support the relationship of age-related loss of normal epigenetic patterns as a mechanism for late onset of common human diseases, but also that losses and gains of DNA methylation observed over time in different individuals could contribute to disease with the example provided of cancer, which is associated with both hypomethylation and hypermethylation through activation of oncogenes and silencing of tumor suppressor genes. The study also showed considerable interindividual age variation, with differences accruing over time within individuals that would be missed by studies that employed group averaging.

The review by Reamone-Buettner and Borlak (2007) provide insight into the role of noncoding RNAs in diseases such as cancer. They report that:

> a large number of noncoding RNAs (ncRNAs) play important role in regulating gene expressions, and advances in the identification and function of eukaryotic ncRNAs, e.g., microRNAs and their function in chromatin organization, gene expression, disease etiology have been recently reviewed. The regulatory pathways mediated by small RNAs are usually collectively referred to as RNA interference (RNAi) or RNA-mediated silencing. RNAi can be triggered by small double-stranded RNA (dsRNA) either introduced exogenously into cells as small interfering siRNAs or that have been produced endogenously from small non-coding RNAs known as microRNAs (miRNAs). The dsRNAs are characteristically cleaved by the ribonuclease III-enzyme Dicer into 21- to 23 nt duplexes and the resulting fragments base-pair with complementary mRNA to target cleavage or to repress translation…Two mechanisms exist of miRNA-mediated gene regulation, degradation of the target mRNA, and translational repression. Whether one or the other of these mechanisms is used depends on the degree of the complementary between the miRNA and target mRNA. For a near perfect match, the Argonaute protein in the RNA-induced silencing complex

(RISC) cleaves the mRNA target, which is destined for subsequent degradation by ribonucleases. In the situation of a less degree of complimentarity, commonly occurring in humans, the translational repression mechanism is used to control gene expression. However, the exact mechanism for translational inhibition is unclear.

The varying degrees in complimentarity would help explain the large number of genes that could be affected by miRNA and pleiotropic response.

The review by Feinberg et al. (2006) specifically addresses the epigenetic progenitor origin of human cancer. They conclude that epigenetic alterations are ubiquitous and serve as surrogate alterations for genetic change (oncogene activation, tumor-suppressor-gene silencing), by mimicking the effect of genetic change. They report that:

Advances in characterizing epigenetic alterations in cancer include global alterations, such as hypomethylation of DNA and hypoacetylation of chromatin, as well as gene-specific hypomethylation and hypermethylation. Global DNA hypomethylation leads to chromosomal instability and increased tumour frequency, which has been shown *in vitro* and *in vivo* in mouse models, as well as gene-specific oncogene activation, such as R-ras in gastric cancer, and cyclin D2 and maspin in pancreatic cancer. In addition, the silencing of tumour-suppressor genes is associated with promoter DNA hypermethylation and chromatin hypoacetylation, which affect divergent genes such as retinoblastoma 1 (RB1), p16 (also known as cyclin-dependent kinase inhibitor 2A (CDKN2A), von Hippel-Lindau tumor suppressor (VHL), and MutL protein homologue (MLH1).

Genetic mechanisms are not the only path to gene disruption in cancer. Pathological epigenetic changes - non-sequence-based alteration that are inherited through cell division - are increasingly being considered as alternatives to mutations and chromosomal alterations in disrupting gene function. These include global DNA hypomethylation, hypermethylation and hypomethylation of specific genes, chromatin alterations and loss of imprinting. All of these can lead to aberrant activation of growth-promoting genes and aberrant silencing of tumour-suppressor genes.

Most CG dinucleotides are methylated on cytosine residues in vertebrate genomes. CG methylation is heritable, because after DNA replication the DNA methyltransferase 1, DNMT1, methylates unmethylated CG on the base-paired strand. CG dinucleotides within promoters within promoters tend to be protected from methylation. Although individual genes vary in hypomethylation, all tumours have shown global reduction of DNA methylation. This is a striking feature of neoplasia.

In addition to global hypomethylation, promoters of individual genes show increased DNA methylation levels. Hypermethylation of tumour-suppressor genes can be tumour-type specific. An increasing number of genes are found to be normally methylated at promoters but hypomethylated and activated in the corresponding tumours. These include R-RAs in gastric cancer, melanoma

antigen family A, 1(MAGE1) in melanoma, maspin in gastric cancer, S100A4 in colon cancer, and various genes in pancreatic cancer.

Our genetic material is complexed with proteins in the form of histones in a one-to-one weight ratio. Core histones H2A, H2B, H3 and H4 form nucleosome particles that package 147 bp of DNA, and the linker histone H1 packages more DNA between core particles, forming chromatin. It is chromatin and not just DNA, that is the substrate for all processes that affect genes and chromosomes. In recent years, it has become increasingly evident that chromatin, like DNA methylation, can impart memory to genetic activity. There are dozens of post-translational histone modifications. Studies in many model systems have shown that particular histone modifications are enriched at sites of active chromatin (histone H3 and H4 hyperacetylation, lysing at 4 and H3 (H3-K4) dimethylation and trimethylation, and H3-K79 methylation) and others are enriched at sites of silent chromatin (H3-K9 and H3-K27 methylation). These and other histone modifications survive mitosis and have been implicated in chromatin memory.

Overproduction of key histone methyltransferases that catalyze the methylation of either H3-K4 or H3-K27 residues are frequent events in neoplasia. Global reductions in monoacetylated H4-K16 and trimethylated H4-K20 are general features of cancer cells.

Genomic imprinting is parent-of –origin-specific gene silencing. It results from a germ-line mark that causes reduced or absent expression of a specific allele of a gene in somatic cells of the offspring. Imprinting is a feature of all mammals affecting genes that regulate cell growth, behaviour, signaling, cell cycle and transport; moreover, imprinting is necessary for normal development. Imprinting is important in neoplasia because both gynogenotes (embryos derived only from the maternal genetic complement) and androgenotes (embryos derived only from the paternal genetic complement) form tumours – ovarian teratomas, and hydtidiform moles/ choriocarcinomas, respectively. Loss of imprinting (LOI) refers to activation of the normally silenced allele, or silencing of the normally active allele, of an imprinted gene. LOI of the insulin-like growth factor 2 gene (IGF2) accounts for half of Wilms tumours in children. LOI of IGF2 is also a common epigenetic variant in adults and is associated with a fivefold increased frequency of colorectal neoplasia. LOI of IGF2 might cause cancer by increasing the progenitor cell population in the kidney in Wilm's tumor and in the gastrointestinal tract in colorectal cancer.

Feinberg et al. (2006) propose that epigenetic changes can provide mechanistic unity to understanding cancer, can occur earlier and set the stage for genetic alterations, and have been linked to the pluripotent precursor cells from which cancers arise. "To integrate the idea of these early epigenetic events, we propose that cancer arises in three steps; an epigenetic disruption of progenitor cells, an initiating mutation and genetic and epigenetic plasticity."

The first step involves an epigenetic disruption of progenitor cells in a given organ or system, which leads to a polyclonal precursor population of neoplasia-

> ready cells. These cells represent a main target of environmental, genetic and age-dependent exposure that largely accounts for the long latency period of cancer. Epigenetic disruption might perturb the normal balance between undifferentiated progenitor cells and differentiated committed cells within a given anatomical compartment, either in number or in their capacity for aberrant differentiation, which provides a common mechanism of neoplasia.
>
> All tumours show global changes in DNA methylation, and DNA methylation is clonally inherited through cell division. Because the conventional genetic changes in cancer are also clonal, global hypomethylation would have to occur universally, at the same moment as the mutational changes, which seems unlikely. This suggests that global DNA hypomethylation (and global reductions of specific histone modifications) precedes genetic change in cancer. Similarly, hypermethylation of tumour-suppressor genes has been observed in the normal tissue of patients in which the same gene is hypermethylated in the tumour tissue. Recent data demonstrate LOI of IGF2 throughout the normal colonic epithelium of patients who have LOI-associated colorectal cancer. LOI is associated with increased risk of intestinal cancers in both humans and mice. A specific change in the epithelium is seen in mice that are engineered to have biallelic expression of IGF2 – a shift in the proportion of progenitor to differentiated cells throughout the epithelium; a similar abnormality was observed in humans with LOI of IGF2.
>
> The proposed existence of the epigenetically disrupted progenitors of cancer implies that the earliest stages in neoplastic progression occur even before what a pathologist would recognize as a benign pre-neoplastic lesion. Such alterations are inherently polyclonal. This is in contrast with the widely accepted model of cancer as a monoclonal disorder that arises from an initiating mutation- a model that was proposed and accepted when little was known about epigenetic phenomena in cancer.

Thus, Feinberg et al. (2006) provide a hypothesis for the latency period of cancer and suggest that epigenetic changes predate mutational ones in cancer. Tissues that look phenotypically "normal" may harbor epigenetic changes and predispositions toward neoplasia. In regard to what cells may be targets or epigenetic changes that can be "progenitor cells" in the case of cancer, Feinberg et al. (2006) define such cell having "capacity for self-renewal and pluripotency—over their tendency toward limited replicative potential and differentiation." Within the liver, there are multiple cell types that would fit such a definition, including those who are considered "mature" (see Section E.3.1.4). Feinberg et al. (2006) also note that epigenetic states can be continuously modified to become heterogeneous at all states of the neoplastic process.

> Telomere erosion results in chromosome shortening and uncapped ends that begin to fuse and the resulting dicentric chromosomes break at anaphase. DNA palindromes have recently been found to form at high levels in cancer cells. Like telomere erosion, DNA palindrome formation can lead to genetic instability by

initiating bridge-breakage-fusion cycles. However, it is not known how or exactly when palindromes form, although they appear early in cancer progression. Epigenetic instability can also promote cancer through pleiotropic alterations in the expression of genes that modify chromatin.

Epigenetic changes are reversible but the changes can initiate irreversible genetic changes. Permanent epigenetic changes can have an epigenetic basis. On a background of cancer-associated epigenetic instability, the effects of mutations in oncogenes and tumour –suppressor genes might be exacerbated. Therefore the risk of developing malignancy would be much higher for a given mutations event if it occurred on the background of epigenetic disruption.

The environmental dependence of cancer fits an epigenetic model generally for human disease – the environment might influence disease onset not simply through mutational mechanisms but in epigenetically modifying genes that are targets for either germline or acquired mutation; that is, by allowing genetic variates to be expressed. Little is known about epigenetic predispositions to cancer, but a recent twin study indicates that, similar to cancer risk, global epigenetic changes show striking increase with age.

Environmental insults might affect the expression of tumour-progenitor genes, leading to both genetic and epigenetic alterations. Liver regeneration after tissue injury leads to widespread hypomethylation and hypermethylation of individual genes; both of these epigenetic changes occur in cancer.

In regard to the implications of epigenomic changes and human susceptibility to toxic insult, the review by Szyf (2007) provided additional insights.

The basic supposition in the field has been that the interindividual variations in response to xenobiotic are defined by genetic differences and that the main hazard anticipated at the genomic level from xenobiotic is mutagenesis or physical damage to DNA. In accordance with this basic hypothesis, the main focus of attention in pharmacogenetics has been on identifying polymorphisms in genes encoding drug metabolizing enzymes and receptors. New xenobiotics were traditionally tested for their genotoxic effects. However, it is becoming clear that epigenetic programming plays an equally important role in generating interindividual phenotypic differences, which could affect drug response. Moreover, the emerging notion of the dynamic nature of the epigenome and its responsibility to multiple cellular signaling pathways suggest that it is potentially vulnerable to the effects of xenobiotics not only during critical period in development but also later in life as well. Thus, non-genotoxic agents might affect gene function through epigenetic mechanisms in a stable and long-term fashion with consequences, which might be indistinguishable from the effects of physical damage to the DNA. Epigenetic programming has the potential to persist and even being transgenerationally transmitted (Anway et al., 2005) and this possibility creates a special challenge for toxicological assessment of safety of xenobiotics. Any analysis of interindividual phenotype diversity should therefore take into account epigenetic variations in addition to genetic sequence

> polymorphisms. Whereas, a germ-line polymorphism is a static property of an individual and might be mapped in any tissue at any point in life, epigenetic differences must be examined at different time points and at diverse cell types.

Karpinets and Foy (2005) proposed that epigenetic alterations precede mutations and that succeeding mutations are not random, but in response to specific types of epigenetic changes the environment has encouraged. This mechanism was also suggested as to explain both the delayed effects of toxicant exposure and the bystander effect of radiation on tumor development, which are inconsistent with the accepted mechanism of direct DNA damage.

> In a study of ionizing radiation, non-irradiated cells acquired mutagenesis through direct contact with cells whose nuclei had previously been irradiated with alpha-particles (Zhou et al., 2003). Molecular mechanisms underlying these experimental findings are not known but it is believed that it may be a consequence of bystander interactions involving intercellular signaling and production of cytokines (Lorimore et al., 2003).

Caldwell and Keshava (2006) reported that:

> aberrant DNA methylation has emerged in recent years as a common hallmark of all types of cancers with hypermethylation of the promoter region of specific tumor suppressor genes and DNA repair genes leading to their silencing (an effect similar to their mutation), and genomic hypomethylation (Pereira et al., 2004a; Ballestar and Esteller, 2002; Berger and Daxenbichler, 2002; Rhee et al., 2002; Herman et al., 1998). Whether DNA methylation is a consequence or cause of cancer is a long-standing issue(Ballestar and Esteller, 2002). Fraga et. al. (2005; 2004) report global loss of monoacetylation and trimethylation of histone H4 as common a hallmark of human tumor cells but suggest genomone-wide loss of 5-methylcytosine (associated with the acquisition of a transformed phenotype) does not exist as a static predefined value throughout the process of carcinogenesis but as a dynamic parameter (i.e., decreases are seen early and become more marked in later stages).

E.3.1.2. Emerging Technologies, DNA and siRNA, miRNA Microarrays—Promise and Limitations for Modes of Action

Currently, new approaches are emerging for the study of changes in gene expression and protein production induced by chemical exposure that could be related to their toxicity and serve as an anchor for determining similar patterns between rodent models and human diseases or risks of chemically-induced health impacts. Such approaches have the promise to extend the definitions of “genotoxic” and “nongenotoxic” effects, which with the advent of epigenomic study have become obsolete as they assume that only alteration of the DNA sequence is important in cancer induction and progression. However, not only is phenotypic anchoring an issue in regard to the differing cell types, regions, and lobes of the liver (see Section E.1.2), it is

also an issue for overall variability of response between animals and is critical for interpretation of microarray and other genomic database approaches.

As shown in the discussions of TCE effects in animal models, TCE treatment resulted in a large variability in response between what are supposed to be relatively homogeneous genetically similar animals, and there was an apparent difference in response between studies using the same paradigm. It is important that as varying microarray approaches and analyses of TCE toxicity or of potential modes of action are published, the issue of phenotypic anchoring at the cellular to animal level is addressed. Several studies of TCE microarray results and those of PPARα agonists have been reported in the literature in an attempt to discern modes of action. Issues related to conduct of these experiments and interpretation of their results are listed below.

Perhaps one of the most important studies of this issue has been reported by Baker et al. (2004). The ILSI HESI formed a hepatotoxicity working group to evaluate and compare biological and gene expression responses in rats exposed to well-studied hepatotoxins (Clofibrate and methapyrilene), using standard experimental protocol and to address the following issues: (1) how comparable the biological and gene expression data are from different laboratories running identical in vivo studies; (2) how reproducible the data are generated across laboratories using the same microarray platform; (3) how data compare using different microarray platforms; (4) how data compare using RNA from pooled and individual animals; and (5) whether the gene expression changes demonstrate time- and dose-dependent responses that correlate with known biological markers of toxicity (Baker et al., 2004).

The rat model studied was the male Sprague-Dawley rat (57 or 60–66 days of age) exposed to 250 or 25 mg/kg-day Clofibrate for 1, 3, or 7 days. Two separate in vivo studies were conducted: one at Abbott Laboratories and one at GlaxoSmithKline (GSK, in United Kingdom). There was a difference in biological response between the two laboratories. The high dose (250 mg/kg-day) group at day 3 had a 15% increase in liver weight relative to body weight in the GSK study, compared with a 3% liver weight increase in the Abbott study. At 7 days, there was a 31% liver weight increase in the GSK study and a 15% increase in the Abbott study. Observed changes in clinical chemistry parameters also indicated differences in the biological response of the in vivo study concordant with difference in liver weight. A significant reduction in total cholesterol levels was seen in the GSK study at the high dose for all time points. However, the Abbott study demonstrated a significant reduction only at one dose and time point. The incidence of mitotic figures also differed between the labs. In both studies, there was a 2–3 times greater Acyl-CoA enzyme (ACOX) activity at the high dose but no difference from control in the low dose. Again, the GSK lab gave greater response. For microarrays, GSK and ULR pooled samples from each treatment group of four animals. U.S. EPA did some of the microarray analyses as well as GSK and ULR (GSK in United Kingdom). It is apparent that although the changes in genes were demonstrated by both laboratories, there were quantitative differences in the fold change values observed between the two sites.

The U.S. EPA analyzed gene expression in individual RNA samples obtained from day 7 high- and low-dose animals that had been treated at Abbot. GSK (United States) and ULR analyzed gene expression in pooled RNA from day 7 high- and low-dose animals treated at GSK (United Kingdom). Gene expression data from individual animal samples indicated that 7 genes were significantly upregulated (maximum of 7.2-fold) and 12 genes were down regulated (maximum of 4.3-fold decrease) in the high-dose group. The low-dose group generated only one statistically significant gene expression change, namely heat shock protein 70 (HSP70). In comparison, expression changes in the 7-day pooled high-dose samples analyzed by GSK (United States) ranged from 43.3-fold to a 3.5-fold decrease. Changes in these same samples analyzed by ULR ranged from a 4.9-fold increase to a 4.3-fold decrease. As an example, the microarray fold change at 7-day 250 mg/kg-day Clofibrate showed a 3.8-fold increase for U.S. EPA individual animals sampled, a 2.2-fold increase for pooled samples by ULR, and a 20.3-fold increase in pooled samples by GSK (United States) for CYP4A1 (Baker et al., 2004). Thus, these results show a very large difference not only between treatment groups, but also between pooled and nonpooled data and between labs analyzing the same RNA.

Not only was there a difference in DNA microarray results but, also a comparison of gene expression data from day 7 high-dose samples obtained using quantitative realtime PCR vs. data generated using cDNA microarrays has shown a quantitative difference but qualitative similar patterns. Although both methods of quantitative real time PCR on the pooled sample showed the PPARα gene to be downregulated, the GSK (United States) pooled sample microarray analysis indicated upregulation; the URL pooled and U.S. EPA individual microarray analyses showed no change. The microarray for PPARα at 7-day 250 mg/kg-day Clofibrate showed no change for individual animals (U.S. EPA), no change for pooled samples (ULR), and upregulation of 1.8-fold value for pooled samples for GSK (United States). The quantitative real time PCR on the pooled sample using Taqman gave a 4.5-fold downregulation and using SYBR Green gave a 1.2-fold downregulation of PPARα.

Baker et al. (2004) reported that the pooling of samples for microarray analysis has been used in the past to defray the cost of microarray experiments, reduce the effect of biological variation, and in some cases, overcome availability of limiting amounts of tissues. Unfortunately, this approach essentially produced a sample size (n) of one animal. Repeated microarray experiments with such pooled RNA produces technical replicates as opposed to true biological replicates, and thus, does not allow calculation of biologically significant changes in gene expression between different dose groups or time points. Another possible consequence of pooling is to mask individual gene changes and leave open the possibility of introducing error due to individual outlier responses.

Woods et al. (2007b) note that:

> because toxicogenomics is a relatively novel technology, there are a number of limitations that must be resolved before array data is widely accepted. Microarray studies have been touted as being highly sensitive for detecting toxic responses at much earlier time points and/or lower doses than histopathology, clinical chemistry or other traditional toxicological assays can detect. However, based on the nature of the assay, measurements of extreme levels of gene expression – low or high –are thought to be unreliable. Also the reproducibility of microarray experiments has raised concerns. "Batch effects" based on the day, user, and laboratory environment have been observed in array datasets. To address these concerns, confirmation of microarray-derived gene expression profiles is typically performed using quantitative real time polymerase chain reaction (RT-PCR) or Northern blot analysis.

In addition to the issues raised above, Waxman and Wurmbach (2007) raise issues regarding how quantitative real time PCR experiments are conducted. They state that cancer development affects almost all pathways and genes including the "housekeeping" genes, which are involved in the cell's common basic functions (e.g., glyceraldehyde-3-phosphate dehydrogenase [GADPH], beta actin [ACTB], TATA-binding protein, ribosomal proteins, and many more). However, "many of these genes are often used to normalize quantitative real-time RT-PCR (qPCR) data to account for experimental differences, such as differences in RNA quantity and quality, the overall transcriptional activity and differences in cDNA synthesis. GADPH and ACTB are most commonly used for normalization, including studies of cancer." Waxman and Wurmbach (2007) suggest that despite the fact that it has been shown that these genes are differentially expressed in cancers, including colorectal-, prostate-, and bladder-cancer, some qPCR studies on HCC used GAPDH or ACTB for normalization. Since many investigations on cancer include multiple comparisons, and analyze different stages of the disease, such as normal tissue, preneoplasm, and consecutive stages of cancer, "it crucial to find an appropriate gene for normalization" whose expression is constant throughout all disease stages and not response to treatment.

For liver cancers associated with exposure to hepatitis C virus (HCV), Waxman and Wurmbach (2007) reported that differing states, including preneoplastic lesions (cirrhosis and dysplasia) and consecutive stages of HCC, had differential expression of "housekeeping" genes and that using them for normalization had an effect on the fold change of qPCR data and on the general direction (up or down) of differentially expressed genes. For example, GAPDH was strongly upregulated in advanced and very advanced stages of HCC (in some samples up to sevenfold) and ACTB was upregulated two- to threefold in many advanced and very advanced tumor samples. Waxman and Wurmbach (2007) concluded that:

> microarray data are known to be highly variable. Due to its higher dynamic range qPCR is thought to be more accurate and therefore is often used to corroborate microarray results. Mostly, general direction (up and down-regulation) and rank

> order of the fold-changes are similar, but the levels of the fold changes of microarray experiments differ compared to qPCR data and show a marked tendency of being smaller. This effect is more pronounced as the fold change is very high.

In relation to use of gene expression and indicators of cancer causation, Volgelstein and Kinzler (2004) made important points regarding their use:

> Levels of gene expression are unreliable indicators of causation because disturbance of any network invariably leads to a multitude of such changes only peripherally related to the phenotype. Without better ways to determine whether an unmutated but interesting candidate gene has a causal role in neoplasia, cancer researchers will likely be spending precious time working on genes only peripherally related to the disease they wish to study.

This is an important caveat for gene expression studies for mode of action that are "snapshots in time" without phenotypic anchoring and even more applicable to experimental paradigms where there is ongoing necrosis or toxicity in addition to gene changes that may or may not be associated with neoplasia.

For an endpoint that is not as complex as neoplasia, there are issues regarding uses of microarray data. In regard to the determination of acute liver toxicity caused by one of the most studied hepatotoxins, acetaminophen, and its correlation with microarray data, Beyer et al. (2007) also have reported the results of a landmark study examining issues regarding use of this approach.

> The biology of liver and other tissues in normal and disease states increasingly is being probed using global approaches such as microarray transcriptional profiling. Acceptance of this technology is based principally on a satisfactory level of reproducibility of data among laboratories and across platforms. The issue of reproducibility and reliability of genomics data obtained from similar (standardized) biological experiments performed in different laboratories is crucial to the generation and utility of large databases of microarray results. While several recent studies uncovered important limitation of expression profiling of chemical injury to cells and tissues (Beekman et al., 2006; Baker et al., 2004; Ulrich et al., 2004), determining the effects of intralaboratory variables on the reproducibility, validity, and general applicability of the results that are generated by different laboratories and deposited into publicly available databases remains a gap…The National Institutes of Environmental Health Sciences (NIEHS) established the Toxicogenomics Research Consortium to apply the collective and specialized expertise from academic institutions to address issues in integrating gene expression profiling, bioinformatics, and general toxicology. Key elements include developing standardized practices for gene expression studies and conducting systematic assessments of the reproducibility of traditional toxicity endpoints and microarray data within and among laboratories. To this end the consortium selected the classical hepatotoxicant acetaminophen (APAP)

> for its proof of concept experiments. Despite more than 30 years of research on APAP, we are far from a complete understanding of the mechanisms of liver injury, risk factors, and molecular markers that predict clinical outcome after poisoning. APAP–induced hepatotoxicity was performed at seven geographically dispersed Centers. Parallel studies with N-acetyl-m-aminophenol (AMAP), the non-hepatotoxic isomer of APAP, provided a method to isolate transcripts associated with hepatotoxicity (Beyer et al., 2007).

Beyer et al. (2007) identified potential sources of interlaboratory variability when microarray analyses were conducted by one laboratory on RNA samples generated in different laboratories but using the same experimental paradigm and source of animals. Toxic injury by APAP showed variability across Centers and between animals (e.g., percent liver affected by necrosis [<20–80% at one time period and 0–60% at another], control animal serum ALT [threefold difference], and in GSH depletion [<5–>60%] between centers). There was concordance between APAP toxicity as measured in individual animals (rather than expressed as just a mean with SE) and transcriptional response. Of course, the variability between gene platforms and processing of the microarrays had been reduced by using the same facility to do all of the microarray analyses. However, the results show that phenotypic anchoring of gene expression data are required for biologically meaningful meta-analysis of genomic experiments.

Woods et al. (2007b) noted that:

> improvements should continue to be made on statistical analysis and presentation of microarray data such that it is easy to interpret. Prior to the current advances in bioinformatics, the most common way of reporting results of microarray studies involved listing differentially expressed genes, with little information about the statistical significance or biological pathways with which the genes are associated.

However, there are issues with the use of "Classifiers" or predictive genomic computer programs based on genes showing altered expression in association with the observed toxicities.

> Although these metrics built on different machine learning algorithms could be useful in estimating the severity of potential toxicities induced by compounds, the applications of these classifiers in understanding the mechanisms of drug-induced toxicity are not straightforward. In particular this approach is unlikely to distinguish the upstream causal genes from the downstream responsive genes among all the genes associated with an induced toxicity. Without knowledge of the causal sufficiency order, designing experiments to test predicted toxicity in animal models remains difficult" (Dai et al., 2007).

Ulrich (2003) stated the limitation of microarray analysis to study nuclear receptors (e.g., PPARα).

> Nuclear receptors comprise a large group of ligand-activated transcription factors that control much of cellular metabolism. Toxicogenomics is the study of the structure and output of the entire genome as it related and responds to adverse xenobiotic exposure. Traditionally, the genes regulated by nuclear receptors in cells exposed to toxins have been explored at the mRNA and protein levels using northern and western blotting techniques. Though effective when studying the expression of individual genes, these approaches do not enable the understanding of the myriad of genes regulated by individual receptors or of the crosstalk between receptors…Discovery of the multiple genes regulated by each receptor type has thus been driven by technological advances in gene expressional analysis, most commonly including differential display, RT-PCR and DNA microarrays., and in the development or receptor transgenic and knockout animal models. There is much cross talk between receptors and many agonists interact with multiple receptors. Off target effects cannot be predicted by target specificity. Though RCR can affect transcription directly, much of its effects are exerted through heterodimeric binging with other nuclear receptors (PXR, CAR, PPARα, PPARγ, FXR, LXR, TR) (Ulrich, 2003).

Another tool recent developed is gene silencing by introduction of siRNA. Dai et al. (2007) noted issues involved in the siRNA to change gene expression for exploration of mode of action etc., to include the potential of off-target effects, incomplete knockdown, and nontargeting of splice variants by the selected siRNA sequence. Using knockdown of PPARα in mice, Dai et al. (2007) report "PPARα knockdown was variable between mice ranging from ~80% knockdown to little or no knockdown and that differing siRNAs gave different patterns of gene expression with some grouped with PPARα -/- null mice but others grouped with expression patterns of mice injected with control siRNA or Ringers buffer alone and showing no PPARα knockdown." Dai et al. (2007) concluded that it is possible that it is the change in PPARα levels that is important for perturbing expression of genes modulated by PPARα rather than the absolute levels of PPARα.

Not only is the finding of variability in knockdowns by siRNA technologies important, but the finding that level of PPAR is not necessarily correlated with function and that it could be the change and not absolute level that matters in modulation in gene expression by PPARα is of importance as well. How an animal responds to decreased PPARα function may also depend on its gender. Dai et al. (2007) observed more dramatic phenotypes in female vs. male mice treated with siRNA. Costet et al. (1998) have reported sexually dimorphic phenotypes including obesity and increased serum triglyceride levels in females, and steatosis and increased hepatic triglyceride levels in male PPARα-null mice. Ramdhan et al. (2010) provided extensive date regarding lipid dysregulation in male PPARα-null mice and humanized mice.

In regard to the emerging science and preliminary reports of the effects of microRNA as oncogenes and tumor suppressors and of possible importance to hypothesized modes of action for liver cancer, the same caveats as described for DNA microarray analyses all apply, along with additional uncertainties. miRNAs repress their targeted mRNAs by complementary base

pairing and induction of the RNA interference pathway. Zhang et al. (2007) reported Northern blot detection of gene expression at the mRNA level and its correlation with miRNA expression in cancer cells as well as realtime PCR. These PCR-based analyses quantify miRNA precursors and not the active mature miRNAs. However, they reported that the relationship between pri-miRNA and mature miRNA expression has not been thoroughly addressed and is critical in order to use real time PCR analysis to study the function of miRNAs in cancers. They go on to state that:

> although Northern Blotting is a widely used method for miRNA analysis, it has some limitations, such as unequal hybridization efficiency of individual probes and difficulty in detecting multiple miRNAs simultaneously. For cancer studies, it is important to be able to compare the expression pattern of all known miRNAs between cancer cells and normal cells. Thus, it is better to have methods which detect all miRNA expression at a single time…Although Northern blot analysis, real-time PCR, and miRNA microarray can detect the expression of certain miRNAs and determine which miRNAs may be associated with cancer formation, it is difficult to determine whether or not miRNAs play a unique role in cancers. Also these techniques cannot directly determine the correlation between mRNA expression levels and whether the up-regulation or down-regulation of certain miRNAs is the cause of cancer or a downstream effect of the disease…Many miRNA genes have been found that are significantly overexpressed in different cancers. All of them appear to function as oncogenes; however, only a few of them have been well characterized.

Zhang et al. (2007) suggested that bioinformatic studies indicate that numerous genes are the targets of miR-17-92: >600 for miR-19a and miR-20, two members of the miR-17-92 cluster.

Cho (2007) stated that:

> though more than 530 miRNAs have been identified in human, much remains to be understood about their precise cellular function and role in the development of diseases…Although each miRNA can control hundreds of target genes, it remains a great challenge to identify the accurate miRNA targets for cancer research.

Thus, miRNAs have multiple targets so, like other transcription factors, may have pleotropic effects that are cell, timing, and context specific.

Vogelstein and Kinzler (2004) stated "in the last decade many important gene responsible for the genesis of various cancers have been discovered." Most importantly, they and others suggest that pathways rather than individual gene expression should be the focus of study. As a specific example, Volgelstein and Kinzler noted:

> another example of the reason for focusing on pathways rather than individual genes has been provided by studies of TP53 tumor-suppressor gene. The p53 protein is a transcription factor that normally inhibits cell growth and stimulates

> cell death when induced by cellular stress. The most common way to disrupt the p53 pathway is through a point mutation that inactivates its capacity to bind specifically to its cognate recognition sequence. However, there are several other ways to achieve the same effects, including amplification of the MDM2 gene and infection with DNA tumor viruses whose products bind to p53 and functionally inactivate it.

In regard to cellular anchoring for gene expression or pathway alterations associated with cancer and the importance of "context" of gene expression changes, Vogelstein and Kinzler (2004) gave several examples.

> In solid tumors the important of the interactions between stroma and epithelium is becoming increasingly recognized (e.g., the importance of the endothelial cell)…One might expect that a specific mutation of a widely expressed gene would have identical or at least similar effects in different mammalian cell types. But this is not in general what is observed. Different effects of the same mutation are not only found in distinct cell types; difference can even be observed in the same cell types, depending on when the mutation occurred during the tumorigenic process. The RAS gene mutations provide informative examples of these complexities. *KRAS2 g*ene mutation in normal pancreatic duct cells seem to initiate the neoplastic process, eventually leading to the development of pancreatic cancer. The same mutations occurring in normal colonic or ovarian epithelial cells lead to self-limiting hyperplastic or borderline lesions that do not progress to malignancy. In many human and experimental cancers, *RAS* genes seem to function as oncogenes. But *RAS* genes can function as suppressor genes under other circumstances, inhibiting tumorigenesis after administration of carcinogens to mice. These and similar observation on other cancer genes are consistent with the emerging notion that signaling molecules play multiple roles at multiple time, even in the same cell type. However, the biochemical bases for such variations among cancer cells are almost unknown.

In regard to the major pathways and mediators involved in cancer, several investigators have reported a coherent set that are involved in many types of cancers. Vogelstein and Kinzler (2004) noted that major pathways and mediators include p53, RB, WNT, E-cadherin, GL1, APC, ERK, RAS:GTP, P13K,SMAD, RTK BAD, BAX, and H1F1. In regard to coherence and site concordance between animal and human data, the disturbance of a pathway in one species may result in the different expression of tumor pattern in another, but both linked to a common endpoint of cancer. Thus, pathways rather than a single mutation should be the focus of mode of action and cancer as several actions can be manifested by one pathway or change at one time that lead to cancer.

Vogelstein and Kinzler (2004) also noted that pathways that are common to "cancer" are also operative in liver cancer where, as a heterogeneous disease, multiple pathways have been implicated in differing manifestations of this disease. Thus, liver cancer may be an example in its multiple forms that are analogous to differing sites being affected by common pathways

leading to "cancer." Pathway concordance may not always show up as site concordance as expression of cancer between species. Liver cancer may be the example where many pathways can lead a cancer that is characterized by its heterogeneity.

E.3.1.3. Etiology, Incidence, and Risk Factors for HCC

The review article of Farazi and DePinho (2006) provides an excellent summary of the current state of human liver cancer in terms of etiology and incidence. The 5-year survival rate of individuals with liver cancer in the United States is only 8.9% despite aggressive conventional therapy with lethality of liver cancer due in part from its resistance to existing anticancer agents, a lack of biomarkers that can detect surgically respectable incipient disease, and underlying liver disease that limits the use of chemotherapeutic drugs. Chen et al. (2002b) reported that surgical resection is considered the only "curative treatment" but >80 of patients have widespread HCC at the time of diagnosis and are not candidates for surgical treatment. Among patients with localized HCC who undergo surgery, 50% suffer a recurrence. Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality. HCC accounts for between 85 and 90% of primary liver cancers (El-Serag and Rudolph, 2007). Seitz and Stickel (2006) report that epidemiological data from the year 2000 indicate that >560,000 new cases of HCC occurred worldwide, accounting for 5.6% of all human cancers and that HCC is the fifth most common malignancy in men and the eighth in women.

Overall, incidence rates of HCC are higher in males compared to females. In almost all populations, males have higher liver cancer rates than females, with male:female ratios usually averaging between 2:1 and 4:1 and the largest discrepancies in rates (>4:1) found in medium-risk European populations (El-Serag and Rudolph, 2007). Experiments showed a 2–8-fold of control HCC development in male mice as well supporting the hypothesis that androgens influence HCC progression rather than sex-specific exposure to risk factors (El-Serag and Rudolph, 2007). El-Serag and Rudolph (2007) also reported that:

> in almost all areas, female rates peak in the age group 5 years older than the peak age group for males. In low risk population (e.g., U.S.) the highest age-specific rates occur among persons aged 75 and older. A similar pattern is seen among most high-risk Asian populations. In contrast male rats in high-risk African populations (e.g., Gambia) ten to peak between ages 60 and 65 before declining, whereas female rates peak between 65 and 70 before declining.

Age-adjusted incidence rates for HCC are extremely high in East and Southeast Asia and in Africa, but in Europe, there is a gradually decreasing prevalence from South to North. HCC incidence rates also vary greatly among different populations living in the same region and vary by race (e.g., for all ages and sexes in the United States, HCC rates are 2 times higher in Asian than in African Americans, whose rates are 2 times higher than those in whites); ethnic

variability is likely to include differences in the prevalence and acquisition time of major risk factors for liver disease and HCC (El-Serag and Rudolph, 2007).

Worldwide HCC incidence rate doubled during the last two decades and younger age groups are increasingly affected (El-Serag, 2004). The high prevalence of HCC in Asia and Africa may be associated with widespread infection with hepatitis B virus (HBV) and HCV but other risk factors include chronic alcohol misuse, nonalcoholic fatty liver disease (NAFLD), tobacco, oral contraceptives, and food contamination with aflatoxins (Seitz and Stickel, 2006). El-Serag and Rudolph (2007) reported HCC to be the fastest growing cause of cancer-related death in men in the United States with age-adjusted HCC incidence rates increasing more than twofold between 1985 and 2002 and that, overall, 15–50% of HCC patients in the United States have no established risk factors.

Although liver cirrhosis is present in a large portion of patients with HCC, it is not always present. Fattovich et al. (2004) reported that:

> differences of geographic area, method of recruitment of the HCC cases (medical or surgical) and the type of material studied (liver biopsy specimens, autopsy, or partial hepatectomies) may account for the variable prevalence of HCC without underlying cirrhosis (7% to 54%) quoted in a series of studies. Percutaneous liver biopsy specimens are subject to sampling error. However, only a small proportion of patients with HCC without cirrhosis have absolutely normal liver histology, the majority of them showing a range of fibrosis intensity from no fibrosis are all to septal and bridging fibrosis, necroinflammation, steatosis, and liver cell dysplasia.

Farazi and DePinho (2006) noted that for diabetes, a higher indices of HCC have been described in diabetic patients with no previous history of liver disease associated with other factors. El-Serag and Rudolph (2007) reported that in their study of VA patients (173,643 patients with and 650,620 patients without diabetes), that HCC incidence doubled among patients with diabetes and was higher among those with a longer follow-up of evaluation. "Although most studies have been conducted in low HCC rate areas, diabetes also has been found to be a significant risk factor in areas of high HCC incidence such as Japan. Taken together, available data suggest that diabetes is a moderately strong risk factor for HCC."

NAFLD and nonalcoholic steatohepatitis contribute to the development of fibrosis and cirrhosis and therefore, might also contribute to HCC development. The pathogenesis of NAFLD includes the accumulation of fat in the liver, which can lead to reactive oxygen species in the liver with necrosis factor α (TNFα) elevated in NAFDL and alcoholic liver disease (Seitz and Stickel, 2006). Abnormal liver enzymes not due to alcohol, viral hepatitis, or iron overload are present in 2.8–5.5% of the U.S. general population and may be due to NAFLD in 66–90% of cases (Adams and Lindor, 2007). Primary NAFLD occurs most commonly and is associated with insulin-resistant states, such as diabetes and obesity, with other conditions associated with

insulin resistance, such as polycystic ovarian syndrome and hypopituitarism also associated with NAFLD (Adams and Lindor, 2007). The steatotic liver appears to be susceptible to further hepatotoxic insults, which may lead to hepatocyte injury, inflammation, and fibrosis, but the mechanisms promoting progressive liver injury are not well defined (Adams and Lindor, 2007). Substrates derived from adipose tissue such as FFA, TNF-α, leptin, and adiponectin have been implicated, with oxidative stress appearing to be important leading to subsequent lipid peroxidation, cytokine induction, and mitochondrial dysfunction. Liver disease was the 3rd leading cause of death among NAFLD patients compared to the 13th leading cause among the general population, suggesting that liver-related mortality is responsible for a proportion of increased mortality risk among NAFLD patients (Adams and Lindor, 2007).

The RR for HCC in type 2 diabetics has been reported to be approximately 4 and increases to almost 10 for consumption of >80 g of alcohol per day (Hassan et al., 2002). El-Serag and Rudolph (2007) reported that:

> it has been suggested that many cryptogenic cirrhosis and HCC cases represent more severe forms of nonalcoholic fatty liver disease (NAFLD), namely nonalcoholic steato hepatitis (NASH). Studies in the United States evaluating risk factors for chronic liver disease or HCC have failed to identify HCV, HBV, or heavy alcohol intake in a large proportion of patients (30-40%). Once cirrhosis and HCC are established, it is difficult to identify pathologic features of NASH. Several clinic-based controlled studies have indicated that HCC patients with cryptogenic cirrhosis tend to have clinical and demographic features suggestive of NASH (predominance of women, diabetes, and obesity) as compared with age- and sex-matched HCC patients of well defined vial or alcoholic etiology. The most compelling evidence for an association between NASH and HCC is indirect and come from studies examining HCC risk with 2 conditions strongly associated with NASH: obesity and diabetes. In a large prospective cohort in the US, followed up for 16 years, liver cancer mortality rates were 5 times greater among men with the greatest baseline body mass index (range 35-40) compared with those with a normal body mass index. In the same study, the risk of liver cancer was not as increase in women, with a relative risk of 1.68. Two other population-based cohort studies from Sweden and Denmark found excess HCC risk (increased 2- to 3-fold) in obese men and women compared with those with a normal body mass index…Finally, liver disease occurs more frequently in those with more severe metabolic disturbances, with insulin resistance itself shown to increase as the disease progresses. Several developed countries most notably the United States, are in the midst of a burgeoning obesity epidemic. Although the evidence linking obesity to HCC is relatively scant, even small increase in risk related to obesity could translate into a large number of HCC cases.

Thus, even a small increase in risk related to obesity could result in a large number of HCC cases. and the latency of HCC may make detection of increased HCC risk not detectable for several years.

Other factors are involved, as not every cirrhotic liver progresses to HCC. Seitz and Stickel (2006) suggested that 90–100% of those who drink heavily suffer from alcoholic fatty liver, 10–35% of those evolve to alcoholic steatohepatitis, 8–20% of those evolve to alcoholic cirrhosis, and 1–2% of those develop HCC. HCV infects approximately 170 million individuals worldwide with approximately 20% of chronic HCV cases developing liver cirrhosis and 2.5% developing HCC.

Infection with HBV, a noncytopathic, partially double-stranded hepatotropic DNA virus classified as a member of the hepadnaviridae family, is also associated with liver cancer risk with several lines of evidence supporting the direct involvement of HBV in the transformation process (Farazi and DePinho, 2006). El-Serag and Rudolph (2007) suggested that:

> Epidemiologic research has shown that the great majority of adult-onset HCC cases are sporadic and that many have at lease 1 established non-genetic risk factor such as alcohol abuse or chronic HCV or HBV infection. However, most people with these known environmental risk factors never develop cirrhosis or HCC, whereas a sizable minority of HCC case develop among individuals without any known risk factors…Genetic epidemiology studies in HCC, similar to several other conditions, have fallen short of early expectations that they rapidly and unequivocally would result in identification of genetic variants conveying substantial excess risk of disease and thereby establish the groundwork for effective genetic screening for primary prevention.

E.3.1.4. Issues Associated with Target Cell Identification

Another outstanding and important question in HCC pathogenesis involves the cellular origin of this cancer. The liver is made up of a number of cell types showing different phenotypes and levels of differentiation. Which cell types are targets of hepatocarcinogens and are those responsible for human HCC is a matter of intense debate. Studies over the last decade provide evidence of several types of cells in the liver that can repopulate the hepatocyte compartment after a toxic insult. "Indeed, although the existence of a liver stem cell is often debated, most experts agree that progenitor liver cells are activated, in response to significant exposure to hepatotoxins. Also, progenitor cells derived from nonhepatic sources, such as bone marrow and pancreas, have been demonstrated recently to be capable of differentiating into mature hepatocytes under correct microenvironmental conditions" (Gandillet et al., 2003).

At present, analyses of human HCCs for oval cell markers, comparison of their gene-expression patterns with rat fetal hepatoblasts and the cellular characteristics of HCC from various animal models have provided contrasting results about the cellular origin of HCC and imply dual origins from either oval cells or mature hepatocytes. The failure to identify a clear cell of origin for HCC might stem from the fact that there are multiple cells of origin, perhaps reflecting the developmental plasticity of the hepatocyte lineage. The resolution of the HCC cell of origin issue could affect the development of useful preventative strategies to target nascent

neoplasms, foster an understanding of how HCC-relevant genetic lesions function in that specific cell-development context, and increase our ability to develop more accurate mouse models in which key genetic events are targeted to the appropriate cellular compartment (Farazi and DePinho, 2006). Two reviews by Librecht (2006) and Wu and Chen (2006) provide excellent summaries of the issues involved in identifying the target cell for HCC and the review by Roskams et al. (2004) provided a current view of the "oval cell" its location and human equivalent. Recent reports by Best and Coleman (2007) suggest another type of liver cell is also capable of proliferation and differentiating into small hepatocytes (i.e., small hepatocyte-like progenitor cell).

The review by Librecht (2006) provides an excellent description of the controversy and data supporting different views of the cells of origin for HCC.

> In recent years, the results of several studies suggest that human liver tumors can be derived from hepatic progenitor cells rather than from mature cell types. The available data indeed strongly suggest that most combined hepatocellular-cholangiocarcinomas arise from hepatic progenitor cells (HPCs) that retained their potential to differentiate into the hepatocyte and biliary lineages. Hepatic progenitor cells could also be the basis for some hepatocellular carcinomas and hepatocellular adenomas, although it is very difficult to determine the origin of an individual hepatocellular carcinoma. There is currently not enough data to make statements regarding a hepatic progenitor cell origin of cholangiocarcinoma. The presence of hepatic progenitor cell markers and the presence and extent of the cholangiocellular component are factors that are related the prognosis of hepatocellular carcinomas and combined hepatocellular-cholangiocarcinomas, respectively…The traditional view that adult human liver tumors arise from mature cell types has been challenged in recent decades…HPCs are small epithelial cells with an oval nucleus, scant cytoplasm and location in the bile ductules and canals of Hering. HPCs can differentiate towards the biliary and hepatocytic lineages. Differentiation towards the biliary lineage occurs via formation of reactive bile ductules, which are anastamosing ductules lined by immature biliary cells with a relatively large and oval nucleus surrounded by a small rim of cytoplasm. Hepatocyte differentiation leads to the formation of intermediate hepatocyte-like cells, which are defined as polygonal cells with a size intermediate between than of HPCs and hepatocytes. In most liver diseases, hepatic progenitor cells are "activated" which means that they proliferate and differentiate towards the hepatocytic and/or biliary lineages. The extent of activation is correlated with disease severity…HPCs and their immediate biliary and hepatocytic progeny not only have a distinct morphology, but they also express several markers, with many also present in bile duct epithelial cells. Immunohistochemistry using antibodies against these markers facilitates the detection of HPCs. The most commonly used markers are cytokeratin (CK) 19 and CK7…The proposal that a human hepatocellular carcinoma does not necessarily arise from mature hepatocyte, but could have HPC origin, has classically been based on three different observations. Each of them, however, gives only indirect evidence that can be disputed…Firstly, it has been shown that

> HPCs are the cells of origin of HCC in some animal models of hepatocarcinogenesis, which has led to the suggestion that this might also be the case in humans. However, in other animal models, the HCCs arise from mature hepatocytes and not from HPCs or reactive bile ductular cells (Bralet et al 2002; Lin et al 1995– DEN treated rats). Since it is currently insufficiently clear which of these animal models accurately mimics human hepatocarcinogenesis, one should be careful about extrapolating data regarding HPC origin of HCC in animal models to the human situation…Secondly, liver diseases that are characterized by the presence of carcinogens and development of dysplastic lesions also show HPC activation. Therefore, the suggestion has been made that HPCs form a "target population" for carcinogens, but this is only a theoretical possibility not supported by experimental data…Thirdly, several studies have shown that a considerable proportion of HCCs express one or more HPC markers that are not present in normal mature hepatocytes. Due to the fact that most HPC markers are also expressed in the biliary lineage, the term "biliary marker" has been used in some of these studies. The "maturation arrest" hypothesis states that genetic alterations occurring in a HPC, or its immediate progeny, cause aberrant proliferation and prevent its normal differentiation. Further accumulation of genetic alterations eventually leads to malignant transformation of these incompletely differentiated cells. The resulting HCC expresses HPC markers as evidence of its origin. However, expression of HPC markers can also be interpreted in the setting of the "dedifferentiation" hypothesis, which suggests that the expression of HPC markers is acquired during tumor progression as a consequence of accumulating mutations. For example, experiments in which human HCC cells lines were transplanted into nude mice have nicely shown that the expression of HPC marker, CK19, steadily increased when the tumors became increasingly aggressive and metastasized to the lung, Thus, the expression of CK19 in a HCC does not necessarily mean that the tumor has a HPC origin, but it can also be mutation-induced, acquired expression associated with tumor progression. Both possibilities are not mutually exclusive. For an individual HCC that expresses a HPC marker, it remains impossible to determine whether this marker reflects the cellular origin and/or is caused by tumor progression. This can only be elucidated by determining whether HCC contains cells that are ultrastructurally identical to HPCs in nontumor liver.

Similarly, the review by Wu and Chen (2006) also presents a valuable analysis of these issues and stated:

> The question of whether hepatocellular carcinomas arises from the differentiation block of stem cells or dedifferentiation of mature cells remains controversial. Cellular events during hepatocarcinogenesis illustrate that HCC may arise for cells at various stages of differentiation in the hepatic stem cell lineage…The role of cancer stem cells has been demonstrated for some cancers, such as cancer of the hematopoietic system, breast and brain. The clear similarities between normal stem cell and cancer stem cell genetic programs are the basis of the a proposal that some cancer stem cells could derived from human adult stem cells. Adult mesenchymal stem cells (MSC) may be targets for malignant transformation and undergo spontaneous transformation following long-term *in vitro* culture,

> supporting the hypothesis of cancer stem cell origin. Stem cells are not only units of biological organization, responsible for the development and the regeneration of tissue and organ systems, but are also targets of carcinogenesis. However, the origin of the cancer stem cell remains elusive…Three levels of cells that can respond to liver tissue renewal or damage have been proved (1) mature liver cells, as "unipotential stem cells," which proliferate under normal liver tissue renewal and respond rapidly to liver injury, (2) oval cells, as bipotential stem cells, which are activated to proliferate when the liver damage is extensive and chronic or if proliferation of hepatocytes is inhibited; and (3) bone marrow stem cells, as multipotent liver stem cells, which have a very long proliferation potential. There are two major nonexclusive hypotheses of the cellular origin of cancer; from stem cells due to maturation arrest or from dedifferentiation of mature cells. Research on hepatic stem cells in hepatocarcinogenesis has entered a new era of controversy, excitement and great expectations…The two major hypotheses about the cellular origination of HCC have been discussed for almost 20 years. Debate has centered on whether or not HCC originates from the differentiation block of stem cells or dedifferentiation of mature cells. Recent research suggests that HCC may originate from the transdifferentiation of bone marrow cells. In fact, there might be more than one type of carcinogen target cell. The argument about the origination of HCC becomes much clearer when viewed from this viewpoint: poorly differentiated HCC originate from bone marrow stem cells and oval cells, while well-differentiated HCC originates from mature hepatocytes…The cellular events during hepatocarcinogenesis illustrate that HCC may arise from cells at various stages of differentiation in the hepatocyte lineage. There are four levels of cells in the hepatic stem cell lineage: bone marrow cell, hepato-pancreas stem cell, oval cell and hepatocyte. HSC and the liver are known to have a close relationship in early development. Bone marrow stem cells could differentiate into oval cells, which could differentiate into heptatocytes and duct cells. The development of pancreatic and liver buds in embryogenesis suggests the existence of a common progenitor cells to both the pancreas and liver. All of the four levels of cells in the stem cell lineage may be targets of hepatocarcinogenesis.

Along with the cell types described as possible targets and participants in HCC, Best and Coleman (2007) described yet another type of cell in the liver that can respond to hepatocellular injury, which they term small hepatocyte-like progenitor cells and conclude that they are not the progeny of oval cells, but represent a distinct liver progenitor cell population. Another potential regenerative cell is the small hepatocyte-like progenitor cell (SHPC). SHPCs share some phenotypes with hepatocytes, fetal hepatoblasts, and oval cells, but are phenotypically distinct. They express markers such as albumin, transferring, and alpha-fetoprotein (AFP) and possess bile canaliculi and store glycogen.

A recent review by Roskams et al. (2004) provided a current view of the "oval cell" its location and human equivalent. They concluded that:

> while similarities exist between the progenitor cell compartment of human and rodent livers, the different rodent models are not entirely comparable with the

> human situation, and use of the same term has created confusion as to what characteristics may be expected in the human ductular reaction. For example, a defining feature of oval cells in many rodent models of injury is production of alpha-fetoprotein, whereas ductular reactions in humans rarely display such expression. Therefore we suggest that the "oval cell" and "oval –like cell" no longer be used in description of human liver.

In the chronic hepatitis and cancer model of Vig et al. (2006), it is not the oval cells or SHPCs that are proliferating but the mature hepatocytes, thus supporting theories that it is not only oval cells that are causing proliferations leading to cancer. Vig et al. (2006) also reported that studies in mice and humans indicate that oval cells also may give rise to liver tumors and that oval cells commonly surround and penetrate human liver tumors, including those caused by hepatitis B. Tarsetti et al. (1993) noted that although some studies have suggested that oval cells are directly involved in the formation of HCC, others assert that HCC originates from preneoplastic foci and nodules derived from hepatocytes and report that HCC evolved in their model of liver damage from hepatocytes, presumably hepatocellular nodules, and not from oval cells. They also suggested that proliferation alone may not lead to cancer. Recent studies that follow the progression of hepatocellular nodules to HCC in humans (see Section E.3.1.8) suggest an evolution from nodule to tumor.

E.3.1.5. Status of Mechanism of Action for Human HCC

The underlying molecular mechanisms leading to hepatocarcinogenesis remain largely unclear (Yeh et al., 2007). Although HCC is multistep, and its appearance in children suggest a genetic predisposition exists, the inability to identify most of the predisposing genes and how their altered expression relates to histological lesions that are the direct precursors to HCC, has made it difficult to identify the rate limiting steps in hepatocarcinogenesis (Feitelson et al., 2002). Calvisi et al. (2007) report that although the major etiological agents have been identified, the molecular pathogenesis of HCC remains unclear and that while deregulation of a number of oncogenes (e.g., c-Myc, cyclin D1 and β-catenin and tumor suppressor genes including $P16^{INK4A}$, p53, E-cadherin, DLC-1, and pRb) have been observed at different frequencies in HCC, the specific genes and the molecular pathways that play pivotal roles in liver tumor development have not been identified. Indeed rather than simple patterns of mutations, pathways that are common to cancer have been identified through study of tumors and through transgenic mouse models. Branda and Wands (2006) stated that the molecular factors and interactions involved in hepatocarcinogenesis are still poorly understood but are particularly true with respect to genomic mutations, "as it has been difficult to identify common genetic changes in >20 to 30% of tumors." As well as phenotypically heterogeneous, "it is becoming clear that HCCs are genetically heterogeneous tumors." The descriptions of heterogeneity of tumors and of pathway disruptions common to cancer are also shown for liver

tumors (see Sections E.3.1.6 and E.3.1.8). However, many of these studies focused on the end process and of examination of the genomic phenotype of the tumor for inferences regarding clinical course, aggressiveness of tumor, and consistency with other forms of cancer. As stated above, the events that produce these tumors from patients with conditions that put them at risk, are not known.

El-Serag and Rudolph (2007) suggested that risk of HCC increases at the cirrhosis stage when liver cell proliferation is decreased and that acceleration of carcinogenesis at this stage may result from telomere shortening (resulting in limitations of regenerative reserve and induction of chromosomal instability), impaired hepatocyte proliferation (resulting in cancer induction by loss of replicative competition), and altered milieu conditions that promote tumor cell proliferation.

> When telomeres reach a critically short length, chromosome uncapping induces DNA damage signals, cell-cycle arrest, senescence, or apoptosis. Telomeres are critically short in human HCC and on the single cell level telomere shortening correlated with increasing aneuploidy in human HCC…Chemicals inhibiting hepatocyte proliferation accelerate carcinogen-induced liver tumor formation in rats as well as the expansion and transformation of transplanted hepatocytes. It is conceivable that abnormally proliferating hepatocytes would not expand in healthy regenerating liver but would expand quickly and eventually transform in the growth restrained cirrhotic liver….Liver mass is controlled by growth factors – mass loss through could provide a growth stimulatory macroenvironment. For the microenvironment, cirrhosis activates stellate cells resulting in increased production of extracellular matrix proteins, cytokines, growth factors, and products of oxidative stress.

Like other cancers, genomic instability is a common feature of human HCC with various mechanisms thought to contribute, including telomere erosion, chromosome segregation defects, and alteration in DNA damage-response pathways. In addition to genetic events associated with the development of HCC (p53 inactivation, mutation in β-catenin, overexpression of ErbB receptor family members, and overexpression of the MET receptor whose ligand is HGF), various cancer-relevant genes seem to be targeted on the epigenetic level (methylation) in human HCC (Farazi and DePinho, 2006). Changes in methylation have been detected in the earliest stages of hepatocarcinogenesis and to a greater extent in tumor progression (Lee et al., 2003). Seitz and Stickel (2006) report that aberrant DNA hypermethylation (a silencing effect on genes) may be associated with genetic instability as determined by the loss of heterozygosity and microsatellite instability in human HCC due to chronic viral hepatitis and that modifications of the degree of hepatic DNA methylation have also been observed in experimental models of chronic alcoholism.

Farazi and DePinho (2006) reported that two of the key molecules that are involved in DNA damage response, p53 and BRCA2, seem to have roles in destabilizing the HCC genome (Gollin, 2005). The inactivation of p53 through mutation or viral oncoprotein sequestration is a

common event in HCC and p53 knock in mouse models containing dominant point mutations have been shown to cause genomic instability. However, Farazi and DePinho (2006) noted that despite documentation of deletions or mutations in these and other DNA damage network genes, their direct roles in the genomic instability of HCC have yet to be established in many genetic model systems.

Telomere shortening has been described as a key feature of chronic hyperproliferative liver disease (Rudolf and DePinho, 2001; Miura et al., 1997; Urabe et al., 1996; Kitada et al., 1995), specifically occurring in the hepatocyte compartment. These observations have fueled speculation that telomere shortening associated with chronic liver disease and hepatocyte turnover contribute to the induction of genomic instability that drives human HCC (Farazi and DePinho, 2006). Defects in chromosome segregation during mitosis result in aneuploidy, a common cytogenetic feature of cancer cell including HCC (Farazi and DePinho, 2006).

Several studies have attempted to categorize genomic changes in relation to tumor state. In general, high levels of chromosomal instability seem to correlate with the de-differentiation and progression of HCC (Wilkens et al., 2004). Several studies have suggested certain chromosomal changes to be specific to dysplastic lesions, early-stage and late-stage HCCs, and metastases. It is important to note that the studies that have attempted to compare genomic profiles and tumor state are few in number, often did not classify HCCs on the basis of etiology, and used relatively low-resolution genome-scanning platforms (Farazi and DePinho, 2006). Farazi and DePinho (2006) noted that it should be emphasized that although genome etiology correlates reported in some studies are intriguing, several studies have failed to uncover significant differences in genomic changes between different etiological groups, although the outcome might related to small sample sizes and the low-resolution, genome scanning platform used.

E.3.1.6. Pathway and Genetic Disruption Associated with HCC and Relationship to Other Forms of Neoplasia

In their landmark paper, Hanahan and Weinberg (2000) suggested that the vast catalog of cancer cell genotypes was a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth signals), elevation of programmed cell death (apoptosis), limitless replication potential, sustained angiogenesis, and tissue invasion and metastasis. They proposed that these six capabilities are shared in common by most, and perhaps all, types of human tumors and, while virtually all cancers must acquire the same six hallmark capabilities, their means of doing so would vary significantly, both mechanistically and chronologically. It was predicted that in some tumors, a particular genetic lesions may confer several capabilities simultaneously, decreasing the number of distinct mutational steps required to complete tumorigenesis. Loss of the p53 tumor suppressor was cited as an example that could facilitate both angiogenesis and

resistance to apoptosis and to enable the characteristic of genomic instability. The paths that cells could take on their way to becoming malignant were predicted to be highly variable, and within a given cancer type, mutation of a particular target genes such as ras or p53 could be found only in a subset of otherwise histologically identical tumors. Furthermore, mutations in certain oncogenes and tumor suppressor genes could occur early in some tumor progression pathways and late in others. Genes known to be functionally altered in "cancer" were identified as including Fas,Bcl2, Decoy R, Bax, Smads, TFGβR, p15, p16, Cycl D, Rb, human papilloma virus E7, ARF, PTEN, Myc, Fos, Jun, Ras, Abl, NF1, RTK, transforming growth factor alpha (TGF-α), Integrins, E-cadherin, Src, β-catenin, APC, and WNT.

Branda and Wands (2006) reported that two signal transduction cascades that appear to be very important are insulin/IFG-1/IRS-1/MAPK and Wnt/Frizzled/β-catenin pathways, which are activated in over 90% of HCC tumors (Branda and Wands, 2006). Feitelson et al. (2002) reported that:

> In addition to NF-κB, up-regulated expression of rhoB has been reported in some HCCs. RhoB is in the *ras* gene family, is associated with cell transformation, and may be a common denominator to both viral and non-viral hepatocarcinogenesis. Activation of ras and NF-κB, combined with down regulation of multiple negative growth regulatory pathways, then, may contribute importantly to early steps in hepatocarcinogenesis. Thus viral proteins may alter the patterns of hepatocellular gene expression by transcriptional trans-regulation…Another early event appears to involve the mutation of β-catenin, which is a component of the Wnt signal transduction pathway whose target genes include c-myc, c-jun, cyclin D1, fibronectin, the connective tissue growth factor WISP, and matrix metaolloproteinases.

Boyault et al. (2007) reported that:

> altogether, the principle carcinogenic pathways known to be deregulated in HCC are inactivation of TP53, Wnt/wingless activation mainly through CTNNB1 mutations activating β-catenin- and AXIN1-inactivating mutations, retinoblastoma inactivation through RB1 and CDKN2A promoter methylation and rare gene mutations, insulin growth factor activation through IGF2 overexpression, and IGF2R-inactiving mutations.

El-Serag and Rudolph suggested that "in general, the activation of oncogenic pathways in human HCC appears to be more heterogeneous compared with other cancer types." El-Serag and Rudolph (2007) reported that the p53 pathway is a major tumor-suppressor pathway that: (1) limits cell survival and proliferation (replicative senescence) in response to telomere shortening; (2) induces cell-cycle arrest in response to oncogene activation (oncogene-induced senescence); (3) protects genome integrity; and (4) is affected at multiple levels in human HCC.

"p53 mutations occur in aflatoxin induced HCC (>50%) and with lower frequency (20–40%) in HCC not associated with aflatoxin." In addition,

> the vast majority of human HCC overexpresses gankyrin, which inhibits both Rb checkpoint and p53 checkpoint function…The p16/Rb checkpoint is another major pathway limiting cell proliferation in response to telomere shortening, DNA damage, and oncogene activation. In human HCC the Rb pathway is disrupted in more than 80% of cases, with repression of p16 by promoter methylation being the most frequent alteration. Moreover, expression of gankyrin (an inhibitor of p53 and Rb checkpoint function) is increased in the vast majority of human HCCs, indicating that the Rb checkpoint is dysfunctional in the vast majority of human HCCs…The frequent inactivation of p53 in human HCC indicates that abrogation of p53-dependent apoptosis could promote hepatocarcinogenesis. The role of impairment of p53-independent apoptosis for hepatocarcinogenesis remains to be defined…Activation of the β-catenin pathway frequently occurs in mouse and human HCC involving somatic mutations, as well as transcriptional repression of negative regulators. An activation of the Akt signaling and impaired expression of phosphatase and tensin homolog (PTEN) (a negative regulator of Akt) have been reported in 40-60% of Human HCC.

They suggested that although Myc is a potent oncongene inducing hepatocarcinogenesis in mouse models, the data on human HCC are heterogeneous and further studies are required.

E.3.1.7. Epigenetic Alterations in HCC

The molecular pathogenesis of HCC remains largely unknown, but it is presumed that the development and progression of HCC are the consequence of cumulative genetic and epigenetic events similar to those described in other solid tumors (Calvisi et al., 2006). Calvisi et al. (2007) provided a good summary of DNA methylation status and cancer as well as its status in regard to HCC:

> Aberrant DNA methylation occurs commonly in human cancers in the forms of genome-wide hypomethylation and regional hypermethylation. Global DNA hypomethylation (also known as demethylation) is associated with activation of protooncogenes, such as c-Jun, c-Myc, and c-HA-Ras, and generation of genomic instability. Hypermethylation on CpG islands located in the promoter regions of tumor suppressor genes results in transcriptional silencing and genomic instability. CpG hypermethylation (also known as de novo methylation) acts as an alternative and/or complementary mechanisms to gene mutations causing gene inactivation, and it is now recognized as an important mechanism in carcinogenesis. Although the mechanism(s) responsible for de novo methylation in cancer are poorly understood, it has been hypothesized that epigenetic silencing depends on activation of a number of proteins known as DNA methyltransferases (DNMTs) that posses de novo methylation activity. The importance of DNMTs in CpG methylation was substantiated by the observation that genetic disruption of both DNMT1 and DNMT3b genes in HCT116 cell lines nearly eliminated

methyltransferase activity. However, more recent findings indicate that the HCT116 cells retain a truncated, biologically active form of DNMT1 and maintain 80% of their genomic methylation. Further reduction of DNMT1 levels by a siRNA approach resulted in decreased cell viability, increased apoptosis, enhanced genomic instability, checkpoint defects, and abrogation of replicative capacity. These data show that DNTM1 is required for cell survival and suggest that DNTM1 has additional functions that are independent of its methyltransferase activity. Concomitant overexpression of DNMT1, -3A, and -3b has been found in various tumors including HCC. However, no changes in the expression of DNMTs were found in other neoplasms, such as colorectal cancer, suggesting the existence of alternative mechanisms. In HCC, a novel DNMT3b splice variant, known as DNMT3b4 is overexpressed. DNMT3b4 lacks DNMT activity and competes with DNMT2b3 for targeting of pericentromeric satellite regions in HCC, resulting in DNA hypomethylation of these regions and induction of chromosomal instability, further linking aberrant methylation and generation of genomic alterations.

It is now well accepted that methylation changes occur early and ubiquitously in cancer development. The case has been made that tumor cell heterogeneity is due, in part, to epigenetic variation in progenitor cells and that epigenetic plasticity together with genetic lesions drive tumor progression (Feinberg et al., 2006).

A growing number of genes undergoing aberrant CpG island hypermethylation in HCC have been discovered, suggesting that de novo methylation is an important mechanism underlying malignant transformation in the liver. However, most of the previous studies have focused on a single or a limited number of genes, and few have attempted to analyze the methylation status of multiple genes in HCC and associated chronic liver diseases. In addition, the functional consequence(s) of global DNA hypomethylation and CpG island hypermethylation in human liver cancer has not been investigated to date. Furthermore, to our knowledge no comprehensive analysis of CpG island hypermethylation involving activation of signaling pathways has been performed.

Calvisi et al. (2007) reported that global gene expression profiles show human HCC to harbor common molecular features that differ greatly from those of nontumorous surrounding tissues, and that human HCC can be subdivided into two broad but distinct subclasses that are associated with length of patient survival. They further suggested that aberrant methylation is a major event in both early and late stages of liver malignant transformation and might constitute a critical target for cancer risk assessment, treatment, and chemoprevention of HCC. Calvisi et al. (2007) conducted analysis of methylation status of genes selected based on their capacity to modulate signaling pathways (Ras, Jak/Stat, Wingless/Wnt, and RELN) and/or biologic features of the tumors (proliferation, apoptosis, angiogenesis, invasion, DNA repair, immune response, and detoxification). Normal livers were reported to show the absence of promoter methylation for all genes examined. At least 1 of the genes involved in inhibition of Ras (ARH1, CLU,

DAB2, hDAB21P, HIN-1, HRASL, LOX, NORE1A, PAR4, RASSF1A, RASSF2, RASSF3, RASSF4, RIG, RRP22, and SPRY2 and -4), Jak/Stat (ARH1,CIS, SHP1, PIAS-1, PIAS-γ, SOCS1, -2, and -3, SYK, and GRIM-19), and Wnt/β-catenin (APC, E-cadherin, γ-catenin, SFRP1, -2, -4, and -5, DKK-1 and -3, WIF-1 and HDPR1) pathways were affected by de novo methylation in all HCC. A number of these genes were also reported to be highly methylated in the surrounding nontumorous liver. In contrast, inactivation of at least one of these genes implicated in the RELN pathway (DAB1, reelin) was detected differentially in HCC of subclasses of tumors that had differences in tumor aggressiveness and progression. Epigenetic silencing of multiple tumor suppressor genes maintains activation of the Ras pathway with a major finding in the Calvisi et al. (2007) study to be the concurrent hypermethylation of multiple inhibitors of the Ras pathway with Ras was significantly more active in HCC than in surrounding or normal livers. Also important was the finding that no significant associations between methylation patterns and specific etiologic agents (i.e., HVB, HVC, ethanol, etc.) were detected, further substantiating the conclusion that aberrant methylation is a ubiquitous phenomenon in hepatocarcinogenesis.

> Current evidence suggests that hypomethylation might promote malignant transformation via multiple mechanisms, including chromosome instability, activation of protooncogenes, reactivation of transposable elements, and loss of imprinting…The degree of DNA hypomethylation progressively increased from nonneoplastic livers to fully malignant HCC, indicating that genomic hypomethylation is an important prognostic factor in HCC, as reported for brain, breast, and ovarian cancer.

Calvisi et al. (2007) also reported that regional CpG hypermethylation was also enhanced during the course of HCC disease and that the study of tumor suppressor gene promoters showed that CpG methylation was frequently detected both in surrounding nontumorous livers and HCC.

E.3.1.8. Heterogeneity of Preneoplastic and HCC Phenotypes

A very important issue for the treatment of HCC in humans is early detection. Research has focused on identification of lesions that will progress to HCC and to also determine from the phenotype of the nodule and genetic expression its cell source, likely survival, and associations with etiologies and modes of action. As with rodent models where preneoplastic foci have been observed to be associated with progression to adenoma and carcinoma, nodules observed in humans with high risk for HCC have been observed to progress to HCC. In humans, histomorphology of HCC is notoriously heterogeneous (Yeh et al., 2007). Although much progress has been made, there is currently not universally accepted staging system for HCC partly because of the natural course of early HCC is unknown and the natural progression of intermediated and advanced HCC are quite heterogeneous (Thorgeirsson, 2006). Nodules are

heterogeneous as well, with differences in potential to progress to HCC. Chen et al. (2002b) reported that standard clinical pathological classification of HCC has limited valued in predicting the outcome of treatment as the phenotypic diversity of cancer is accompanied by a corresponding diversity in gene expression patterns. There is also histopathological variability in the presentation of HCC in geographically diverse regions of the world with some slow growing, differentiated HCC nodules surrounded by a fibrous capsule are common among Japanese but, in contrast, a "febrile" form of HCC, characterized by leukocytosis, fever, and necrosis within a poorly differentiated tumor to be common in South African blacks (Feitelson et al., 2002).

A multistep process is suggested histologically, where HCC appears within the context of chronic hepatitis and/or cirrhosis within regions of the liver cell dysplasia or adenomatous hyperplasia (Feitelson et al., 2002). Kobayashi et al. (2006) reported that the higher the grade of the nodule, the higher the percentage that will progress to HCC with 18.8% of all nodules and regenerative lesions going on to become HCC, 53.3% remaining unchanged, and 27.9% disappearing in the observation period of 0.1–8.9 years. Borzio et al. (2003) reported that the rate of liver malignant transformation was 40% in larger regenerative nodules, low-grade dysplastic, and high-grade dysplastic nodules with higher grade of dysplasia extranodular detection of large cell change and hyperchronic pattern associated with progression to HCC. Yeh et al. (2007) reported that nuclear staining for Ki-67 and Topo II-α (a nuclear protein targeted by several chemotherapeutic agents) significantly increased in the progression from cirrhosis, through high-grade dysplastic nodules to HCC, whereas the scores for TGF-α in these lesions showed an inverse relationship. "In comparison with 18 HCC arising in noncirrhotic livers, the expression of TGF-α is significantly stronger in cirrhotic liver than in noncirrhotic parenchyma and its expression is also stronger in HCC arising in cirrhosis than in HCC arising in noncirrhotic patients." They concluded that initiation in cirrhotic and noncirrhotic liver may have different pathways with transforming growth factor-α (a mitogen activated the EFGR) playing a relative more important role in HCC from cirrhotic liver. Overexpression of TGF-α in the liver of transgenic mice induced increased proliferation, dysplasia, adenoma, and carcinoma. Yeh et al. (2007) concluded that such high-grade dysplastic nodules are precursor lesions in hepatocarcinogenesis and that TGF-α may play an important role in the early events of liver carcinogenesis.

Moinzadeh et al. (2005) reported in a meta-analysis of all available (n = 785) HCCs that gains and losses of chromosomal material were most prevalent in a number of chromosomes and that amplifications and deletions occurred on chromosomal arms in which oncogenes (e.g., MYC and 8q24) and tumor suppressor genes (e.g., RB1 on 13q14) are located as well as modulators of the WNT-signaling pathway. However, in multifocal HCC, nodules arising de novo within a single liver have a different spectrum of genetic lesions. "Hence, there are likely to be many paths to HCC, and this is why it has been difficult to assign specific molecular alterations to

changes in hepatocellular phenotype, clinical, or histopathological changes that accompany tumor development" (Feitelson et al., 2002).

Serum AFP is commonly used as a tumor marker for HCC. Several reports have linked HCC to cytokines in an attempt to find more specific markers of HCC. Jia et al. (2007) reported that AFP marker allows for identification of a small set of HCC patients with smaller tumors, and these patients have a relatively long-term survival rate following curative treatment.

> Presently the only approach to screen for the presence of HCC in high-risk populations is the combination of serum AFP and ultrasonagraphy. However, elevated AFP is only observed in about 60 to 70% of HCC patients and to a lesser extent (33-65%) in patients with smaller HCCs. Moreover, nonspecific elevation of serum AFP has been found in 15% to 58% of patients with chronic hepatitis and 11% to 47% of patients with liver cirrhosis.

Soresi et al. (2006) reported that serum IL-6 levels are low in physiological conditions, but increase considerably in pathological conditions such as trauma, inflammation, and neoplasia. In tumors, IL-6 may be involved in promoting the differentiation and growth of target cells. "Many works have reported high serum IL-6 levels in various lifer diseases such as acute hepatitis, primary biliary cirrhosis, chronic hepatitis (hepatitis C) and HCV-correlated liver cirrhosis and in hepatocellular carcinoma." Soresi et al. (2006) reported that patients with HCC group had higher IL-6 values than those with cirrhosis and that "higher-staged" patients had the highest IL-6 levels. Hsia et al. (2007) also examined IL-6, IL-10 and hepatocyte growth factor (HGF) as potential markers for HCC.

> The expression of IL-6 or IL-10 or higher level of HGF or AFP was observed only 0-3% of normal subjects. Patients with HCC more frequently had higher IL-6 and IL-10 levels, where as HGF levels in HCC patients were not significantly elevated compared to patients with chronic hepatitis or non-HCC tumors (but greater than controls). Among patients with low AFP level, IL-6 or IL-10 expression was significantly associated with the existence of HCC. Patients with large HCC (>5 cm) more often had increased IL-6, IL-10 or AFP levels. Serum levels of IL-6 and IL-10 are frequently elevated in patients with HCC but not in benign liver disease or non-HCC tumors.

Nuclear DNA content and ploidy have also been the subjects of several studies through the years for identification of pathways for prediction of survival or origin of tumors. Nakajima et al. (2004) report that p53 loss can contribute to the propagation of damaged DNA in daughter cells through the inability to prevent the transmission of inaccurate genetic material, considered to be one of the major mechanisms for the emergence of aneuploidy in tumors with inactivated p53 protein and the increasing ploidy in HCC was associated with disturbance in p53. McEntee et al. (1992) reported that specimens from 74 patients who underwent curative resection for

primary HCC and analyzed for DNA content, (i.e., tumors were classified as DNA aneuploid if a separate peak was present from its standard large diploid peak [2C] and tetraploid peak [4C]) 33% were DNA diploid, 30% were DNA tetraploid/polyploidy, and 37% were aneuploid of the primary tumors examined. Nontumor controls were diploid and survival was not different between patients with diploid vs. nondiploid tumors. Zeppa et al. (1998) reported ploidy in 84 HCCs diagnosed by fine-needle aspiration biopsy to have 68 cases that were aneuploid and 16 euploid (9 diploid and 7 polyploid), with median survival of 38 months for patients with diploid HCC and 13 months for aneuploid HCC. Lin et al. (2003) reported in their study of fine-needle aspiration of HCC that:

> the ratio of S and G2/M periods of DNA, which reflect cell hyperproliferation, in the group with HCC tumors>3 cm in diameter were markedly higher than those of the group with nodules<3 cm in diameter and the group with hyperplastic nodules…DNA analysis of aspiration biopsy tissues acquired from intrahepatic benign hyperplastic nodules showed steady diploid (2c) peak that stayed in G1 period. DNA analysis of aspiration biopsy tissues acquired from HCC nodules showed S period of hyperproliferation and G2/M period. The DNA analysis of HCC nodules showed aneuploid peak.

They concluded that in regard to the biological behavior of the cell itself, that the normal tissue, reactive tissue, and benign tumor all have normal diploid DNA but, like most other malignant tumors, "HCC appears to have polyploid DNA, especially aneuploid DNA."

Attallah et al. (1999) reported small needle liver biopsy data to show HCC to be 21.4% diploid, 50% aneuploid, and 28.6% tetraploid and that higher ploidies (aneuploid and tetraploid) were observed in human liver cancer than residual tissues, although in some cases, there was increased aneuploidy (cirrhosis, 37%, hepatitis ~50%). Of note for the study is the lack of appropriate control tissue and uncertainty as to how some of their diploid cells could have been binucleate tetraploid cells. Anti et al. (1994) reported reduction in binuclearity in the chronic hepatitis and cirrhosis groups that was significantly correlated with a rise in the diploid/ polyploidy ratio and that precancerous and cancerous nodules within cirrhotic liver show an increased tendency toward diploidy or the emergence of aneuploid populations. They noted that a number of investigators have reported significantly increased hepatocyte diploidization during the early stages of chemically induced carcinogenesis in rat liver, but other experimental findings indicate that malignant transformation can occur after any type of alteration in ploidy distribution.

On the other hand, Melchiorri et al. (1994) noted that several studies using flow cytometric or image cytometric methods reported high DNA ploidy values in 50–77% of the examined HCCs and that the presence of aneuploidy was significantly related to a poor patient prognosis. They reported that the DNA content of mononucleated and binucleated hepatocytes, obtained by ultrasound-guided biopsies of 10 macroregenerative nodules without histologic signs

of atypia from the lesions with the greater fraction of mononucleated hepatocytes were diagnosed as HCCs during the clinical follow-up, with results also suggesting that diploid and tetraploid stem cell lines are the main lines of the HCCs as well as a reduction in the percentage of binucleated hepatocytes in HCC. Gramantieri et al. (1996) reported that the percentage of binucleated cells was reduced in most of the HCC that they studied (i.e., the mean percentage of binucleated cells 9% in comparison to 24% found in normal liver) and that most HCC, as many other solid neoplasms, showed altered nuclear parameters.

Along with reporting pathways that are perturbed in HCC, emerging evidence also shows that signatures of pathway are predictive of clinical characteristics of HCC. A number of studies have examined gene expression in tumors to try to determine which pathways may have been disturbed in an attempt to predict survival and treatment options for the patients and to investigate possible modes of action for the tumor induction and progression. Chen et al. (2002b) described a systematic characterization of gene expression patterns in human liver cancers using cDNA microarrays to study tumor and nontumor liver tissues in HCC patients, and of note did quality assurance on their microarray chips (many studies do not report that they have done so), and examined the effects of hepatitis virus on its subject and identified people with it. Most importantly, Chen et al. (2002b) provided phenotypic anchoring of each tumor with its genetic profile rather than pooling data.

The hierarchical analysis demonstrated that clinical samples could be divided into two major clusters, one representing HCC samples and the other with a few exceptions, representing nontumor liver tissues. Most importantly, expression patterns varied significantly among the HCC and nontumor liver samples and that samples from HBV-infected, hepatitis C virus infected, and noninfected individuals were interspersed in the HCC branch. Thus, tumors from people infected with HVB, HVC, and noninfected people with HCC were interspersed in the HCC pattern and could be discerned based on etiology. One cluster of genes was highly expressed in HCC samples compared with nontumor liver tissues included a “proliferation cluster” comprised of genes whose functions are required for cell-cycle progression and whose expression levels correlate with cellular proliferation rates with most of the genes in this cluster are specifically expressed in the G2/M phase. Gene profiles for HCC were consistent with fewer molecular features of differentiated normal hepatocytes.

Chen et al. (2002b) noted that both normal and liver tumors are complex tissue compose of diverse cells and that distinct patterns of gene expression seemed to provide molecular signatures of several specific cell types including expression of two clusters of genes associated with T and B lymphocytes, presumably reflecting lymphocytic infiltration into liver tissues, and genes associated with stellate cell activation. This important finding acknowledges that HCC is not only heterogeneous in hepatocyte phenotype but is also made up of many other nonparenchymal cell types and that gene expression patterns reflect that heterogeneity. A gene cluster was also identified at a higher level in HCC that included several genes typically

expressed in endothelial cells, including CD34, which is expressed in endothelial cells in veins and arteries but not in the endothelial cells of the sinusoids in nontumor liver and which may reflect disruption of the molecular program that normally regulate blood vessel morphogenesis in the liver.

Of great importance was the investigation by Chen et al. (2002b) of whether samples from multiple sites in a single HCC tumor, or multiple separate tumor nodules in one patient, would share a recognizable gene expression signature. With a few instructive exceptions, all of the tumor samples from each patient clustered were reported to cluster together. To further examine the relationship among multiple tumor samples from individual patients, they calculated the pairwise comparison for all pairs of samples and samples some primary tumors multiple times. Tumor patterns of gene expression were more highly correlated with those seen in samples from the same patient than other patients but every tumor had a distinctive and characteristic gene expression pattern, recognizable in all samples taken from different areas of the same tumor.

For multiple discrete tumor masses obtained from six patients, three of these patients had multiple tumors with a shared distinctive gene expression pattern but in three other patients, expression patterns varied between tumor nodules and the difference providing new insights into the sources of variation in molecular and biological characteristics of cancers. Thus, in some patients, multiple tumors were from the same clone, as demonstrated by a similar gene expression profile, but for some patients, multiple tumors were arising from differing clones within the same liver. In regard to whether the distinctive expression patterns characteristic of each tumor reflect the individuality of the tumor or are determined by the patient in whom the tumor arose, analysis of the expression patterns observed in the two tumor nodules from one patient showed that the two tumors were not more similar than those of an arbitrary pair of tumors from different patients. These results show the heterogeneity of HCC and that “one gene pattern” will not be characteristic of the disease.

However, HCC did have a pattern that differed from other cancers. Chen et al. (2002b) analyzed the expression patterns of 10 randomly selected HCC samples and 10 liver metastases of other cancers and reported that the HCC samples and the metastatic cancers clustered into two distinct groups, based on difference in their patterns of gene expression. Although some of the HCC samples were poorly differentiated and expressed the genes of the liver-specific cluster at very low levels compared to with either normal liver or well-differentiated HCC, the genes of the liver-specific cluster were reported to be consistently expressed at higher levels in HCC than in tumors of nonliver origin. Metastatic cancers originating from the same tissue typically clustered together, expressing gene characteristic of the cell types of origin. Thus, liver cancer was distinguishable from other cancer even though very variable in expression and differentiation state.

In an attempt to create molecular prognostic indices that can be used for identification of distinct subclasses of HCC that could predict outcome, Lee et al. (2004a) reported two subclasses of HCC patients characterized by significant differences in the length of survival. They also identified expression profiles of a limited number of genes that accurately predicted the length of survival. Total RNAs from the 19 normal livers, including "normal liver in HCC patients," were pooled and used as a reference for all microarray experiments and thus variations between patients, and especially differences due to conditions predisposing HCC, were not determined. DNA microarray data using hierarchical clustering was reported to yield two major clusters, one representing HCC tumors, and the other representing nontumor tissues with a few exceptions that were not characterized by the authors. Lee et al. (2004a) reported that, along with two distinctive subtypes of gene expression patterns in HCC, there was heterogeneity among HCC gene expression profiles and that one group had an overall survival time of 30.8 months and the other 83.7 months. Only about half the patients in each group were reported to have cirrhosis. Expression of typical cell proliferation markers such as PCNA and cell cycle regulators such as *CDK4*, *CCNB1*, *CCNA2*, and *CKS2* was greater in one class than the other of HCC.

The report by Boyault et al. (2007) attempted to compare etiology and genetic characterization of the tumors they produce and confirmed the heterogeneity of HCC, some without attendant genomic instability. Boyault et al. (2007) reported that genetic alterations are indeed closely associated with clinical characteristics of HCC that define two mechanisms of hepatocarcinogenesis.

> The first type of HCC was associated with not only a high level of chromosome instability and frequent TP53 and AXIN1 mutations but also was closely linked to HBV infections and a poor prognosis. Conversely, the second subgroup of HCC tumors was chromosome-stable, having a high incidence of activating β-catenin alteration and was not associated with viral infection.

Boyault et al. (2007) reported that in a series of 123 tumors, mutations in the CTNNB1 (encoding β-catenin), TP53, ACIN1, TCF1, PIK3CA and KRAS genes in 34, 31, 13, 5, 2, and 1 tumors were identified, respectively. No mutations were found in NRAS, HRAS, or EGFR. Hypermethylation of the CDKN2A and CDH1 promoter was identified in 35 and 16% of the tumors, respectively. Boyault et al. (2007) grouped tumors by genomic expression as well as other factors. HCC groups associated with high rate of chromosomal instability were reported to be enriched with overexpression of cell-cycle/proliferation/DNA metabolism genes. They concluded that "the primary clinical determinant of class membership is HBV infection and the other main determinants are genetic and epigenetic alterations, including chromosome instability, CTNNB1 and TP53 mutations, and parental imprinting. Tumors related to HCV and alcohol abuse were interspersed across subgroups G3-G6." Boyault et al. (2007) suggested that their results indicated that HBV infection early in life leads to a specific type of HCC that has

immature features with abnormal parental gene imprinting selections, possibly through the persistence of fetal hepatocytes or alternatively through partial dedifferentiation of adult hepatocytes. "These G1 tumors are related to high-risk populations found in epidemiological studies."

E.3.2. Animal Models of Liver Cancer

There are obvious differences between rodents and primate and human liver, and there is a difference in background rates of susceptibility to hepatocarcinogenesis. With strains of mice, there are large differences in responses to hepatotoxins (e.g., acetaminophen) and to hepatocarcinogens as well as background rates of hepatocarcinogenecity. Boyault et al. (2007) reported that modulators of murine hepatocarcinogenesis, such as diet, hormones, oncogenes, methylation, imprinting, and cell proliferation/apoptosis are among multiple mechanistically associated factors that impact this target organ response in control as well as in treated mice, and suggested that there is no one simple paradigm to explain the differential strain sensitivity to hepatocarcinogenesis. Because of the variety of studies with differing protocols used to generate susceptibility data, direct comparisons among strains and stocks is problematic but in regard to susceptibility to carcinogenicity, the C3H/HeJ and C57BL/6J mouse have been reported to have up to a 40-fold difference in liver tumor multiplicity (Boyault et al., (2007).

However, as noted above, TCE causes liver tumors in C6C3F1 and Swiss mice with studies of TCE metabolites DCA, TCA, and CH suggesting that both DCA and TCA are involved in TCE-induced liver tumorigenesis. Many effects reported in mice after DCA exposure are consistent with conditions that increase the risk of liver cancer in humans and can involve GST Xi, histone methylation, and overexpression of insulin-like growth factor-II (IGF-II) (Caldwell and Keshava, 2006). The heterogeneity of liver phenotype observed in mouse models is also consistent with human HCC. These data lend support to the qualitative relevance of the mouse model for TCE-induced cancer risk.

Bannasch et al. (2003) made important observations that have implications regarding the differences in susceptibility between rodent and human liver cancer. They stated that:

> Although the classification of such nodular liver lesions in rodents as hyperplastic or neoplastic has remained controversial, persistent nodules of this type are considered neoplasms, designated as adenomas. In human pathology, the situation appears to be paradoxical because adenomas are only diagnosed in the noncirrhotic liver, yet a confusing variety terms avoiding the clearcut classification as an adenoma has been created for nodular lesions in liver cirrhoses, not withstanding that the vast majority hepatocellular carcinomas develop in cirrhotic livers. Even if a portion of these nodular lesions would be regarded as adenomas, being integrated into an adenoma-carcinoma sequence as observed in many animal experiments, clinical and epidemiological records of liver neoplasms, including both benign and malignant forms, would increase

> considerably. This would not only bring hepatic neoplasia further into focus of human neoplasia in general, but also shed new light on the classification of some chemicals producing high incidence of liver neoplasms in rodents, but appearing harmless to humans according to epidemiological evaluations solely based on the incidence of hepatocellular carcinoma in exposed populations.

Thus, in humans, only HCCs are recorded, but in animals, adenomas are counted as neoplasms, indicating that the scope of the problem of liver cancer in humans may be underestimated.

Tumor phenotype differences have been reported for several decades through the work of Bannasch et al. The predominant cell line of foci of altered hepatocytes (FAH) have excess glycogen storage early in development that appears to be similar to that shown by DCA treatment. Bannasch et al. (2003) reported that "the predominant glycogenotic-basophilic cell line FAH reveals that there is an overexpression of the insulin receptor, the IGF-1 receptor, the insulin receptor substrates-1/2 and other components of the insulin-stimulated signal transduction pathway." Bannasch et al. (2003) stated that foci of this type have increased expression of GST-π and insulin has also been shown to induce the expression of GST-pi, but that hyperinsulin-induced foci do not show increased GST-π. Cellular dedifferentiation during progression from glycogenotic to basophilic cell populations is associated with downregulation in insulin signaling. The amphophilic-basophilic cell lineage of peroxisome proliferators and hepadnaviridae were reported to have foci that mimic effects of thyroid hormone with mitochondrial proliferation and activation of mitochondrial enzymes. Bannasch et al. (2003) stated that:

> the unequivocal separation of 2 types of compounds, usually classified as initiators and promoters, remains a problem at the level of the foci because at least the majority of chemical hepatocarcinogens seem to have both initiating and promoting activity, which may differ in quantitative rather than qualitative terms from one compound to another…Whereas genetic mutations have been predominantly postulated to initiate hepatocarcinogenesis for many years, more recently epigenetic changes have been increasingly discussed as a plausible cause of the evolution of preneoplastic foci characterized by metabolic changes including the expression of GSTpi.

Su and Bannasch (2003) reported that glycogen-storing foci represent early lesions with the potential to progress to more advance glycogen-poor basophilic lesions through mixed-cell foci and resulting hyperproliferative lesions and are associated with HCC in man. Small-cell change (SCC) of liver parenchyma (originally called liver cell dysplasia of small cell size) is reported to share cytological and histological similarities to early well defined HCC. Close association between SCC and more advanced (basophilic) foci indicates that foci often progress to HCC through SCC in humans. SCC was reported to be present in all basophilic foci.

Previous studies were cited that showed that the biochemical phenotype of human FAH, mainly including glycogen storing clear cell foci and clear cell-predominated mixed cell foci, were observed in >50% of cirrhotic livers with or without HCC. FAH of clear and mixed cell types were observed in almost all livers bearing HCC, and in chronic liver diseases without HCC but at a lower frequency. Su and Bannasch (2003) reported that:

> the finding of mixed cell foci (MCF) mainly in livers with high-risk or cryptogenenic cirrhosis indicates that these are more advanced precursor lesions in man, in line with earlier observations in experimental animals. Considering their preferential emergence in cirrhotic livers of the high-risk group, their unequivocally elevated proliferative activity, and the resulting large size with frequent nodular transformation, we suggest that mixed cell populations are endowed with a high potential to progress to HCC in humans, as previously shown in rats.

In human HCC, irregular areas of liver parenchyma with marked cytoplasmic amphophilia, phenotypically similar to the amphophilic preneoplastic foci in rodent liver exposed to different hepatocarcinogenic chemicals (e.g., DHEA a peroxisome proliferator) or the hepadnaviruses, were reported to present in 45% of the specimens from cirrhotic livers examined. "However, more data are needed to elucidate the nature of the oncocytic and amphophilic lesions regarding their role in HCC development."

With respect to the ability respond to a mitogenic stimulus, differences between primate and rodent liver response to a powerful stimulus, such as partial hepatectomy, have been noted that indicate that primate and human liver respond differently (and much more slowly) to such a stimulus. Gaglio et al. (2002) reported after 60% partial hepatectomy in Rhesus macaques (*Macaca mulatto*), the surface area of the liver remnant was restored to its original preoperative value over a 30-day period. The maximal liver regeneration occurred between days 14 and 21, with thickening of liver cell plates, binucleation of hepatocytes, Ki-67 and PCNA expression (occurring in hepatocytes throughout the lobule at a maximum labeling index of 30%), and mitoses parallel increased most prominently between posthepatectomy days 14 and 30.

However, cytokines associated with inducing proliferation were elevated much earlier. TGF-α, IL-6, HGF, IL-6, and TNF-α mRNA persisted until Day 14, with peak elevations of IL-6 and TNF-α, occurring 24 hours later surgery, and IL-6 reduced to control levels by day 14. Gaglio et al. (2002) suggested that their results clearly indicate that the pattern and timing of liver regeneration observed in this nonhuman primate model are significantly different when comparing different species (e.g., peak expression of Ki-67 in a 60% partial hepatectomy model in rats occurs within hours following partial hepatectomy) and that the difference in timing and pattern of maximal hepatocellular regeneration cannot be explained simply by differences in size of animals (e.g., 60% partial hepatectomy in dogs produced liver regeneration peaks at 72 hours with weights approximating the weights of the Rhesus macaques). They noted that previous

studies in humans, who underwent 40–80% partial hepatectomy, reveal a similar delay in peak liver regeneration based on changes in serum levels of ornithine decarboxylase and thymidine kinase, further highlighting significant interspecies differences in liver regeneration.

For C57BL/6 X 129 mice, Fujita et al. (2001) reported that after partial hepatectomy, the liver had recovered >90% of its weight within 1 week. This difference in response to a mitogenic stimulus has impacts on the interpretations of comparisons between rodent and primate liver responses to chemical exposures which give a transient increases in DNA synthesis or cell proliferation such as PPARα agonists. Also, as stated above, the primate and human liver, while having a significant polyploidy compartment, do not have the extent of polyploidization and the early onset of that has been observed in the rodent. However, as noted by Lapis et al. (1995), exposure to DEN has proven to be a highly potent hepatocarcinogen in nonhuman primates, inducing malignant tumors in 100% of animals with an average latent period of 16 months when administered at 40 mg/kg i.p. every 2 weeks.

In regard to species extrapolation of epigenomic changes between humans and rodents, Weidman et al. (2007) cautioned that:

> Although we do predict some overlap between mouse and human candidate imprinted genes identified through our machine-learning approach, it is likely that the most significant criterion in species-specific identification will differ. This difference underscored the importance for increased caution when assessing human risk from environmental agents that alter the epigenome using rodent models; the molecular pathways targeted may be independent.

Despite species differences, the genome of the mouse has been sequenced and many transgenic mouse models are being used to study the consequences of gene expression modulation and pathway perturbation to study human diseases and treatments. However, the use of transgenic models must be used with caution in trying to determine to determine modes of action and the background effects of the transgene (including background levels of toxicity) and specificity of effects must be taken into account for interpretation of mode-of-action data, especially in cases where the knockout in the mouse causes significant liver necrosis or steatosis (Caldwell et al., 2008b; Caldwell and Keshava, 2006; Keshava and Caldwell, 2006). For the determination of effects of pathway perturbation and similarity to human HCC phenotype, mouse transgenic models have been particularly useful with tumors produced in such models shown to correlate with tumor aggressiveness and survival to human counterparts.

E.3.2.1. Similarities with Human and Animal Transgenic Models

Mice transgenic for transforming growth factor α (a member of the EGF family and a ligand for the ErfB receptors) develop HCCs (Farazi and DePinho, 2006). Compound TGFα and MYC transgenic mice show increase hepatocarcinogenesis that is associated with the disruption

of TGF-β1 signaling and chromosomal losses, some of which are syntenic to those in human HCCs that include the retinoblastoma (RB) tumor suppressor locus (Sargent et al., 1999).

Lee et al. (2004b) investigated whether comparison of global expression patterns of orthologous genes in human and mouse HCCs would identify similar and dissimilar tumor phenotypes, and thus allow the identification of the best-fit mouse models for human HCC. The molecular classification of HCC on the basis of prognosis in Lee et al. (2004a) was further compared with gene-expression profiles of HCCs from seven different mouse models (Lee et al., 2004b). Lee et al. (2004b) characterized the gene expression patters of 68 HCC from seven different mouse models; two chemically induced (Ciprofibrate and diethylnitrosamine), and four transgenic (targeted overexpression of Myc, E2F1, Myc and E2F1, and Myc and Tgfa in the liver). HCCs from some of these mice (MYC, E2F1, and MYC-E2F1 transgenics) showed similar gene-expression patterns to the ones of HCCs from patients with better survival. Murine HCCs derived for MYC-TGF-α transgenic model or diethylnitrosamine-treated mice showed similar gene-expression patterns to HCCs from patients with poor survival. The authors reported that Myc Tgfa transgenic mice typically have a poor prognosis, including earlier and higher incident rates of HCC development, higher mortality, higher genomic instability and higher expression of poor prognostic markers (e.g., AFP) and that Myc and Myc/E2f1 transgenic mice have relatively higher frequency of mutation in β-catenin (*Catnb*) and nuclear accumulation of β-catenin that are indicative of lower genomic instability and better prognosis in human HCC.

Lee et al. (2004b) indentified three distinctive HCC clusters, indicating that gene expression pattern of mouse HCC are clearly heterogeneous and reported that Ciprofibrate-induced HCCs and HCCs from Acox -/- mice were closely clustered and well separated from other mouse models. However, there are several issues regarding this study that give limitations to some of its conclusions regarding the Acox -/- mouse and Ciprofibrate treatment. The Acox -/- mouse is characterized by profound hepatonecrosis, which confounds conclusions regarding gene expression related to PPARα agonism made by the authors. There was very limited reporting of the animal models (DEN and Clofibrate) protocols used. Only three tumors were examined for Clofibrate treatment and it is unknown if the tumors were from the same animals. Similarly, only three tumors were examined from DEN treatment, which has been shown to produce heterogeneous tumors and to produce necrosis in some paradigms of exposure. Myc/E2F1 and E2F1 mice were split in both clusters that were compared with human HCCs. The authors used previously published data from Meyer et al. (2003) for tumors from $Acox1^{-1-}$ null mice, DENA-treated mice, and Ciprofibrate-treated mice.

Meyer et al. (2003) examined three tumors from two C57BL/6j mice fed Ciprofibrate for 19 months and three tumors from two C57BL/6j mice injected with DEN at 2–3 months, but the age at which tumors appeared was not given by the authors. Pooled mRNA from animals of varying age (5–15 months old) was used for controls. mRNAs that differed by twofold in tumors were reported to have: 60 genes upregulated and 105 genes downregulated in $Acox1^{-1-}$ null mice

tumors; 136 genes upregulated and 156 genes downregulated in Ciprofibrate-induced tumors; and 61 genes upregulated and 105 genes downregulated in DEN-induced tumors. The authors stated that "Each tumor class revealed a somewhat different unique expression pattern." There were "genes that were general liver tumor markers in all three types of tumors" with 38 genes commonly deregulated in all three tumor types. Of note, the cell cycle genes (CDK4, CDC25Am CDC7, and MAPK3) cited by Lee et al. (2004b) as being more highly expressed in DEN-induced tumors were not reported to be changed in DEN tumors in Meyer et al. (2003) or to be altered in the $Acox1^{-1-}$ null mice or mice treated with Ciprofibrate. Finally, the distinction between groups may be dominated by gene expression changes in a large number of genes that are related to PPAR activation, but not related to hepatocarcinogenesis.

Calvisi et al. (2004a) used transgenic mice to study pathway alterations and tumor phenotype and to further examine the premise that genomic alterations (genetic and epigenetic) characteristic of HCC can describe tumors into two broad categories, the first category characterized by activation of the Wnt/Wingless pathway via disruption of β-catenin function and chromosomal stability and the second by chromosomal instability. Increased coexpression of c-Myc with TGF-α or E2F-1 transgenic mice was reported to result in a dramatic synergistic effect on liver tumor development when compared with respective monotransgenic lines, including shorter latency period, and more aggressive phenotype. β-catenin activation is relatively common in HCCs developed in c-Myc and c-Myc/TGF-β1 transgenic mice and rare in the c-Myc/TGF-α transgenic line which also has genomic instability.

Calvisi et al. (2004a) also reported that β-catenin staining correlated with histopathologic type of liver tumors. Eosinophilic tumors with abnormal nuclear staining of β-catenin were predominant in neoplastic lesions characteristic of c-Myc and c-Myc/E2F1 lesions. Poorly differentiated HCCs with basophilic or clear-cell phenotypes developed more frequently in c-Myc/TGF-α and TGF-α mice and often showed a reduction or loss of β-catenin immunoreactivity. β-catenin mutation was associated with a more benign phenotype. These observations regarding tincture and aggressiveness are consistent with those of Bannasch (1996) and Carter et al. (2003). Calvisi et al. (2004a) noted that the relationship between β-catenin activation, tumor grade, and clinical outcome in human HCC remains controversial.

> There are studies that show a significant correlation between β-catenin nuclear accumulation, a high grade of HCC tumor differentiation, and a better prognosis, whereas others find that nuclear accumulation of β-catenin may be associated with poor survival or that it does not affect clinical outcome.

Calvisi et al. (2004b) reported that for E-cadherin, a variety of morphologenetic events, including cell migration, separation, and formation of boundaries between cell layers and differentiation of each cell layer into functionally distinct structures. Loss of expression of E-cadherin was reported to result in dedifferentiation, invasiveness, lymph node, or distant

metastasis in a variety of human neoplasms including HCC and that the role of E-cadherin might be more complex than previously believed.

> In order to elucidate the role of E-cadherin in the sequential steps of liver carcinogenesis, we have analyzed the expression patterns of E-cadherin in a collection of preneoplastic and neoplastic liver lesions from c-Myc, E2F1, c-Myc/TGF-α and c-Myc/E2F1 transgenic mice. In particular, we have investigated the relevance of genetic, epigenetic, and transcriptional mechanisms on E-cadherin protein expression levels. Our data indicate that loss of E-cadherin contributes to HCC progression in c-Myc transgenic mice by promoting cell proliferation and angiogenesis, presumably through the upregulation of HIF-1α and VEGF proteins.

The c-Myc line was most like wild-type and lost E-cadherin in the tumors. c-Myc/TGF-α dysplastic lesions were reported to show overexpression of E-cadherin mainly in pericentral areas with E2F1 clear cell carcinoma showed intense staining of E-cadherin. Reduction or loss of E-cadherin expression is primarily determined by loss of heterozygosity at the E-cadherin locus or by its promoter hypermethylation in human HCC. Calvisi et al. (2004b) determined the status of the E-cadherin locus and promoter methylation in wild-type livers and tumors from transgenic mice by microsatellite analysis and methylation specific PCR, respectively.

> Wild-type livers and HCCs, regardless of their origins, showed the absence of LOH at the E-cadherin locus. E-cadherin promoter was not hypermethylated in wild-type, c-Myc/TGF-α and E2F1 livers. No E-cadherin promoter hypermethylation was detected in c-Myc and c-Myc/E2F1 HCCs with normal levels of E-cadherin protein. In striking contrast, seven of 20 (35%) of c-Myc and two of four (50%) c-Myc/E2F1 HCCs with downregulation of E-cadherin displayed E-cadherin promoter hypermethylation. These results suggest that promoter hypermethylation might be responsible for E-cadherin downregulation in a subset of c-Myc and c-Myc/E2F1 HCCs…The molecular mechanisms underlying down-regulation of E-cadherin in c-Myc tumors remain poorly understood at present. No LOH at the E-cadherin locus was detected in the c-Myc HCCs whereas only a subset of c-Myc tumors displayed hypermethylation of the E-cadherin promoter. Furthermore, no association was detected between E-cadherin downregulation and protein levels of transcriptional repressors, Snail, Slug or the tumor suppressor WT1, in disagreement with the finding that overexpression of Snail suppresses E-cadherin in human HCC…E-cadherin might play different and apparently opposite roles, which depend on specific tumor requirements in both human and murine liver carcinogenesis.

Importantly, the results of Calvisi et al. (2004b) showed that hypermethylation of promoters can be associated with downregulation of a gene in mouse liver tumors similar to human HCC and that tumors can have the same behavior with methylation change as with loss of hetererozygosity.

This report also gave evidence of the usefulness of the mouse model to study human liver cancer as it shows the similarity of dysfunctional regulation in mouse and human cancer and the heterogeneity within and between mouse lines tumors with differing dysfunctions in gene expression. These findings parallel human cancer where there is heterogeneity in tumors from one person and every tumor has its own signature. Finally, this report correlates differing pathway perturbations with mouse liver phenotypes similar to those reported in experimental carcinogenesis models and for TCE and its metabolites.

Farazi and DePinho (2006) suggested that:

> as comparative array CGH analysis of various murine cancers has shown that such aberrations often target syntenic loci in the analogous human cancer type, we further suggest that comparative genomic analysis of available mouse model of mouse HCC might be particularly helpful in filtering through the complex human cancer genome. Ultimately, mouse models that share features with human HCCs could serve as valuable tools for gene identification and drug development. However, one needs to keep in mind key differences between mice and humans. For example, as noted in certain human HCC cases, telomere shortening might drive the genomic instability that enables the accumulation of cancer-relevant changes for hepatocarcinogenesis. As mice have long telomeres, this aspect of hepatocarcinogenesis might be fundamentally different between the species and provide additional opportunities for model refinement and testing of this mechanism through use of a telomere deficient mouse model. These and other cross-species difference, and limitations in the use of human cell-culture systems, must be considered in any interpretation of data from various model systems (Farazi and DePinho, 2006).

Thus, these mouse models of liver cancer inductions are qualitatively able to mimic human liver cancer and support the usefulness of mouse models of cancer.

E.3.3. Hypothesized Key Events in HCC Using Animal Models

E.3.3.1. Changes in Ploidy

As stated in Section E.1.1, increased polyploidization has been associated with numerous types of liver injury and appears to result from exposure to TCE and its metabolites as well as changes in the number of binucleate cells. Hortelano et al. (1995) reported that cytokines and NO can affect ploidy and further suggest a role of these changes for carcinogenesis in general. Vickers and Lucier (1996) noted that while both DEN and 17 α-ethinylestradiol have been reported to enhance the proportion of diploid hepatocytes, initiators like *N*-nitrosomorpholine are reported to increase the proportion of hypertrophied and polyploidy hepatocytes. The relationship of such changes to cancer induction has been studied in transgenic mouse models and in models involved with mitogens of differing natures.

Melchiorri et al. (1993) reported the response pattern of the liver to acute treatment with primary mitogens in regard to ploidy changes occurring in rat liver following two different types

of cell proliferation: compensatory regeneration induced by surgical partial hepatectomy (PH) and direct hyperplasia induced by the mitogens lead nitrate and Nafenopin (a PPARα agonist) in 8-week-old male Wistar rats. Feulgen stain was used and DNA content was quantified by image cytometry in mononucleated and binucleated cells. Mitotic index was determined in the same samples. The term "diploid" was used to identify cells with a single, diploid nucleus and tetraploid for cells containing two diploid nuclei or one tetraploid nucleus referred (bi- and mononucleated, respectively). Octoploid cells were identified as either binucleate or mononucleate.

> During liver regeneration following surgical PH an increase in the mitotic index with a peak at 24 hours was observed. The most striking effect associated with the regenerative response was the almost complete disappearance of binucleate cells, tetraploid (2 X 2c) as well as octoploid (4 X 2c) with only < 10% of the control values being present 3 days after PH…Concomitantly, an increase in mononucleate tetraploid (4c) as well as mononucleate octoploid (8c) cells was observed, resulting at 3 days after PH in a population made up of almost entirely (98%) by mononucleated cells.

Lead nitrate treatment was reported to induce rapid increases in the formation of binucleated cells occurring 3 days after treatment, their number accounting for 40% of the total cell population vs. 22% binucleate cells in control rats and 2% in PH animals killed at the same time point. The increased binuclearity was reported to be observed only in the 4 × 2c cells (25 vs. 6% of the controls) and in 8 × 2c cells (3.7 vs. 0.1% of controls). The increase in 4 × 2c and 8 × 2c cells was reported to be accompanied by a concomitant reduction in 2 × 2c cells with the change induced in cellular ploidy by lead nitrate resulting in 37% of cells being either 8c or 16c. However, at the same time point, cells having a ploidy higher than 4c were reported to account for only 11% in PH rats and 9% in control animals. Changes in the ploidy pattern were reported to be preceded by an increased mitotic activity, which was maximal 48 hours after treatment with lead nitrate. The increase in mitotic index in lead nitrate-treated rats was associated with a striking increase in the labeling index of hepatocytes (60.1 vs. 3% of control rats) and to an almost doubling of hepatic DNA content in 3 days after lead nitrate.

Melchiorri et al. (1993) concluded that the entire cell cycle appeared to be induced by lead nitrate but that the finding of a high increase of binucleated cells suggested that lead nitrate-induced liver growth, unlike liver regeneration induced by partial hepatectomy, was characterized by an uncoupling between cell cycle and cytokinesis. This raised questions on whether lead nitrate-induced liver growth resulted in a true increase in cell number or is only the expression of an increased hepatocyte ploidy. They reported that part of the increase in DNA content observed 3 days after lead nitrate was indeed expression of polyploidizing process due to acytokinetic mitoses, but that a consistent increase in cells number (+26%) was also induced by lead nitrate treatment.

After Nafenopin treatment, Melchiorri et al. (1993) reported that the increase in DNA content was increased 22% over controls and was much lower than induced by lead nitrate and that Nafenopin did not induce significant changes in binucleated cell number. However, a shift towards a higher ploidy class (8c) was reported to be observed following Nafenopin and the 21% increase in DNA content seen after Nafenopin treatment was almost entirely due to increase in the ploidy state with only 7% increase in cell number.

Melchiorri et al. (1993) examined whether hepatocytes characterized by high ploidy content (highly differentiated cells) would be preferentially eliminated by apoptosis. An increase in apoptotic bodies was reported to be associated with the regression phase after lead nitrate treatment (when liver mass is reduced) but despite the elimination of excess DNA, the changes in ploidy distribution induced by lead nitrate were found to persist suggested that polyploidy cells were not preferentially eliminated by apoptosis during the regression phase of the liver. Melchiorri et al. (1993) noted that other studies in rats exposed to the mitogen, cyproterone acetate (CPA), and the peroxisome proliferator, MCP, also reported a very strong decline in binucleated cells with a concomitant increase in mononucleated tetraploid cells in the liver similar to the pattern described after partial hepatectomy.

Lalwani et al. (1997) reported the results of 1,000 ppm WY-14,643 exposure in male Wistar rats after 1, 2, and 4 weeks and suggested that an early wave of nuclear division occurred at the early stages of exposure without cumulative effects on cell proliferation. Consistent with hepatomegaly, WY-14,643-treated rats were reported to exhibit multifocal hepatocellular hypertrophy and karyomegaly by routine microscopic analysis. For binucleated hepatocytes, there were no reported differences between WY-14,643-treated and control groups for days 4 and 11 but an increase in the number at day 25 in WY-14,643-treated animals compared to controls. Increases in the diameter of nuclei were shown by WY-14,643-treatment from days 11 and 25 with increasing numbers of cells displaying larger nuclear diameters. The mitotic index was reported not to be significantly changed in WY-14,643-treated rats compared to controls. Mitotic figures did not appear to survive the treatment necessary for flow cytometric analyses. PCNA was increased on day 4 in WY-14,643-treated animals compared to controls whereas no differences were found on days 11 and 25.

However, immunohistochemistry was reported to show remarkable increases in BrdU-labeled nuclei in liver sections after 4 days of labeling, with the populations of BrdU-labeled cell declining over the course of treatment. The labeling index was high and approximately 80% of the BrdU-labeled cells were in periportal areas. PCNA-expressing cells were increased in the periportal area of the liver. Intense nuclear staining of PCNA was evident as an indicator of DNA replication in S phase. Microscopic examination showed BrdU labeling only in periportal hepatocytes, whereas no significant labeling was observed in nonparenchymal cells, indicating that the replicative activity was confined to the liver cells.

Lalwani et al. (1997) suggested that their results showed that events related to cell proliferation occur in the initial phase of WY-14,643 treatment in rats but not followed by changes in the rate of DNA synthesis as the treatment progressed. They note that Marsman et al. (1988) observed constant increases in DNA synthesis by [^{3}H]-thymidine authoradiography with up to 1 year of continuous administration of WY-14,643, whereas the rate of DNA synthesis or the BrdU labeling index in their study declined after the first 4 weeks of treatment. They suggest that the increased percentage of cells appearing in G2-M phase and the analysis of liver nuclear profiles suggest that the progression of these additional cells (i.e., cells that are stimulated to enter the cell cycle by the test agent) through the cell cycle is arrested in the late stages of the cell cycle. They state:

> Unlike BrdU labeling, which demonstrated DNA synthesis activity over the 4-day labeling period, the PCNA labeling index represents levels of the protein product at an interval post treatment. PCNA expression in cells exposed to chemicals or to WY may not provide true representation of S phase or proliferative activity because PCNA-expressing nuclei were also found in G0=G1 and G2-M phases.

Lalwani et al. (1997) concluded that cell proliferation alone does not appear to constitute a determining process leading to tumors in most tissues and sustained cell replication may not be a primary feature of peroxisome proliferator-induced hepatocarcinogenesis.

Miller et al. (1996) noted that studies with MCP in Alpk:AP rats indicate that DNA synthesis occurs primarily in one hepatocyte subpopulation as defined by ploidy status, the binucleated tetraploid (2 × 2N) hepatocytes, and that this preferential hepatocyte DNA synthesis is manifested by dramatic alterations in hepatocyte ploidy subclasses (i.e., significant increases in mononucleate tetraploid [4N] hepatocytes concomitant with decreases in 2 × 2N hepatocytes).

They reported results in male F344 rats that were 13 weeks old (an age in which polyploidization had reached a plateau) exposed to 1,000 ppm WY-14,643 and MCP (gavage via corn oil at 8 mg/mL or 25 mg/kg MCP once daily) for 2, 5, and 10 days (n = 4). WY-14,643 and MCP were reported to induce significant increases in the octoploid hepatocyte class that coincided with decreases in the tetraploid hepatocyte class. However, MCP did not induce this shift until day 5 of exposure. These results showed an approximate doubling of mononuclear octoploid (8N) hepatoctyes but still a very low number of the total hepatocyte population that did not reach >7% and was still only approximately twice that of control values. Thus, this finding does not indicate a very large target population. There was no real effect on 4N hepatocytes due to these treatments and the percent of hepatocytes that were 4N stayed ~70% and were thus the major cell type in the liver. Miller et al. (1996) noted the importance of maturation and/or strain for these analyses; there are maturation-dependent differences in the distribution and mitogenic sensitivity of hepatoctyes in the various subclasses.

Hasmall and Roberts (2000) noted that despite their differing abilities to induce liver cancer, both DCB (a nonhepatocarcinogen in F344 rats) and DEHP, at the doses and routes used in the NTP bioassays, induced similar profiles of S-phase LI. A large and rapid peak during the first 7 days (1,115 and 1,151% of control for DEHP and DCB, respectively) was followed by a return to control levels. They suggested that the size of the S-phase response does not necessarily determine hepatocarcinogenic risk and that the subpopulation in which S-phase is induced may be a better correlate with subsequent hepatocarcinogenecity.

They compared the effects on polyploidy/nuclearity and on the distribution of S-phase labeled cells with ETU, the peroxisome proliferator: MCP and phenobarbitone. Male F334 rats 7–9 weeks old were exposed to MCP (0.1% in diet), ETU (83 ppm in diet), or phenobarbitone (500 mg/mL in drinking water) for 7 days. The number of rats for the 7-day study was not given by the authors. Hasmall and Roberts (2000) reported that treatment of rats with MCP, ETU, or phenobarbitone for 7 days had no significant effect on the ploidy profile as compared with corn oil controls (data not shown) but that MCP and phenobarbitone did induce significant changes in nuclearity. MCP reduced the 2 × 2N population and increased the 8N population. Phenobarbitone similarly increased the proportion of cells in the 4N population. ETU had no effect on the nuclearity profile as compared with control. However, what the authors describe for their results in polidy and nuclearity are different than those presented in their figures. There were significant differences between controls that the authors did not characterize and there appeared to be a greater difference between controls than some of the treatments.

Gupta (2000) reported that in transgenic mice with overexpression of TGF-α, liver-cell turnover increases, along with the onset of hepatic polyploidy, whereas HCC originating in these animals contain more diploid cells. Coexpression of c-Myc and TGF-α transgenes in mouse hepatocytes was associated with greater degrees of polyploidy as well as increased development of HCC. Gupta (2000) noted that in the presence of ongoing liver injury and continuous depletion of parenchymal cells, hepatic progenitor cells (including oval cells) are eventually activated but what roles polyploid cells play in this process requires further study. In the working model by Gupta (2000), sustained disease by chronic hepatitis, metabolic disease, toxins, etc., may lead to hepatocyte polyploidy and loss, and the emergence of rapidly cycling progenitor or escape cell clones with the onset of liver cancer.

Conner et al. (2003) described the development of transgenic mouse models in which E2F1 and/or c-Myc was overexpressed in mouse liver. The E2F1 and c-Myc transcription factors are both involved in regulating key cellular activities including growth and death and, when overexpressed, are capable of driving quiescent cells into S-phase in the absence of other mitogenic stimuli and are potent inducers of apoptosis operating at least through one common pathway involving p53. Deregulation of their expression is also frequently found in cancer cells (Conner et al., 2003). Conner et al. (2003) reported that although both c-Myc and E2F1 mono-transgenic mice were prone to liver cancer, E2F1 mice developed HCC more rapidly and with a

higher frequency and that the combined expression of these two transcription factors dramatically accelerated HCC growth compared to either E2F1 or c-Myc mono-transgenic mice. All three transgenic lines were reported to show a low but persistent elevation of hepatocyte proliferation before an onset of tumor growth. Ploidy was shown to be affected differently by c-Myc and E2F1, and suggested distinct differences by which these two transcription factors control liver proliferation/maturation. Both transgenic alterations induced liver cancer but had differing effects on polyploidization suggestive that liver cancer can arise from either type of mature hepatocyte.

c-Myc single-transgenic mice showed a continuous high cell proliferation that preceded the appearance of preneoplastic lesions, which was also true, although to a lesser extent, in the E2F1 mice. At 15 weeks of age, all of the transgenic mouse lines were reported to have a high incidence (>60%) of hepatic dysplasia with mitotic indices equivalent in c-Myc/E2F1, and c-Myc livers, but twofold higher than the mitotic index in E2F1 and very low in wild-type mice. Thus, the combination of the two transgenes did not have an additive effect on proliferation. An analysis of the DNA content in hepatocyte nuclei isolated from 4- to 15-week-old mice was reported to show that in young wild-type livers, the majority of nuclei had a diploid DNA content with a smaller proportion of tetraploid nuclei. As the mice aged, the number of tetraploid and octoploid nuclei increased consistent with the previous findings of others.

However, c-Myc mice were reported to demonstrate a premature polyploidization with the number of 2N nuclei in c-Myc livers almost 2-fold less, while the proportion of 4N nuclei increased >2.5-fold at 4 weeks of age. The most prominent ploidy alteration was an increase in the fraction of hepatocytes with octaploid nuclei (~200-fold higher). The percentage of polyploidy cells was reported to continue to rise in 15-week-old c-Myc livers. The majority of hepatocytes had nuclei with 4N and 8N DNA content, with an attendant increase in binucleated hepatocytes and increase in average cell size.

In striking contrast, E2F1 hepatocytes were reported not to undergo normal polyploidization with aging. The majority of E2F1 nuclei were reported to remain in the diploid state and to be almost identical in E2F1 mice at 4 and 15 weeks of age. The percentage of binucleated hepatocytes was also reduced. In c-Myc/E2F1 mice, the age-related changes in ploidy distribution were reported to resemble those found in both c-Myc and in E2F1 single transgenic mice.

At a young age, c-Myc/E2F1 mice, similar to E2F1 mice, were reported to retain significantly more diploid nuclei than c-Myc mice. However, as mice aged, the majority of c-Myc/E2F1 hepatocytes, similar to c-Myc cells but in contrast to findings in E2F1 cells, became polyploid. Consistent with a more progressive polyploidization, the DNA content was significantly higher in both c-Myc/E2F1 and c-Myc livers. Conner et al. (2003) reported that other known modulators of ploidy in the liver are the tumor suppressor p53, pRb, and the cell

cycle inhibitor p21 as well as genes involved in the control of the cell cycle progression such as cyclin A, cyclin B, cyclin D3, and cyclin E.

Along with increased liver cancer, Conner et al. (2003) noted that the C-Myc mice also experienced a persistent liver injury as evidenced by significant elevation of circulating levels of AST, ALT, and ALP along with the appearance of a frequent oval/ductular proliferation. However, oval cell proliferation may be a marker of hepatocyte damage but not be the cells responsible for tumor induction (Tarsetti et al., 1993). Conner et al. (2000) reported that if E2F1 is overexpressed in the liver, there is both oncogenic and tumor-suppressive properties. In regard to liver morphological changes, E2F1 transgenic mice were reported to uniformly develop pericentral dysplasia and foci adjacent to portal tracts followed by the abrupt appearance of adenomas and subsequent malignant conversion with all of the animals having foci by 2–4 months, and by 8–10 months, most having adenomas with dysplastic changes remaining confined to the pericentral regions of the liver lobule.

In regard to phenotype, the majority of the foci were composed of small round cells, with clear-cell phenotype but eosinophilic, mixed, and basophilic foci were also seen. In adenomas with malignant transformation to HCC, there appeared to be high mitotic indices, blood vessel invasion, and central collection of deeply basophilic cells with large nuclei giving a "nodule-in-nodule" appearance. Macrovesicular hepatic steatosis was first noted in some E2F1 transgenic livers at 6–8 months, and by 10–12 months, 60% of animals had developed prominent fatty change. Hepatic steatosis has been noted in several transgenic mouse models of liver carcinogenesis (Conner et al., 2000). These results raise interesting points of regional difference in tumor formation which can be lost in analyses using whole liver and that the phenotype of foci and tumors are similar to those seen from chemical carcinogenesis. The occurrence of hepatotoxicity in these transgenic mice is also of note.

E.3.3.2. Hepatocellular Proliferation and Increased DNA Synthesis

Caldwell et al. (2008b) presented a discussion of the role of proliferation in cancer induction. They stated that:

> in the case of CCl4 exposure, hepatocyte proliferation may be related to its ability to induce liver cancer at necrogenic exposure levels, but the nature of this proliferation is fundamentally different from peroxisome proliferators or other primary mitogens that cause hepatocyte proliferation without causing cell death (Columbano and Ledda-Columbano, 2003; Ledda-Columbano et al., 2003; Ledda-Columbano et al., 1998; Menegazzi et al., 1997; Coni et al., 1993; Ledda-Columbano et al., 1993). After initiation with a mutagenic agent, the transient proliferation induced by primary mitogens has not been shown to lead to cancer-induction, while partial hepatectomy or necrogenic treatments of CCl4 result in the development of tumors (Gelderblom et al., 2001; Ledda-Columbano et al., 1993).

Roskams et al. (2003) noted that partial hepatectomy does not cause HCC in normal mice without initiation. Melchiorri et al. (1993) reported that a series of studies has shown that acute proliferative stimuli provided by primary mitogens, unlike those of the regenerative type such as those elicited by surgical or chemical partial hepatectomy, do not support the initiation phase and do not effectively promote the growth of initiated cells (Columbano et al., 1990; Ledda-Columbano et al., 1989; Columbano et al., 1987). They noted that the finding that most of these chemicals, with the exception of WY, induce only a very transient increase in cell proliferation raises the question whether such a transient induction of liver cell proliferation might be related to liver cancer appearing 1–2 years later. They noted that mitogen-induced liver growth differs from compensatory regeneration in several aspects: (1) it does not require an increased expression of hepatocyte growth factor mRNA in the liver; (2) it is not necessarily associated with an immediate early genes such as c-Fos and c-Jun; and (3) it results in an excess of tissue and hepatic DNA content that is rapidly eliminated by apoptotic cell death following withdrawals of the stimulus.

Other studies have questioned the importance of a brief wave of DNA synthesis in induction of liver cancer. Chen et al. (1995) noted that Jirtle et al. (1991) and Schulte-Hermann et al. (1986) reported that during a 2-week period of treatment with lead, DNA synthesis was increased most in centrolobular hepatocytes and that the predominantly centrilobular distribution of the labeled nuclei may have been due largely to the brief wave of mitogenic response, because from the fifth day onward, DNA synthesis activity returned to control level even though lead nitrate treatment continued. They concluded that sustained cell proliferation may be more important than a brief wave of increased DNA synthesis. Chen et al. (1995) also noted that a number of different agents acting via differing modes of action will induce periportal proliferation.

Vickers and Lucier (1996) reported that mitogenic response induced by acute 17 α-ethinylestradiol administration is randomly distributed throughout the hepatic lobule, while continuous administration increases the proportion of diploid cells. Richardson et al. (1986) reported that the lobular distribution of the correlation of hepatocyte initiation and akylation reported in their model of carcinogenicity did “not support that early proliferation is associated with cancer as at 7 days there is a transient increase in the lobes least likely to get a tumor and no difference between the lobes at 14 and 28 days DEN although there is a difference in tumor formation between the lobes.” Thus, cells undergoing DNA synthesis may not be in the same zone of the liver where other hypothesized “key events” take place.

Tanaka et al. (1992) noted that the distribution of hepatocyte proliferation in the periportal area was in contrast to the distribution of peroxisome proliferation in the centrilobular area of Clofibrate-treated rats. Melnick et al. (1996) noted that replicative DNA synthesis commonly has been evaluated by measurement of the fraction of cells incorporating BrdU or

tritiated thymidine into DNA during S-phase of the cell cycle (S-phase labeling index), but that the S-phase labeling index would not be identical to the cell division rate when replication of DNA does not progress to formation of two viable daughter cells. “The general view at an international symposium on cell proliferations and chemical carcinogenesis was that although cell replication is involved inextricably in the development of cancers, chemically enhanced cell division does not reliably predict carcinogenicity” (Melnick et al., 1993). They noted that the finding that enzyme-altered hepatic foci were not induced in rats fed WY-14,643 for 3 weeks followed by partial hepatectomy indicates that early high levels of replicative DNA synthesis and peroxisome proliferation are not sufficient activities for initiation of hepatocarcinogenesis.

Baker et al. (2004) reported that, similar to the pattern of transient increases in DNA synthesis reported for TCE metabolites, Clofibrate exposure induced the upregulation of a variety of cell proliferation-associated genes (e.g., G2/M specific cyclin B1, cyclin-dependent kinase 1, DNA topoisomerase II alpha, c-Myc protooncogene, pololike serien-threonine protein kinase, and cell divisions control protein 20) began on or before day 1 and peaked at some point between days 3 and 7. By day 7, cell proliferation genes were downregulated. The chronology of this gene expression agrees with the histologic diagnosis of mitotic figures in the tissue, where an increase in mitotic figures was detected in the day 1 and most notably day 3 high and low-dose groups. However, by day 7, the incidence of mitotic figures had decreased. The clustering of genes associated with the G2/M transition point suggests that in the rats, the polyploid cells arrested at G2/M are those that are proceeding through the cell cycle.

A dose-response for increased DNA-synthesis also seems to be lacking for the model PPARα agonist, WY-14,643 suggesting that the transient increases in DNA synthesis reported by Eacho et al. (1991) for this compound at lower levels that then increase later at necrogenic exposure levels, are not related to its carcinogenic potential. Wada et al. (1992) reported that in male F344 rats exposed to a range of WY-14,643 concentrations (5–1,000 ppm), liver weight gain occurred at the lowest dose that gave a sustained response for many weeks but gave increased cell labeling only in the first week. Peroxisomes proliferation, as measure by electron microscopy, increases started at 50 ppm exposures. By enzymatic means, peroxisomal activities were elevated at the 5 ppm dose. Of note is the reported difference in distribution in hepatocellular proliferation, which was not where the hypertrophy or where the lipofuscin increases were observed. The authors noted that these data suggest that 50 and 1,000 ppm WY-14,643 should give the same carcinogenicity if peroxisome proliferation or sustained proliferation are the “key events.”

The study of (Marsman et al., 1992) is very important in that it not only shows that clofibric acid (another PPAR α agonist) does not have sustained proliferation, but it also shows that it and WY-14,643 at 50 ppm did not induce apoptosis in rats. It is probable that use of WY-14,643 at high concentrations may induce apoptosis in a manner not applicable to other peroxisome proliferators or to treatment with WY-14,643 at 50 ppm. This study also confirmed

that exposure to WY-14,643 at 50 ppm and WY-14,643 at 1,000 ppm induces similar effects in regards to hepatocyte proliferation and peroxisomal proliferation.

The study by Eacho et al. (1991) also gave a reference point for the degree of hepatocytes undergoing transient DNA synthesis from WY-14,643 and Clofibrate and how much smaller it is for TCE and its metabolites, which generally involve <1% of hepatocytes.

> The labeling index of BrdU was 7.2% on day 3 and 15.5% on day 6 after clofibric acid but by day 10 and 30 labeling index was the same as controls at ~1-2%.…For WY the labeling index was 34.1% at day 3 and 18.6% at day 6. At day 10 the labeling index was 3.3% and at day 30 was 6%, representing 6.6- and 15-fold of respective controls. Control levels were ~0.5 to 1%.…The labeling index was increased to 32% by 0.3% LY171883 and to 52% by 0.05% Nafenopin. The 0.005% and 0.1% dietary doses of WY increased the 7 day labeling index to a comparable level (55% - 58%).

Yeldandi et al. (1989) reported that until foci appear, cell proliferation has ceased to increase over controls after the first week for Ciprofibrate-induced hepatocarcinogenesis. The results also showed the importance of using age-matched controls and not pooled controls for comparative purposes of proliferation as well as how low proliferative rates are in control animals.

The results of Barrass et al. (1993) are important in suggesting that age of animals is important when doing quantitation of labeling indexes. Studies such as that conducted by Pogribny et al. (2007) that only give the replication rate as a ratio to control will make the proliferation levels look progressive when, in fact, they are more stable with time as it is just the controls that change with age as a comparison point.

E.3.3.3. Nonparenchymal Cell Involvement in Disease States Including Cancer

The recognition that not only parenchymal cells but also nonparenchymal cells play a role in HCC has resulted in studies of their role in initiation as well as progression of neoplasia. The role of the endothelial cell in controlling angiogenesis, a prerequisite for neoplastic progression, and the role of the Kupffer cell and its regulation of the cytokine milieu that controls many hepatocyte functions and responses have been reported. However, as pointed out by Pikarsky et al. (2004) and by the review by Nickoloff et al. (2005), the roles of inflammatory cytokines in cancer are context- and timing-specific and not simple. For TCE, nonparenchymal cell proliferation has been observed after inhalation (Kjellstrand et al., 1983a) and gavage (Goel et al., 1992) exposures of ~4 weeks duration.

E.3.3.3.1. Epithelial Cell Control of Liver Size and Cancer—Angiogenesis

The epithelium is key in controlling restoration after partial hepatectomy and not surprisingly HCC growth. Greene et al. (2003) hypothesized that the control of physiologic

organ mass was similar to the control of tumor mass in the liver and that specifically, the proliferation of hepatocytes after partial hepatectomy, like the proliferations of neoplastic cells in tumors, requires the synthesis of new blood vessels to support the rapidly increasing mass. They reported that a peak in hepatocyte production of vascular endothelial growth factor (VEGF), an endothelial mitogen, corresponds to an increase of VEGF receptor expression on endothelial cells after partial hepatectomy and the rate of endothelial proliferation. Fibroblast growth factor and transforming growth factor-alpha (TGfox), which stimulate endothelial cells, are secreted by hepatoctyes 24 hours after partial hepatectomy. However, endothelial cells were reported to secrete hepatocyte growth factor, a potent hepatocyte mitogen, that is also proangiogenic. The secretion of transforming growth factor-beta by (TGfox) endothelial cells 72 hours after partial hepatectomy was reported to inhibit hepatocyte proliferation. Thus, Greene et al. (2003) suggested that endothelial cells and hepatocytes of the regenerating liver influence each other, and both populations are required for the regulation of the regenerative process.

E.3.3.3.2. Kupffer Cell Control of Proliferation and Cell Signals, Role in Early and Late Effects

Vickers and Lucier (1996) have reported that Kupffer cells are increased in number in preneoplastic foci but are decreased in HCC, and that other studies have demonstrated that both sinusoidal endothelial cells and Kupffer cells within HCC cells in humans stain positive for mitotic activity although the number of nonparenchymal cells compared to parenchymal cells may be reduced. Lapis et al. (1995) reported that Kupffer cells contain lysozyme in their cytoplasmic granules, vacuoles, and phagosomes, some cells show a positive reaction in the rough endoplasmic reticulum, perinuclear cisternae, and the Golgi zone, and that in human monocytes, the lysozyme is colocalized with the CD68 antigen and myeloperoxidase. They also reported that, in rodent hepatocarcinogenesis, increased numbers of Kupffer cells were observed in preneoplastic foci, whereas abnormally low numbers were present following progression to HCC. They also noted that "the Kupffer cell count in human HCC has also been shown to be very low and varies with different histological form." They reported that for monkey HCCs, the proportion of endothelial elements remained constant (the parenchymal/endothelial cell ratio); however, there was a striking reduction in the areas occupied by Kupffer cells. While healthy control livers contained the highest number of Kupffer cells, in the tumor-bearing cases, the nonneoplastic, noncirrhotic liver adjacent to the HCC nodules had a significantly lower number of Kupffer cells and the number decreased further in the nonneoplastic portions of cirrhotic livers. Within HCC nodules, the Kupffer cell count was greatly reduced with no significant changes observed between the cirrhotic areas and the carcinomas; however, the tumors contained fewer lysozyme and CD68 positive cells. Lapis et al. (1995) noted that:

since other cell types within the liver sinusoids (monocytes and polypmorphs) and portal macrophage were also positive, it was important to identify the star-like morphology of the Kupffer cells. The results of the two independent observers assessment of the morphology and enumeration of Kupffer cells were quite consistent and differed by only 3%." "The loss of Kupffer cells in the HCC may possibly result from capillarization of the sinusoids, which has been observed during the process of liver cirrhosis and carcinogenesis. Capillarization entails the sinusoidal lining endothelial cells losing their fenestrations.

E.3.3.3.3. Nf-kB and TNF-α—Context, Timing and Source of Cell Signaling Molecules

A large body of literature has been devoted to the study of nuclear factor κ B for its role not only in inflammation and a large number of other processes, but also in carcinogenesis. However, the effects of these cytokines are very much dependent on their cellular context and the timing of their modulation. As described by Adli and Baldwin (2006):

> The classic form of NF-kB is composed of a heterodimer of the p50 and p65 subunits, which is preferentially localized in the cytoplasm as an inactive complex with inhibitor proteins of the IkB family. Following exposure to a variety of stimuli, including inflammatory cytokines and LPS, IkBs are phosphorylated by the IKKα/β complexes then accumulate in the nucleus, where they transcriptionally regulate the expression of genes involved in immune and inflammatory responses.

The five members of the mammalian NF-kB family, p65 (RelA), RelB, c-Rel, P50/p105 (NF-KB1), and p52/p100 (NF-kB2), exist in unstimulated cells as homo- or heterodimers bound to IkB family proteins. Transcriptional specificity is partially regulated by the ability of specific NF-kB dimmers to preferentially associate with certain members of the IkB family. Individual NF-kB responses can be characterized as consisting of waves of activation and inactivation of the various NF-kB members (Hayden and Ghosh, 2004). While the function of NF-kB in many contexts have been established, it is also clear that there is great diversity in the effects and consequences of NF-kB activation with NF-kB subunits not necessarily regulating the same genes in an identical manner and in all of the different circumstances in which they are induced. The context within which NF-kB is activated, be it the cell type or the other stimuli to which the cell is exposed, is therefore, a critical determinant of the NF-kB behavior (Perkins and Gilmore, 2006).

Balkwill et al. (2005) reported that:

> the NF-κB pathway has dual actions in tumor promotion: first by preventing cell death of cells with malignant potential, and second by stimulating production of proinflammatory cytokines in cells of infiltrating myeloid and lymphoid cells. The proinflammatory cytokines signal to initiated and/or otherwise damaged epithelial cells to promote neoplastic cell proliferation and enhance cell survival.

> However, the tumor promoting role of NF-κB may not always predominate. In some cases, especially early cancers, activation of this pathway may be tumor suppressive (2004). Inhibiting NF-κB in keratinocytes promotes squamous cell carcinogenesis by reducing growth arrest and terminal differentiation of initiated keratinocytes (Seitz et al., 1998).

Other inflammatory mediators have also been associated with oncogenesis. Balkwill et al. (2005) reported that TNFα is frequently detected in human cancers (produced by epithelial tumor cells, as in for instance, ovarian and renal cancer) or stromal cells (as in breast cancer). They also report that the loss of hormonal regulation of IL-6 is implicated in the pathogenesis of several chronic diseases, including B cell malignancies, RCC, and prostate, breast, lung, colon, and ovarian cancers. Over 100 agents, such as antioxidants, proteosome inhibitors, NSAIDs, and immunosuppressive agents are NF-κB inhibitors with none being entirely specific (Balkwill et al., 2005). Thus, alterations in these cytokines, and the cells that produce them, are implicated as features of "cancer" rather than specific to HCC.

Balkwill et al. (2005) reported that:

> Two mouse models of inflammation-associated cancer now implicate the gene transcription factor NF-κB and the inflammatory mediator known as tumor-necrosis factor α (TNF- α) in cancer progression. Using a mouse model of inflammatory hepatitis that predisposes mice to liver cancers, Pikarsky et al. present evidence that the survival of hepatocytes - liver cells - and their progression to malignancy are regulated by NF-κB. NF-κB is an important transcription factor that controls cell survival by regulating programmed cell death, proliferation, and growth arrest. Pikarsky et al. find that the activation state of NF-κB, and its localization in the cell, can be controlled by TNF-α produced by neighboring inflammatory cells (collectively known as stromal cells).

Pikarsky et al. (2004) reported that that the inflammatory process triggers hepatocyte NF-κB through upregulation of TNF-α in adjacent endothelial and inflammatory cells. Switching off NF-κB in mice from birth to 7 months of age, using hepatocyte-specific inducible IκB-super repressor transgene, had no effect on the course of hepatitis, nor did it affect early phases of hepatocyte transformation. By contrast, suppressing NF-κB inhibition through anti-TNF-α treatment or induction of the IκB-super repressor in later stages of tumor development resulted in apoptosis of transformed hepatocytes and failure to progress to HCC. The Mdr2 knockout hepatocytes in Pikarsky's model of hepatocarcinogenicity were distinguishable from wild-type cells by several abnormal features: high proliferation rate, accelerated hyperploidy and dysplasia. Pikarsky et al. (2004) reported that NF-κB knockout and double mutant mice displayed comparable degrees of proliferation, hyperploidy, and dysplasia, implying that NF-κB is not required for early neoplastic events. Thus, activation of NF-κB was not important in the early stages of tumor development, but was crucial for malignant conversion.

It was noted that:

> Greten et al. reporting in Cell, come to a similar conclusion by studying a mouse colitis-associated cancer model. Their work does not directly implicate TNF-α, but instead found enhanced production of several pro-inflammatory mediators (cytokines) including TNF-α, in the tumor microenvironment during the development of cancer. An important feature of both studies is that NF-κB activation was selectively ablated in different cell compartments in developing tumor masses, and at different stages of cancer development.

Balkwill et al. (2005) also noted that TNF-α and NF-κB have many different effects, depending on the context in which they are called into play and the cell type and environment.

In contrast, El-Serag and Rudolph (2007) noted that "the influence of inflammatory signaling on hepatocarcinogenesis can be context dependent; deletion of Nf-κB-dependent inflammatory responses enhanced HCC formation in carcinogen treated mice (Sakurai et al., 2006)." Similarly, deletion of Nf-κB essential modulator/I kappa β kinase (NEMO/IKK), an activator of Nf-κB, induced steatohepatitis and HCC in mice (Luedde et al., 2007).

Maeda et al. (2005) reported that hepatocyte-specific deletion of IKKβ (which prevents NF-kB activation) increased DEN-induced hepatocarcinogenesis and that a deletion of IKKβ in both hepatocytes and hematopoietic-derived cells, however, had the opposite effect, decreasing compensatory proliferation and carcinogenesis. They suggested that these results differ from previous suggestion that the tumor-promoting function of NF-kB is excreted in hepatocytes (Pikarsky et al., 2004), and suggest that chemicals or viruses that interfere with NF-kB activation in hepatocytes may promote HCC development.

Alterations in NF-kB levels have been suggested as a key event for the hepatocarcinogenicity by PPARα agonists. The event associated with PPAR effects has been the extent of NF-kB activation as determined through DNA binding. As reported by Tharappel (2001), NF-kB activity is assayed with electrophoretic mobility shift assay with nuclear extracts prepared from frozen liver tissue as a measure of DNA binding of NF-kB. Increased transcription of downstream targets of NF-kB activity has also been measured. It has been suggested that PPARα may act as a protective mechanism against liver toxicity. Ito et al. (2007) cite repression of NF-kB by PPARα to be the rationale for their hypothesis that PPARα-null mice may be more vulnerable to tumorigenesis induced by exposure to environmental carcinogens. However, as shown in Section E.3.4.1.2, although DEHP was reported to also induce glomerulonephritis more often in PPARα-null mice, as suggested (Kamijima et al., 2007) to be due of the absence of PPARα-dependent anti-inflammatory effect of antagonizing the oxidative stress and NF-κB pathway, there was no greater or lesser susceptibility to DEHP-induced liver carcinogenicity in the PPARα null mice.

Because PPARα is known to exert anti-inflammatory effects by inducing expression of IκBα, which antagonizes NFκB signaling, the expression of IκBα has been measured in some studies (Kamijima et al., 2007), as well as expression of TNR1 mRNA to evaluate the sensitivity to the inflammatory response. Ito et al. (2007) reported that in wild-type mice, there did not appear to be a difference between controls and DEHP treatment for p65 immunoblot results. DEHP treatment was also reported to not induce p65 or p52 mRNA either or influence the expression levels of TNFα, IkBα, IkBβ, and IL-6 mRNA in wild-type mice.

Tharappel et al. (2001) treated rats with WY-14,643, Gemfibrozil, or dibutyl phthalate and reported elevated NF-kB DNA binding in rats with WY-14,642 to have sustained response but not others. WY-14,643 increased DNA binding activity of NF-kB at 6, 34, or 90 days. Gemfibrozil and DEHP increased NF-kB activity to a lesser extent and not at all times in rats. For Gemfibrozil, there was only a twofold increase in binding at 6 days with no increase at 34 days and an increase only in low dose at 90 days. In rats treated with dibutyl phthalate, there was no change at 6 days; at 34 days, there was an increase at high and low dose and at 90 days, only low-dose animals showed a change. In pooled tissue from WY-14,643-treated animals, the complex that bound the radiolabeled NF-kB fragment did contain both p50 and p65. Both WY-14,643 and Gemfibrozil were reported to produce tumors in rats with dibutyl phthalate untested in rats for carcinogenicity. Thus, early changes in NF-kB were not supported as a key event and WY-14,643 to have a pattern that differed from the other PPARα agonists examined.

In regard to the links between inflammation and cancer, Nickoloff et al. (2005), in their review of the issue, cautioned that such a link is not simple. They noted that:

> dissecting the mediators of inflammation in cutaneous carcinogenic pathways has revealed key roles for prostaglandins, cyclooxygenase-2, tumor necrosis factor-α, AP-1, NF-κB, signal transducer and activator of transcription (STAT)3, and others. Several clinical conditions associated with inflammation appear to predispose patients to increased susceptibility for skin cancer including discoid lupus erythematosus, dystrophic epidermolysis bullosa, and chronic wound sites. Despite this vast collection of data and clinical observations, however, there are several dermatological setting associated with inflammation that do not predispose to conversion to lesions into malaignancies such as psoriasis, atopic dermatitis, and Darier's disease.

Nickoloff et al. (2005) suggested that such a

> link may not be as simple as currently portrayed because certain types of inflammatory processes in skin (and possibly other tissues as well) may also serve a tumor suppressor function. Over the past few months, several publications in leading biomedical journals grappled with an important issue in oncology, namely defining potential links between chronic tissue damage, inflammation, and the development of cancer. Balkwill and Coussens (2004) reviewed the role of the NF-κB signal transduction pathway that can regulate inflammation and also

promote malignancy. Their review summarized the latest findings revealed in a letter to Nature by Pikarsky et al. (2004). Using Mdr2 knockout mice in which hepatitis is followed by hepatocellular carcinoma, Pikarsky et al. implicated TNFα upregulation in tumor promotion of HCC, and suggest that TNFα and NF-κB are potential targets for cancer prevention in the context of chronic inflammation. A similar conclusion was reached with respect to NF-κB by an independent group of investigators using a model of experimental dextran sulfate-induced colitis, in which inactivation of the IκB kinase resulted in reduced colorectal tumors (Greten et al., 2004). Although there are many other clinical condition supporting the concept of inflammation is a critical component of tumor progression (e.g., reflux esophagitis/esophageal cancer; inflammatory bowel disease/colorectal cancer), there is at least one notable example that does not fit this paradigm. As described below, psoriasis is a chronic cutaneous inflammatory disease, which is seldom if ever accompanied by cancer suggesting the relationship between tissue repair, inflammation, and development may not be as simple as portrayed by the aforementioned reviews and experimental results. Besides psoriasis, other noteworthy observations pointing to more complexity include the observation that in the Mdr2 knockout mice, we rarely detect bile duct tumors despite extensive inflammation, NF-κB activation, and abundant proliferation of bile ducts in portal spaces (Pikarsky et al., 2004). Moreover, in a skin-cancer mouse model, NF-κB was shown to inhibit tumor formation (Dajee et al., 2003). Thus, the composition of inflammatory mediators, or the properties of the responding epithelial cells (e.g., signaling machinery, metabolic status), may dictate either tumor promotion or tumor suppression. Chronic inflammation and tissue repair can trigger pro-oncogenic events, but also that tumor suppressor pathways may be upregulated at various sites of injury and chronic cytokine networking.

One cannot easily dismiss the many dilemmas raised by the psoriatic plaque that confound a simple link between the tissue repair, inflammation, and carcinogenesis. Since it is easily visible to the naked eye, and patients may suffer from such lesions for decades, it is difficult to argue that various skin cancers such as squamous cell carcinoma, basal cell carcinoma, or melanoma actually do develop within plaques by are being overlooked by patients and dermatologists. Remarkably, psoriatic plaques are intentionally exposed to mutagenic agents including excessive sunlight, topical administration of crude coal tar, or parenteral DNA cross-linking agent –psoralen followed by ultraviolet light. Moreover these treatments are known to induce skin cancer in nonlesional skin. Thus since psoriatic skin is characterized by altered differentiation, angiogenesis, increased telomerase activity, proliferative changes, and apoptosis resistance, one would expect that each and every psoriatic plaque would be converted to cancer, or at least serve as fertile soil for the presence of non-epithelial skin cancers over time.…In conclusion, it would seem prudent to remember the paradigm proposed by Weiss (1971) in which he suggested that premalignant cells do not comprise an isolated island, but are a focus of intense tissue interactions. The myriad inflammatory effects of the tumor microenvironment are important for understanding tumor development, as well as tumor suppression and senescence, and for the design for efficacious prevention strategies against inflammation-associate cancer (Nickoloff et al., 2005).

E.3.3.4. Gender Influences on Susceptibility

As discussed previously, male humans and rodents are generally more likely to get HCC. The increased risk of liver tumors from estrogen supplements in women has been documented. In mice, TCE exposure has been shown not only to have greater variability in response and greater effects on body weight in males (Kjellstrand et al., 1983a; Kjellstrand et al., 1983b) but also to induce dose-related increases in liver weight and carcinogenic response in female mice as well as males (see Section E.2.2). Recent studies have attempted to link differences in inflammatory cytokines and gender differences in susceptibility.

Lawrence et al. (2007) suggested that:

> studies of Naugler et al. (2007) and Rakoff-Nahoum and Medzhitov (2007), advance our understanding of the mechanisms of cancer-related inflammation. They describe an important role for an intracellular signaling protein called MyD88 in the development of experimental liver and colon cancers in mice. MyD88 function has been well characterized in the innate immune response (Akira and Takeda, 2004), relaying signals elicited by pathogen-associated molecules and by the inflammatory cytokine interleukin-1 (IL-1).…The conclusion from Naugler et al. (2007) and Rakoff-Nahoun and Medzhitov is that MyD88 may function upstream of NF-κB in cells involved in inflammation-associated cancer. Immune cells infiltrate the microenvironment of a tumor. Naugler et al. (2007) and Rakoff-Nahoun and Medzhitov (2007) suggest that the development of liver and intestinal cancers in mice may depend on a signaling pathway in infiltrating immune cells that involved the protein MyD88, the transcription factor NF-κB, and the pro-inflammatory cytokine Il-6. TLR binds a ligand which acts on MyD88 which acts on NF-κB which leads to secretion of inflammatory cytokine IL-6 which leads to promotion of tumor cell survival and proliferation.

Naugler et al. (2007) suggested gender disparity in MyD88-dependent IL-6 production was linked to differences in cancer susceptibility using the DEN model (a mutagen with concurrent regenerative proliferation at a single high dose) with a single injection of DEN. Partial hepatectomy was reported to induce no gender-related difference in IL-6 increase. After DEN treatment, the male mouse had 275 ng/mL as the peak IL-6 levels 12 hours after DEN, and for female mice, the peak was reported to be 100 ng/mL 12 hours after DEN administration. This is only about a 2.5-fold difference between genders. IL-6 mRNA induction was reported for mice 4 hours after DEN, at a time when there was no difference in serum IL-6 between male and female mice. It was not established that the 4-hour results in mRNA translated to the differences in serum at 12 hours between the sexes. The magnitude of mRNA differences does not necessarily hold the same relationship as the magnitude in serum protein. In fact, there was not a linear correlation between mRNA induction and IL-6 serum levels.

A number of issues complicate the interpretation of the results of the study. The study examined an acute response for the chronic endpoint of cancer and may not explain the differences in gender susceptibility for agents that do not cause necrosis. The DEN was administered in 15-day-old mice (which had not reached sexual maturity) for tumor information at a much lower dose than used in short-term studies of inflammation and liver injury in which mature mice were used. If large elevations of IL-6 are the reason for liver cancer, why does not a partial hepatectomy induce liver cancer in itself?

The percentage of proliferation at 36 and 48 hours after partial hepatectomy was the same between the sexes. If a 2.5-fold difference in IL-6 confers gender susceptibility, it should do so after partial hepatectomy and lead to cancer. For female mice, partial hepatectomy showed alterations in a number of parameters. However, partial hepatectomy does not cause cancer alone. The 5-fold increase 4 hours after DEN induction of IL-6 mRNA in male mice is in sharp contrast to the 27-fold induction of IL-6 1 hour after partial hepatectomy (in which at 4 hours, the IL-6 had diminished to 6-fold). There appeared to be variability between experiments. For example, the difference in males between experiments appears to be the same magnitude as the difference between male and female in one experiment and the baseline of IL-6 mRNA induction appeared to be highly variable between experiments as well as absolute units of ALT in serum 24 and 48 hours after DEN treatment that tended to be greater that the effects of treatments. The experiments used very few animals (n = 3) for most treatment groups. Of note is that the MyD88 -/- male mice still had a background level of necrosis similar to that of WT mice at 48 hours after DEN treatment, a time, long after the peak of IL-6 mRNA induction and IL-6 serum levels were reported to have peaked.

One of the key issues regarding this study is whether difference in IL-6 reported here lead to an increase in proliferation and does that difference within 48 hours of a necrotizing dose of a carcinogen change the susceptibility to cancer? This report shows that male and female mice have a difference in necrosis after carbon tetrachloride and a difference in proliferation. Are early differences in IL-6 at 4 hours related to the same kind of stimulus that leads to necrosis and concurrent proliferation? The amount of proliferation (as measured by DNA synthesis) between male and female mice 48 hours after DEN was very small and the study was conducted in a very few mice (n = 3). At 36 hours, the degree of proliferation was almost the same between the genders and about 0.6% of cells. The baseline of proliferation also differed between genders, but the variation and small number of animals made it insignificant statistically. At 48 hours the differences in proliferation between the male and female mouse were more pronounced, but were still quite low (2% for males and ~1% for females). Is the change in proliferation just a change in damage by the agent? Given the large variation in serum ALT and by inference necrosis, is there an equal amount of variability in proliferation? This study gives only limited information for DEN treatment.

The difference in incidence of HCC was reported to be greater than that of "proliferation" between genders and of other parameters, although differences in tumor multiplicity or size between the genders are never given in the paper. Most importantly, comparisons between the short-term changes in cytokines and indices of acute damage are for adult animals that are sexually mature and at doses that are 4 times (100 vs. 25 mg/kg) that of the sexually immature animals that are going through a period of rapid hepatocyte proliferation (15-day-old animals).

It is therefore difficult to extrapolate between the two paradigms to distinguish the effects of hormones and gender on the response. Finally, the work of Rakoff-Nahoum and Medzhitov (2007) showed that it is the effect of tumor progression and not initiation that is affected by MyD88 (a signaling adaptor to Toll-like receptors). Thus, examination of parameters at the initiation phase at necrotic doses for liver tumors may not be relevant.

E.3.3.5. Epigenomic Modification

There are several examples of chemical exposure to differing carcinogens that have led to progressive loss of DNA methylation (i.e., DNA hypomethylation) including TCE and its metabolites. The evidence for TCE and its metabolites is specifically discussed in Section E.3.4.2.2. Other examples of carcinogen exposures or conditions that have been noted to change DNA methylation are early stages of tumor development include ethionine feeding, phenobarbitol, arsenic, dibromoacetic acid, and stress. However, it has not yet been established whether epigenetic changes induced by carcinogens and found in tumors play a causative role in carcinogenesis or are merely a consequence of the transformed state (Tryndyak et al., 2006).

Pogribny et al. (2007) reported the effects of WY-14,643 on global mouse DNA hypomethylation exposed at 1,000 ppm for 1 week, 5 weeks, or 5 months. What is of particular note in this study is that at this exposure level, one commonly used for mode-of-action studies using WY-14,643 to characterize the effects of PPARα agonists as a class, there was significant hepatonecrosis and mortality reported by Woods et al. (2007a).

Both wild-type and PPARα -/- null mice were examined. In wild-type mice DNA syntheses was elevated 3-, 13-, and 22-fold of time-matched controls after 1 week, 5 weeks, and 5 months of WY 14,543 treatment. Changes in ploidy were not examined. After 5 weeks of exposure, the ratio of unmethylated CpG cites in whole-liver DNA was the same for WY-14,643 treatment and control but by 5 months, there was an increase in hypomethylation in WY-14,643 treated wild-type mice. The authors did not report whether foci were present or not, which could have affected this result. The similarity in hypomethylation at 5 days and 5 weeks, a time point that also had a small probability of foci development, is suggestive of foci affecting the result at 5 months.

For PPAR -/- mice, there was increased hypomethylation reported at 1 and 5 weeks after WY-14,643 treatment that was not statistically significant with so few animals studied. At 5 months, the null mice had decreased hypomethylation compared to 1 and 5 weeks. The authors

noted that methylation of c-Myc genes was reported to not be affected by long-term dietary treatment with WY-14,643 even though WY-14,643-related hypomethylation of c-Myc gene early after a single dose of WY-14,643 has been observed (Ge et al., 2001a). The authors concluded "thus, alterations in the genome methylation patterns with continuous exposure to nongenotoxic liver carcinogens, such as WY, may not be confined to specific cell proliferation-related genes."

Pogribny et al. (2007) reported Histone H3 and H4 trimethylation status in wild-type and PPAR null mice to show a rapid and sustained loss of histone H3K9 and histone H4K20 trimethylation in wild-type mice fed WY-14,643 from 1 week to 5 months. There was no progressive loss in histone hypomethylation, with the same amount of demethylation occurring at 5 days, 5 weeks, and 5 months in wild-type mice fed WY-14,643. The change from control was ~60% reduction. The control values with time were not reported and all controls were pooled to give one value (n = 15). For PPAR -/- l mice, there was a slight decrease with WY-14,643 treatment (~15%) reported. In wild-type mice, WY-14,643 treatment was reported to have no effect on the major histone methyltransferase, Suv39h1, while expression of another (PRDM/Riz1) increased significantly as early as one week of treatment and remained elevated for up to 5 months. The effect on expression of Suv420h2 (responsible for histone H4K20 trimethylation) was more gradual and the amounts of this protein in livers of mice fed WY-14,643 were reported to be lower than in control.

The authors did not examine these parameters in the null mice, so the relationship of these effects to receptor activation cannot be determined. Pogribny et al. (2007) reported hypomethylation of retroelements (LTR IAP, LINE1, and LINE2 retrotransposons) following long-term exposure to WY-14,643, which the authors concluded can have effects on the stability of the genome. Again, these results are for whole liver that may contain foci.

Nevertheless, these findings raise questions about other target organs and a more general mechanism for WY-14,643 effects than a receptor mediated one. The lack of effects on c-Myc and the irrelevance of the transient proliferation through it reported here gives more evidence of the irrelevance of a mode of action dependent on transient proliferation. The authors noted that studies show that a sustained loss of DNA methylation in liver is an early and indispensable event in hepatocarcinogenesis induced by long-term exposure of both genotoxic and nongenotoxic carcinogens in rodents. Thus, this statement argues against making such a distinction in mode of action for "genotoxic" and "nongenotoxic" carcinogens. Finally, the use of a dose that Woods et al. (2007a) demonstrate to have significant hepatonecrosis and mortality, limits the interpretation of these results and their relevance to models of carcinogenesis without concurrent necrosis.

Strain sensitivity to hepatocarcinogenicity has been investigated in terms of short-term changes in methylation. Bombail et al. (2004) reported that a tumor-inducing dose of phenobarbital reduced the overall level of liver DNA methylation in a tumor-sensitive ($B6C3F_1$)

mouse strain but that the same dose of phenobarbital did not alter the global methylation level in a more tumor-resistant strain (C57BL/6), although the compound increased hepatocyte proliferation as measured by increased DNA synthesis in both strains (Counts et al., 1996). Bombail et al. reported that "In a similar study, Watson and Goodman (2002) used a PCR-based technique to measure DNA methylation changes specifically in GC-rich regions of the mouse genome." Watson and Goodman (2002) found that, that in these areas of the genome, exposure to phenobarbital caused an increase in methylation in dosed animals compared with control animals. Again, the change was more pronounced in tumor-prone C3H/He and $B6C3F_1$ strains than in the less sensitive C57BL/6 strain. They also reported increased DNA synthesis in C57BL/6 mice but decreased global methylation in the $B6C3F_1$ strain after phenobarbitol administration for 1–2 weeks. The lifetime spontaneous tumor rates were reported to be <5% in C57BL/6 mice but up to 80% in C3H/He mice.

Counts et al. (1996) reported cell proliferation and global hepatic methylation status in relatively liver tumor susceptible $B6C3F_1$ with relatively resistant C57BL6 mice following exposure to phenobarbitol and/or chlorine/methionine deficient (CMD) diet. Cell proliferation (i.e., DNA synthesis) was reported to be higher in C57BL/6 mice, while transient hypomethylation occurred to a greater extent in $B6C3F_1$ mice after phenobarbital treatment. Dual administration of CMD and phenobarbitol led to enhanced cell proliferation and greater global hypomethylation with similar trends in terms of strain sensitivities in comparison to with either treatment alone (i.e., greater increase in cell proliferation in C57BL/6 and greater levels of hypomethylation in $B6C3F_1$). Thus, the authors concluded that $B6C3F_1$ mice have relatively low capacity to maintain the nascent methylation status of their hepatic DNA.

However, on the whole, the control values for methylation for the C57BL/6 mice appear to be slightly higher than the $B6C3F_1$ mice. Claims that the liver tumor sensitive $B6C3F_1$ had more global hypomethylation after a promoting stimulus, which could be related to tumor sensitivity, are tempered by the fact that the resistant strain had a higher control baseline of methylation. The baseline level of LI or hepatocyte proliferation also appears to be slightly higher in the C57BL/6 mouse. In addition, the largest strain difference in hypomethylation after a CMD diet was at week 12 (135% of control for the $B6C3F_1$ strain and 151% of control for the C57BL/6 strain) and this pattern was opposite that for the 1-week time point. Thus, the suggestion by Counts et al. (1996), that the inability to maintain methylation status by the $B6C3F_1$ strain, is also not supported by the longer duration data for CMD diet.

E.3.4. Specific Hypothesis for Mode of Action of TCE Hepatocarcinogenicity in Rodents

E.3.4.1. PPARα Agonism as the Mode of Action for Liver Tumor Induction—The State of the Hypothesis

PPARα receptor activation has been suggested to be the mode of action for TCA liver tumor induction and for TCE liver tumor induction to occur primarily as a result of the presence

of its metabolite TCA (NRC, 2006). However, as discussed previously (see Section E.2.1.10), TCE-induced increases in liver weight have been reported in male and female mice that do not have a functional PPARα receptor (Ramdhan et al., 2010; Nakajima et al., 2000). The dose-response for TCE-induced liver weight increases differs from that of TCA (see Section E.2.4.2). The phenotype of the tumors induced by TCE have been described to differ from those by TCA and to be more like those occurring spontaneously in mice, those induced by DCA, or those resulting from a combination of exposures to both DCA and TCA (see Section E.2.4.4). As to whether TCA-induced tumors are induced through activation of the PPARα receptor, the tumor phenotype of TCA-induced mouse liver tumors has been reported to have a pattern of H-ras mutation frequency that is opposite to that reported for other peroxisome proliferators (see Section E.2.4.4.; Bull et al., 2002; Stanley et al., 1994; Hegi et al., 1993; Fox et al., 1990). While TCE, DCA, and TCA are weak peroxisome proliferators, liver weight induction from exposure to these agents has not correlated with increases in peroxisomal enzyme activity (e.g., PCO activity) or changes in peroxisomal number or volume. However, liver weight induction from subchronic exposures appears to be a more accurate predictor of carcinogenic response for DCA, TCA, and TCE in mice (see Section E.2.4.4). The database for cancer induction in rats is much more limited than that of mice for determination of a carcinogenic response to these chemicals in the liver and the nature of such a response.

The mode of action for peroxisome proliferators has been the subject of research and debate for several decades. It has evolved from an "oxidative damage" due to increased peroxisomal activity to a mode-of-action framework example developed by Klaunig et al. (2003) that described causal inferences for hepatocarcinogenesis after a chemical exposure was shown to activate of the PPAR-α receptor with concurrent perturbation of cell proliferation and apoptosis, and selective clonal expansion. Of note, although inhibition of apoptosis was proposed as part of the sequellae of PPARα activation, as noted in Section E.2.4.1, no changes in apoptosis in mice exposed to TCE have been reported, with the exception of mild enhanced apoptosis at a 1,000 mg/kg-day dose. More importantly, for mice, the rate of apoptosis decreases as mice age and appears to be lower than that of rats. While DCA exposure has been noted to reduce apoptosis, the significance of DCA-induced reduction in apoptosis from a level that is already inherently low in the mouse, is difficult to apply as the mode of action for DCA-induced liver cancer.

Klaunig et al. (2003)based causal inferences on the attenuation of these events in PPAR-α-null mice in response to the prototypical agonist WY-14,643 with a number of intermediary events considered to be associative (e.g., expression of peroxisomal and nonperoxisome genes, peroxisome proliferation, inhibition of gap junction intracellular communication, hepatocyte oxidative stress as well as Kupffer cell-mediated events). The data set for DEHP was prominently featured as an example of "PPAR-α induced hepatocarcinogenesis." For DEHP, PPAR-α activation was described as the initial key event with evidence lacking for a direct effect

but primarily supported by evidence from PPAR-α-knockout mice treated with WY-14,643. Klaunig et al. (2003) concluded that "…all of the effects observed are due only to the activation of this receptor and the downstream events resulting from this activation and that no other modes of action are operant"

Although that PPARα receptor activation is the sole mode of action for DEHP has been cited by several reports (including IARC, 2000), several articles have questioned the adequacy of this proposed mode of action (Guyton et al., 2009; Caldwell et al., 2008b; Melnick et al., 2008; Keshava et al., 2007; Caldwell and Keshava, 2006; Keshava and Caldwell, 2006); FIFRA SAP, 2004). New information is now available that also questions several of the assumptions inherent in the proposed mode of action by Klaunig et al. (2003) and the dismissal of PPARα agonists as posing a health risk to humans. These issues were recently examined in Guyton et al. (2009) and are discussed below. Furthermore, IARC has recently concluded that additional mechanistic information has become available, including studies with DEHP in PPAR-α-null mice, studies with several transgenic mouse strains, carrying human PPARα or with hepatocyte-specific constitutively activated PPARα and a study in humans exposed to DEHP from the environment that has changed its conclusions regarding the relevance of rodent tumor data to human risk (Grosse et al., 2011). Data from these new studies suggest that many molecular signals and pathways in several cell types in the liver, rather than a single molecular event, contribute to cancer development in rodents, with IARC concluding that the human relevance of the molecular events leading to DEHP-induced cancer in several target tissues (e.g., liver and testis) in rats or mice could not be ruled out, resulting in the evaluation of DEHP as a Group-2B agent, rather than Group 3.

Specific questions have been raised about the use of WY-14,643 as a prototype for PPARα (especially at necrogenic doses) and use of the PPARα -/- null mouse in abbreviated bioassays to determine carcinogenic hazard.

E.3.4.1.1. Heterogeneity of PPARα Agonist Effects and Inadequacy of WY-14,643 Paradigm as Prototype for Class

Inferences regarding the carcinogenic risk posed to humans by PPARα agonists have been based on limited epidemiology studies in humans that were not designed to detect such effects. However, as noted by Nissen et al. (2007), the PPARα receptor is pleiotropic, highly conserved, has "cross talk" with a number of other nuclear receptors, and plays a role in several disease states. "The fibrate class of drugs, which are PPARα agonists intended to treat dyslipidemia and hypercholesterolemia, have recently been associated with a number of serious side effects." While these reports of clinical side effects are for acute or subchronic conditions and are not (and would not be expected to be) able to detect liver cancer from fibrate treatment, they clearly demonstrate that compounds activating the PPAR receptors may produce a spectrum of effects in humans and the difficulty in studying and predicting the effects from PPAR

agonism. Graham et al. (2004) recently reported significantly increased incidence of hospitalized rhabdomyolysis in patients treated with fibrates both alone and in combination with statins. Even though pharmaceutical companies have spent a great deal of effort to develop agonists that are selective for desired effects, the pleiotropic nature of the receptor continues to be an obstacle.

Also, fibrates, WY-14,643, and other PPARα agonists are pan agonists for other PPARs. Shearer and Hoekstra (2003) noted that fibrates, including Fenofibrate, Clofibrate, Bezafibrate, Ciprofibrate, Gemfibrozil, and Beclofibrate are all drugs that were discovered prior to the cloning of PPARα and without knowledge of their mechanism of action but with optimization of lipid lowering activity carried out by administration of candidates to rodents. They report that many PPARα ligands, including most of the common fibrate ligands, show only modest selectivity over the other subtypes with, for example, fenofibric acid and WY-14,643 showing <10-fold selectivity for activation of human PPARα compared to PPARγ and/or PPARδ. In human receptor transactivation assays, they report:

> Human receptor transactivation assays of median effective concentration (EC_{50}):
> WY-14,643 = 5.0 μm for PPARα, 60 μm for PPAR γ, 35 μm for PPARδ.
> Clofibrate = 55 μm for PPARα, ~500 μm for PPAR γ, inactive at 100 μm for PPARδ.
> Fenofibrate = 30 μm for PPARα, 300 μm for PPAR γ, inactive at 100 μm for PPARδ.
> Bezafibrate = 50 μm for PPARα, 60 μm for PPAR γ, 20 μm for PPARδ.
>
> Murine receptor transactivation assay of EC_{50}:
> WY = 0.63 μm for PPARα, 32 μm for PPAR γ, inactive at 100 μm for PPARδ.
> Clofibrate = 50 μm for PPARα, ~500 μm for PPAR γ, inactive at 100 μm for PPARδ.
> Fenofibrate = 18 μm for PPARα, 250 μm for PPAR γ, inactive at 100 μm for PPARδ.
> Bezafibrate = 90 μm for PPARα, 55 μm for PPAR γ, 110 μm for PPARδ.

Thus, these data show the relative effective concentrations and "potency for PPAR activity" of various agonists in humans and rodents, rodent and human responses may vary depending on agonist, agonists vary in what they activate between the differing receptors, and there is a great deal of transactivation of these drugs.

For fibrates specifically, a study by Nissen et al. (2007) reported that in current practice, two fibrates, Gemfibrozil and Fenobibrate, are still widely used to treat a constellation of lipid abnormalities known as atherogenic dyslipidemia and note that currently available fibrates are weak ligands for the PPARα receptor and may interact with other PPAR systems. They noted that the pharmaceutical industry has sought to develop new, more potent and selective agents within this class but, most importantly, that none of the novel PPARα agonists has achieved regulatory approval and that according to a former safety officer in the U.S. Food and Drug Administration (El-Hage, 2007) that >50 PPAR modulating agents have been discontinued due

to various types of toxicity (e.g., elevations in serum creatinine, rhabdomylosis, "multi-species, multi-site increases in tumor with no safety margin for clinical exposures," and adverse cardiovascular outcomes) but without scientific publications describing the reasons for termination of the development programs. Nissen et al. (2007) reported differences in effect between a more highly selective and potent PPARα agonist and the less potent and specific one in humans. They noted:

> a recent large study of Fenofibrate in patients with diabetes showed no significant reduction in morbidity but a trend toward increased all-cause mortality (Keech et al., 2006; Keech et al., 2005). Whether this potential increase in mortality is derived from compound specific toxicity of Fenofibrate or is an adverse effect of PPARα activation remains uncertain."
>
> In addition to the lack of publication of effects from PPAR agonists in human trials in which toxicity can be examined as noted by Nissen et al., the Keech study is illustrative of the problem in trying to ascertain liver effects from fibrate treatment in humans as the focus of the outcomes was coronary events in a study of 5 years duration in a older diabetic population. As stated above, the challenges the pharmaceutical industry and the risk assessor face in determining the effects of PPAR agonists is "that these compounds and drugs modulate the activity of a large number of genes, some of which produce unknown effects."

Nissen et al. further noted that:

> Accordingly, the beneficial effects of PPAR activation appear to be associated with a variety of untoward effects which may include, oncogenesis, renal dysfunction, rhabdomylosis, and cardiovascular toxicity. Recently, the FDA began requiring 2-year preclinical oncogenicity studies for all PPAR-modulating agents prior to exposure of patients for durations of longer than 6 months (El-Hage, 2007).

Guyton et al. (2009) further explored the status of the PPARα epidemiological database and describe its inability to discern a cancer hazard from the available data. Thus, while existing evidence for liver cancer in humans is null rather than negative, there remains a concern for oncogenicity and many obstacles for determining such effects through human study. The heterogeneity in response to PPARα agonists and the heterogeneity of effects they cause (Keshava and Caldwell, 2006) are evident from these reports.

Many studies have used the effects of WY-14,643 at a very high dose and extrapolated those findings to PPARα agonists as a class. However, this diverse group of chemicals has varying potencies and effects for the "key events" described by Klaunig et al. (Keshava and Caldwell, 2006; 2003). The standard paradigm used with WY-14,643 to induced liver tumors in all mice exposed to 1 year (an abbreviated bioassay), uses a large dose that has also has been

reported to produced liver necrosis, which can have an effect of cell proliferation and gene expression patterns, and to also induce premature mortality (Woods et al., 2007a).

As stated above, WY-14,643 also has a short peak of DNA synthesis that peaks after a few days of exposure, recedes, and then unlike most PPARα agonists studied (e.g., Clofibrate, clofibric acid, Nafenopin, Ciprofibrate, DEHP, DCA, TCA and LY-171883), has a sustained proliferation at the doses studied (David et al., 1999; Carter et al., 1995; Barrass et al., 1993; Lake et al., 1993; Marsman et al., 1992; Tanaka et al., 1992; Eacho et al., 1991; Sanchez and Bull, 1990; Yeldandi et al., 1989; Marsman et al., 1988). Clofibrate has been shown to have a decrease in proliferation gene expression shortly after its peak (see Section E.3.3.2).

As shown above for WY-14,643, hepatocellular increases in DNA synthesis did not appear to have a dose-response (see Section E.3.3.2), only WY-14,643 had a sustained elevation of Nf-κB (gem and dibutyl phthalate did not) (see Section E.3.3.3.3). The effects on DNA methylation occurred at 5 months and not earlier time points (when foci were probably present) and effects of histone trimethylation were observed to be the same from 1 weeks to 5 months (see Section E.3.3.5). Such effects on the epigenome suggest that other effects of WY-14,643, other than receptor activation, are not specific to just WY-14,643 and are found in a number of conditions leading to cancer and in tumor progression (see Sections E.3.1.1 and E.3.1.7).

In their study of PPARα-independent short-term production of reactive oxygen species from induced by large concentrations of WY-14,643 and DEHP in the diet, Woods et al. (2007a) examined short-term exposures to 0.6% w/w DEHP or 0.05% or 500 pm WY-14,643 for 3 days, 1 weeks or 3 weeks and reported that WY-14,643 induced a dramatic increase in bile flow that was not observed from DEHP exposure. By 1 week of exposure, there was a 5% increase in bile flow for DEHP treatment but a 240% increase in bile flow for WY-14,643 treatment. By 3 weeks, the difference in bile volume between treated and control was 12% for DEHP and 1,100% for WY-14,643 treated animals.

In this study, oxygen radical formation, as measured by spin trapping in the bile, was reported to be decreased after 3 days of DEHP and WY-14,643 treatment. However, the large changes in bile flow by WY-14,643 treatment limit the interpretation of these data along with a small number of animals examined in this study (e.g., six control and DEHP animals and three animals exposed to WY-14,643 at 3 days), a 30% variation in percent liver/body weight ratios between control groups, and the insensitivity of the technique. In an earlier study, oxidative stress appears to be correlated with neither cell proliferation nor carcinogenic potency (Woods et al., 2006). Woods et al. (2006) reported WY-14,643Y or DEHP to induce an increase in free radicals at 2 hours, a decrease at 3 days then an increase at 3 weeks for both. However, radical formation did not correlate with the proliferative response, as DEHP fails to produce a sustained induction of proliferative response in rodent liver but WY-14,643 does, and both WY-14,643 and DEHP gave a similar pattern of radical formation that did not vary much from controls, which is in contrast to their carcinogenic potency.

Although assumed to be a reflection of cell proliferation in many studies of WY-14,643 and by Klaunig et al. (2003), DNA synthesis recorded using the standard exposure paradigm for WY-14,643 can also be a reflection of hepatocyte, nonparenchymal cell, or inflammatory cell mitogenesis (in the case of necrosis induced inflammation), from changes in hepatocyte ploidy, or a combination of all. Other peroxisome proliferators have been shown to have a decrease in proliferation gene expression shortly after their peaks (e.g., Clofibrate, see Section E.3.3.2) and both Methylclofenapate and Nafenopin have been shown to increase cell ploidy, with Nafenopin having the majority of its DNA synthesis as a reflection of increased ploidy with only a small percentage as increases in cell number (see Section E.3.4.1). Several authors have also noted increases in ploidy for WY-14,643 (see Section E.3.4.1).

The Tg.AC genetically modified mouse was used to study 14 chemicals administered by the topical and oral (gavage and/or diet) routes by Eastin et al. (2001). Clofibrate was considered clearly positive in the topical studies but not WY-14,643, regardless of route of administration. Based on the observed responses, it was concluded by the workgroup (Assay Working Groups) that the Tg.AC model was not overly sensitive and possesses utility as an adjunct to the battery of toxicity studies used to establish human carcinogenic risk. The difference in result between Clofibrate and WY-14,643 is indicative of a different mode of action for the two compounds.

Similarly, at large exposure concentrations, Boerrigter (2004) investigated the response of male and female lacZ-plasmid transgenic mice treated at 4 months of age with 6 doses of 2,333 mg/kg DEHP, 200 mg/kg WY-14,643, or 90 mg/kg Clofibrate over a 2-week period. Mutation frequencies were assayed at 21 days following the last exposure. DEHP and WY-14,643 were shown to significantly elevate the mutant frequency in both male and female liver DNA, while Clofibrate, at the dose level studied, was apparently nonmutagenic in male and female liver (i.e., six-dose exposure to DEHP or WY-14,643 over a 2-week period significantly increased the mutant frequency in liver of both female and male mice by approximately 40%). The author noted that:

> the laxZ plasmid-based transgenic mouse mutation assay is somewhat unique among other commercially available models (e.g. mutamouse and big blue), by virtue of its ability to accurately quantify both point mutations and large deletions including those which originate in the lacZ plasmid catamer and extend into the 3' flanking genomic region. It should be noted that to date there is no single, agreed upon protocol for conducting mutagenicity assays with transgenic rodents although several aspects have been upon by the Transgenic Mutation Assays workgroup of the International Workshop on Genotoxicity Procedures.

For several chemicals, both rats and mice demonstrate evidence of receptor activation through peroxisome proliferation and peroxisome-related gene expression, but only one develops cancer. The herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), is a striking example of the problems that would be associated with only using evidence of PPARα receptor activation to

make conclusions about the mode of action of liver tumors. 2,4-D is structurally similar to the PPARα agonist Clofibrate and has been shown at similar concentrations to increase peroxisome number and size, increase hepatic carnitine acetyltransferase activity and catalase, and decrease serum triglycerides and cholesterol in rats (Vainio et al., 1983). Peroxisome number was also increased in Chinese hamsters to a similar level as with Clofibrate at the same exposure concentration after 9 days of exposure to 2,4-D (Vainio et al., 1982). In mice, Lundgren et al. (1987) reported that 2,4-D exposure statistically increased the liver-somatic index over controls after a few days of exposure and increased mitochondrial protein, microsomal protein, carnitine acetyltransferase, PCO activity, cytochrome oxidase, cytosolic epoxide hydrolase, microsomal epoxide hydrolase, microsomal P450 content, and hepatic cytosolic epoxide hydrolase in mouse liver. Thus, 2,4-D activates the PPARα receptor, with associated changes in peroxisome-related gene expression, in multiple species and at similar doses to Clofibrate. However, Charles et al. (1996) and Charles and Leeming (1998) reported that in several 2-year studies, there were no 2,4-D-induced increases in liver tumors in F344 rats, CD-1 rats, B6C3F_1 mice, or CD-1 mice.

Another example, is provided by Gemfibrozil, known as (5-2[2,5-dimethylphenoxy] 2,2-dimethylpentanoic acid) and [2,2-dimethyl-5-(2,5-xylyoxy) valeric acid], a therapeutic agent that activates the PPARα receptor and is a peroxisome proliferator, but is carcinogenic only in male rats but not female rats, nor in either gender of mouse (Contrera et al., 1997). Gemfibrozil causes tumors in pancreas, liver, adrenal, and testes of male rats and causes increases in absolute and relative liver weights in both rats and mice (Fitzgerald et al., 1981). Gemfibrozil is a highly effective lipid and cholesterol lowering drugs in humans and in mice (Olivier et al., 1988). However, although Gemfibrozil activates the PPARα receptor and induces peroxisome proliferation in mice, it does not induce liver tumors in that species.

In the long-term study of Bezafibrate, Hays et al. (2005) noted that the role of this receptor in hepatocarcinogenesis has only been examined using one relatively specific PPARα agonist (WY-14,643) and report that Bezafibrate can induce the expression of a number of PPARα target genes (acyl CoA oxidase and CYP4a) and increased liver weight in PPARα knockout mice that is not dependent on activation of PPARβ or PPARγ. As noted by Boerrigter (2004):

> In contrast to DEHP and WY-14,643, Clofibrate produced hepatocellular carcinomas in rats only while no increase in the incidence of tumors was reported in mice (Gold and Zeiger, 1997). However, Clofibrate induces peroxisome proliferation in both rats and mice (Lundgren and DePierre, 1989) but only produced hepatocellular carcinomas in rats (Gold and Zeiger, 1997).

Melnick et al. (1996) noted that similar levels of peroxisomal induction were observed in rats exposed to DEHP and di(2-ethylhexyl) adipate (DEHA) at doses comparable to those used in the bioassays of these chemicals. However, DEHP but not DEHA gave a positive liver tumor

response in 2-year studies in rats. In an evaluation of the carcinogenicity of tetrachloroethylene, an expert panel of the IARC concluded that the weak induction of peroxisome proliferation by this chemical in mice was not sufficient to explain the high incidence of liver tumors observed in an inhalation bioassay.

In adult animals, apoptosis acts as a safeguard to prevent cells with damaged DNA from progressing to tumors, but like cell proliferation, alterations in apoptosis are common to many modes of action. In addition, only short-term data are available on changes in apoptosis due to PPARα agonists, and long-term changes have not been investigated (Rusyn et al., 2006). For example, although a decrease in apoptosis has also been suggested to be an important additional molecular event that may affect the number of cells in rodent liver following exposure to the peroxisome proliferator DEHP, apoptosis rates have not investigated past 4 days of exposure and thus, the time-course of this event is uncertain. The antiapoptotic effects of PPAR agonists appear to be also dependent on nonparenchymal cells (i.e., Kupffer cells), which do not express PPARα and could be a transient event (Rusyn et al., 2006). Morimura et al. (2006) reported evidence for exposure to WY-14,643 that does not support a role for PPARα–mediated apoptosis in tumor formation (see Section E.3.4.1.3) as well as appearing to be specific to WY-14,643 (see Section E.3.3.3.3).

The lack of a causal relationship of transient DNA synthesis increases and hepatocarcinogenesis has been raised by many (Caldwell et al., 2008b) and is discussed in Section E.3.4.2 as well as the changes in ploidy (see Section E.3.4.1). In regard to gene expression profiles, many studies have focused on gene profiles during the early transient proliferative phase or have identified genes primarily associated with peroxisome proliferation as "characteristic" or relevant to those associated with tumor induction. Several have focused on the number of genes whose expression "goes up" or "goes down" from a small number of animals. Caldwell and Keshava (2006) presented information on WY-14,643, dibutyl phthalate, Gemfibrozil, and DEHP, and noted inconsistent results between PPARα agonists, paradoxes between mRNA and protein expression, strain, gender, and species differences in response to the same chemical, and time-dependent differences in response for several enzymes and GSH.

E.3.4.1.2. New Information on Causality and Sufficiency for PPARα Receptor Activation

In its review of the U.S. EPA's draft risk assessment of perfluorooctanoic acid (PFOA), the Science Advisory Panel (FIFRA SAP, 2004) expressed concerns about whether PPARα agonism constitutes the sole mode of action for PFOA effects in the liver and the relevance to exposed fetuses, infants, and children. In part based on uncertainties regarding the Klaunig et al. (2003) proposed mode of action, they concluded that the tumors induced by PFOA were relevant to human risk assessment. The hypothesis that activation of the PPARα receptor is the sole mode-of-action of hepatocarcinogenesis induced by DEHP and many other chemicals is further

called into question by recent studies. In the case of DEHP, Klaunig et al. (2003) assumed that WY-14,643 and DEHP would operate through the same key events and that long-term bioassays of DEHP in PPARα -/- knockout mice would be negative and hence demonstrate the need for receptor activation for hepatocarcinogenesis from DEHP.

The fallacy of these assumptions is illustrated by the recent report of the first 2-year bioassay of DEHP in PPARα -/- knockout mice (Sv/129 background strain) that reported DEHP-induced hepatocarcinogenesis (Ito et al., 2007). Further discussion was provided by Guyton et al. (Guyton et al., 2009). Similar to other studies, the PPAR -/- mice had slightly increased liver weights in comparison to controls and treated wild-type mice (~12% increase over controls). In fact, statistical analysis of the incidence data show that adenomas were significantly increased in PPARα -/- mice compared with wild-type mice exposed to 500 ppm DEHP and that a significant dose-response trend for adenomas and adenomas plus carcinomas was observed in PPARα -/- mice (Figure E-5). Overall, the cancer incidences were consistent with a previous study of DEHP (David et al., 1999) in $B6C3F_1$ mice at the same doses for nearly the same exposure duration. A strength of this study is that it was conducted at much lower, more environmentally relevant doses that did not significantly increase liver enzymes as indications of toxicity.

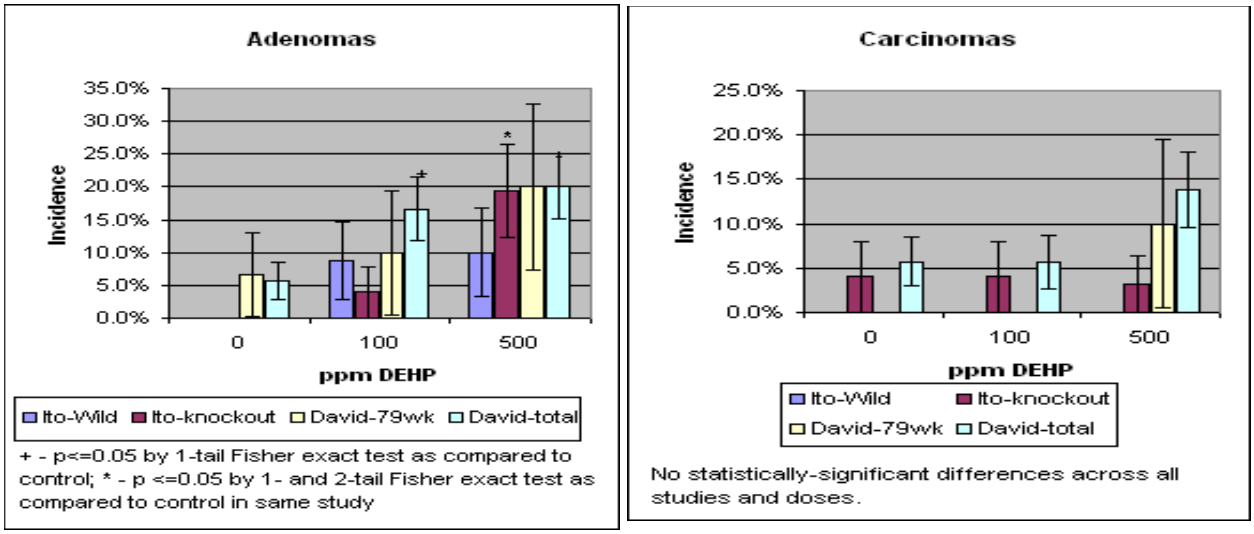


Figure E-5. Comparison of Ito et al. and David et al. data for DEHP tumor induction from (Guyton et al., 2009).

As noted by Kamijo et al. (2007), DEHP was reported also to induce glomerulonephritis more often in PPARα-null mice because of the absence of PPARα-dependent anti-inflammatory effect of antagonizing the oxidative stress and NF-κB pathway (Kamijo et al., 2007). Thus, these data support that hypothesis that there is no difference in liver tumor incidences between PPARα -/- mice and wild-type mice in a standard nonabbreviated exposure bioassay that does

not exceed the maximal tolerated doses and that DEHP can induce hepatotoxicity as well as other effects independent of action of the PPARα receptor.

The study of Yang et al. (2007) informs as to the sufficiency of PPARα receptor activation and subsequent molecular event for hepatocarcinogenesis in mice. The study used a VP16PPARα transgene under control of the liver-enriched activator protein (LAP) promoter to activate constitutively the PPARα receptor in mouse hepatocytes. LAP-VP16PPARα transgenic mice showed a number of effects associated with PPARα receptor activation including decreased serum triglycerides and free fatty acids, peroxisome proliferation, enhanced hepatocyte DNA synthesis, and induction of cell-cycle genes and those described as "PPARα targets" to comparable levels reported for WY-14,643 exposure. Hepatocyte proliferation, as determined by the labeling index of hepatocyte nuclei, was increased after 2 weeks of WY-14,643 treatment over controls (20.5 vs. 1.6% in control livers) with the LAP-VP16PPARα mice giving a similar results (20.8 vs. 1.0% in control livers).

The authors noted that transgenic mice did not appear to have positive labeling of nonparenchymal cell nuclei that were present in the WY-14,643 treated animals. The transferase-mediated dUTP nick end-labeling assay results were reported to show that there was no difference in apoptosis in wild-type mice treated with WY-14,643, the transgenic mice, or controls. In a small number of animals, microsomal genes (CYP4A) and peroxisomal (Acox, BIEN—the bifunctional enzyme) and mitochondrial fatty oxidation genes (LCAD—long chain acyl CoA dehydrogenase and VLCAD) were expressed in the transgenic mice, with WY-14,643 also increasing expression of these genes in wild-type mice but with less lipoprotein lipase (LPL) than the transgenic mice. Hepatic CoA oxidation was increased to a similar level in wild-type mice treated with WY-14,643 and the transgenic mice (n = 3–4) and was statistically different than controls. LAP-VP16PPARα transgenic mice (8 weeks of age) exhibited hepatomegaly (~50 increase percent body/liver weight over controls) and an accumulation of lipid due to triglycerides but not cholesterol.

However, compared to wild-type mice exposed to WY-14,643 for 2 weeks, the extent of hepatomegaly was reduced (i.e., percent liver/body weight increase of ~2.5-fold with WY-14,643 treatment), no hepatocellular hypertrophy or eosinophilic cytoplasms were noted, and no evidence of nonparenchymal cell proliferation was observed in the LAP-VP16PPARα transgenic mice.

At ~1 year of age, Yang et al. (2007) reported there to be no evidence of preneoplastic lesions or hepatocellular neoplasia in LAP-VP16PPARα transgenic mice, in contrast to results after 11 months of exposure to WY-14,643 in wild-type mice. Microscopic examination of liver sections were consistent with the gross findings, as HCCs and hepatic lesions were observed in the long-term WY-14,643 treated wild-type mice, but not in >20 LAP-VP16PPARα mice at the age of over 1 year in the absence of DOX. There was no quantitative information on tumors given nor of foci development in the WY-14,643 mice. As noted by Yang et al. (2007), PPARα

activation only in mouse hepatocytes is sufficient to induce peroxisome proliferation and increased DNA synthesis, but not to induce liver tumors.

Thus, "hepatocyte proliferation" indentified by Klaunig et al. (2003) as a "causal event" in their PPARα mode of action is not sufficient to induce hepatocarcinogenesis. These data not only call into question the adequacy of the mode-of-action hypothesis proposed by Klaunig et al. (2003), but also suggest that multiple mechanisms and multiple cell types may be involved in hepatocarcinogenicity caused by chemicals that are also PPARα agonists.

E.3.4.1.3. Use of the PPAR -/- Knockout and Humanized Mouse

Great importance has been attached to the results reported for PPARα -/- mice and their humanized counterparts with respect to inferences regarding the mode of action or peroxisome proliferators and whether short-term chemical exposures or abbreviated bioassays conducted with these mice can show that a PPARα mode of action is involved. Consequently, the use of these models warrants scrutiny.

Compared to untreated wild-type mice, liver weights in knockout mice or humanized mice have been reported to be elevated (Morimura et al., 2006; Voss et al., 2006; Laughter et al., 2004) and within 10% of each other (Peters et al., 1997). In order to be able to assign effects to a chemical tested in knockout mice, a better characterization is needed of the baseline differences between PPARα -/- knockout and wild-type mice. This is particularly important for examining weak agonists because the changes they induce may be small and need to be confidently distinguished from differences due to the loss of the receptor alone. As shown by the Ito et al. (2007) study and as noted by Maronpot et al. (2004), there is a need for lifetime studies to characterize background or spontaneous tumor patterns and lifespans (including those of the background strain). While the original work by Lee et al. (1995) describes "the mice homozygous for the mutation were viable, healthy, and fertile and appeared normal," the authors did not describe the survival curves for this model nor their background tumor rate. In fact, further work has shown that they carry a background of chronic conditions, including: (1) chronic diseases such as obesity and steatosis (Akiyama et al., 2001; Costet et al., 1998); (2) altered hepatic of hepatocellular structure and function, such as vacuolated hepatocytes (Voss et al., 2006; Anderson et al., 2004), also seen in "humanized" mice (Cheung et al., 2004); and (3) altered lipid metabolism, including reduced glycogen stores, blunted hepatic and cardiac fatty acid oxidation enzyme system response to fasting, elevated plasma free fatty acids, fatty liver (steatosis), impaired gluconeogenesis, and significant hepatic insulin resistance (Lewitt et al., 2001). Howroyd et al. (2004) reported decreased longevity and enhancement of age-dependent lesions in PPARα -/- mice.

These baseline differences from wild-type mice may render them more susceptible to toxic responses or shorten their lifespans with chemical exposure. For example, after administration of 250 μL carbon tetrachloride/kg, all male and 40% of female PPARα knockout

mice were dead or moribund after 2 days of treatment, whereas 25% of male wild-type mice and none of the female wild-type mice exhibited outward signs of toxicity (Anderson et al., 2004). Hays et al. (2005) reported that 100% of PPARα knockout have cholestasis after 1 year of Bezafibrate treatment with higher bile acid concentration than wild-type mice. As described in Section E.2.1.15, Ramdhan et al. (2010) have provided data not only that indicated greater susceptibility of TCE liver toxicity in PPARα-null mice and humanized null mice, but also that there is a background dysregulation of the number of gene and protein expressions and triglyceride accumulation in the liver in these strains.

Lewitt et al. (2001) noted that male knockout mice have more marked accumulation of hepatic fat and hypercholesterolemia and are particularly sensitive to fasting, with some dying if fasted for >24 hours. Sexual dimorphism, especially increased susceptibility of the male mouse, has been reported for knockout mice with pure Sv/129 backgrounds (Anderson et al., 2004; Lewitt et al., 2001) as well as those with a suggested C57BL/6N background (Costet et al., 1998; Djouadi et al., 1998). Akiyama et al. (2001) showed an apparent greater sexual dimorphism in mice with a pure Sv/129 background than C57BL/6N in regard to weight gain from 2 to 9 months, but not in changes in body weight or liver weight between wild-type and knockout animals. Adipose tissue, serum triglycerides, and cholesterol were altered in the knockout animals. Given that the experiment was only carried out for 9 months, changes in body fat, liver weight, and lipid levels may be greater as the animals get older and steatosis is more prevalent.

The dramatic effect on survival as well as gender difference by the increased expression of lipoprotein lipase in the PPARα knockout mouse with further genetic modification is demonstrated by Nöhammer et al. (2003), who reported 50% mortality in 6 months and 100% mortality within 11 months of age while females survived. These differences could affect the results of tumor induction for PPARα agonists with less potency than WY-14,643 that do not produce tumors so rapidly.

In addition, these studies suggest the need for careful consideration of the effects of use of different background strains for the knockout and the need for careful characterization of the background responses of the mouse model and the effects of the use of different background strains for the knockout. Morimura et al. (2006) reported that, using the B6 background strain, there were only foci at time periods but knockouts with the SV129 background had multiple tumors after WY-14,643 treatment.

PPARα knockout mice have also been used to examine the dependence of PPARα on changes in cell signaling, protein production, or liver weight. However, to be useful, the changes incurred just by loss of the PPARα should also be well described. Reported differenced between PPARα-knockout and wild-type mice can impact the sensitivity and specificity of these markers of for the hypothesized mode of action.

In regards to altered cell signaling, Wheeler et al. (2003) note that in normal cells, $p21^{waf}$ and $p27^{kip1}$ inhibit the Cdk/cyclin complexes responsible for cell cycle progression through G1/S

transition. While these cellular signaling molecules are downregulated in response to partial hepatectomy in normal mice, they remain elevated in PPARα knockout mice along with decreased DNA synthesis.

Fumonisins are hepatocarcinogens that have been associated changes in apoptosis and tissue generation, and increased acyl-CoA oxidase and CYP4A (markers of PPARα activation) (Martinez-Larrañaga et al., 1996). Voss et al. (2006) report that the average number of hepatic apoptotic foci per mouse induced by Fumonisins were threefold higher and liver mitotic figures counts were twofold lower in PPARα knockout in comparison to wild-type mice, thus illustrating a difference in proliferative response in the mice. PPARα-null mice have been reported to have increased apoptosis and decreased mitosis with fumonisin treatment.

Voss et al. (2006) also report several differences in gene expression in wild-type and PPARα knockout mice that ranged from 0.3 to 483% of the activity of wild-type mice. The complex expression patterns of gene expression and determination of their mechanistic implications in regard to hepatotoxicity and carcinogenicity are difficult. Certainly the large number of genes whose expression is affected by WY-14,643 (1,012 genes as cited by Voss et al., 2006) illustrates such complexity. Voss et al. (2006) concluded that studies should consider dose- and time course-related effects as well as species and strain-related differences in the expression of gene products.

The "humanized" PPARα mouse has a human copy of PPARα inserted into a PPARα knockout mouse. It is inserted in a tetracycline response system so that in the absence of DOX, only human PPARα is transcribed in humanized mouse liver and not in other tissues. A rigorous examination of newly emerging studies regarding the "humanized" mouse is warranted. The humanized PPARα mouse has been studied in the reports of Cheung et al. (2004), Morimura et al. (2006), and Ramdhan et al. (2010) (see Section E.2.1.15). Many of the issues described above for PPARα -/- mice are of concern for the humanized knockout mouse. In addition, the placement of the humanized PPAR gene is a potential confounding factor, as discussed by Morimura et al. (2006):

> It also cannot be ruled out that the hPPARα mice are resistant to the hepatotoxic effects of peroxisome proliferators due to the site of expression of the human receptor. The cDNA was placed under control of the tetracycline regulatory system and the liver-specific Cebp/B promoter that is preferentially expressed in hepatocytes.

In the Cheung (2004) report, **t**he humanized mouse was fed WY-14,643 for 2 or 8 weeks (age not given for the mice). WY-14,643 and Fenobrate were reported to decrease serum total triglyceride levels in wild and humanized mice to about the level seen in PPARα -/- mice (which were already suppressed without treatment). Hepatomegaly and increase in hepatocyte size were observed in the PPARα-humanized mice fed WY-14,643 for 2 weeks but less than that of wild

mice. By contrast, Morimura et al. (2006) stated that the humanized mice did not exhibit hepatomegaly after treatment with WY-14,643.

Cheung et al. (2004) present figures that showed increased vacuolization of hepatocytes in a control humanized mouse in comparison to wild-type mice. Vacuolization increased with WY-14,643 treatment in the humanized mouse. Therefore, there was a background level of liver dysfunction in these mice even with humanized PPARα. Vacuolization is consistent with fatty liver observed in the nonhumanized PPARα -/- mouse. As reported by Ramdhan et al. (2010), untreated humanized mice had increased triglyceride levels in their livers in comparison to untreated wild type mice.

The authors reported that the humanized mouse did not have increased numbers of peroxisomes after WY treatment. However, they present a figure for genes encoding peroxisomal, mitochondrial, and microsomal fatty acid oxidation enzymes that shows they were still markedly increased in PPARα -humanized mice following 8 weeks of exposure to WY-14,643. Therefore, there is a paradox in these reported results.

Morimura et al. (2006) provided a useful example to illustrate the many issues associated with interpreting studies with genetically-altered animals. While this study is suggestive of a difference in susceptibility to tumor induction between wild-type and PPARα humanized mice, a conclusion that human PPARα is refractory to liver tumor induction is not sufficiently supported.

This study had uneven durations of exposure and follow-up and reported substantial toxicity or mortality that limit the interpretation of the observed tumor rates. For example, the 6-week-old male "humanized" mice had a 44-week experimental period, but for wild-type mice, that period was 38 weeks. In addition, for humanized mice, 10 mice were treated with 0.1% WY-14,643 with 20 controls, but for wild-type mice, 9 mice were given 0.1% WY with 10 controls. Furthermore, wild-type, WY-14,643-treated animals had suppressed growth and only a 50% survival to 38 weeks, so an effective LD_{50} has been used for this length of exposure. Specifically, of the 10 wild-type WY-14,643 treated mice, 3 died of toxicity and 2 were killed due to morbidity and their tissues were examined. Humanized mice had similar growth for animals treated with WY-14,643 or controls with only one mouse killed because of morbidity. Therefore, the reported results, including tumor numbers, are for a mixture of different exposure durations and ages of animals. In addition, the results of the study were reported for only on exposure level.

Furthermore, it is interesting that while control humanized mice had no adenomas, WY-14,643 treated humanized mice had one. Morimura et al. (2006) noted that this adenoma had a morphology "similar to spontaneous mouse liver tumor with basophilic and clear hepatocytes," whereas the tumors in wild-type mice treated with WY-14,643 were more diffusely basophilic. If the humanized animals were allowed to live their natural lifespan, this raises the possibility that WY-14,643 may induce tumors that are similar to other carcinogens rather than those that have been described as "characteristic" of peroxisome proliferators (see

Section E.3.4.1.5) when human PPARα is present. Therefore, the humanized PPARα rather than mouse PPARα may have an association with a tumor phenotype characteristic of other modes of action but this study needed to be carried out for a longer period of exposure and with more animals to make that determination.

The baseline tumor response of PPARα humanized mice needs to be characterized as well as the tumor response following exposure to WY-14,643 or other carcinogens acting through differing modes of action. The numbers of foci were not reported, but "altered foci" were detected in one humanized mouse with WY-14,643 treatment and one without treatment. The phenotypes of the foci were not given by the authors.

As discussed above, changes in liver weights have been associated with susceptibility to liver tumor induction and the issues regarding baseline differences in PPARα -/- mice are equally relevant for PPARα humanized mice. Morimura et al. (2006) reported that absolute liver weight for control humanized mice at 44 weeks was 1.57 g (n = 10). The absolute liver weight for wild control mice was 1.1 g (n = 9) at 38 weeks. The final body weights differed by 14% but liver weights differed by 30%. Therefore, even though comparing different aged mice, the control humanized mice had greater liver size than the wild-type control mice on an absolute and relative basis. This is consistent with humanized knockout mice having greater sized livers and a baseline of hepatomegaly. Ramdhan et al. (2010) reported significantly elevated liver/body weight ratios in untreated humanized mice.

With treatment, Morimura et al. (2006) reported that PPARα humanized mice treated with WY-14,643 had greater absolute and relative liver weights than controls but less elevations than wild-type treated animals. However, because half of the wild-type animals died, it is difficult to discern if liver weights were reported for moribund animals sacrificed as well as animals that survived to 38 weeks for wild-type mice treated with WY-14,643. However, it appears that moribund animals were included that were sacrificed early for treated groups and that values from the animal killed at 27 weeks were added in with those surviving until 45 weeks in the PPARα humanized mice treated with WY-14,643.

With respect to the gene expression results reported by Morimura et al. (2006), it is important to note that they are for liver homogenates with a significant portion of the nuclei from nonparenchymal cell of the liver (e.g., Kupffer and stellate cells). Thus, the results represent changes resulting from a mixture of cell types and from differing zones of the liver lobule, with potentially different gene changes merged together. Livers without macroscopic nodules were used for western blot, but could have contained small foci in the homogenate as well. The gene expression results were also reported for an exposure level of WY-14,643 that is an LD_{50} in wild-type mice and could reflect toxicity responses rather than carcinogenic ones. The samples were also obtained at the end of the experiment (with a mix of durations of exposure) and may not reflect key events in the causation of the cancer but events that are downstream.

These limitations notwithstanding, it is interesting that expression of p53 gene was reported by Morimura et al. (2006) to be increased in PPARα humanized mice treated with WY-14,643 compared to all other groups. Furthermore, of the cell cycle genes that were tested (i.e., CD-1, Cyclin-dependent Kinases 1 and 4, and c-Myc), there was a slightly greater level of c-Myc and CD-1 in control PPARα humanized mice than control wild-type mice as a baseline. This could indicate that there was already increased cell cycling going on in the control PPARα humanized mouse and could be related to the increased liver size. Treatment with WY-14,643 induced an increase in cycling genes in wild-type mice in relation to its control, but whether that induction was greater than control levels for PPARα humanized mice for c-Myc and CDk4 was not reported by the authors.

Apoptosis genes were reported to have little difference between control PPARα humanized and wild-type mice but to have a greater response induced by WY-14,643 in humanized mice for p53 and p21. There was no consistent or large change in apoptosis genes in response to exposure to WY-14,643 in wild-type mice. The increased response of apoptosis genes in PPARα humanized mice without corresponding tumor formation does not support that response as a key event in the mode of action (neither does the lack of response from WY-14,643 in wild-type mice). For genes associated with PPARα peroxisomal (Acox), microsomal (CYP4a), mitochondrial fatty oxidation (Mcad) and especially malic enzyme, there was a greater response in wild-type than PPARα humanized mouse after treatment with WY-14,643. However, this is somewhat in contrast to Cheung et al. (2004), who reported increases in some genes encoding peroxisomal, mitochondrial, and microsomal fatty oxidation enzymes in the PPARα humanized mouse after treatment with WY-14,643.

The results reported by Yang et al. (2007) use another type of "humanized" mouse to study PPARα effects. Yang et al. (2007) used a PPARα humanized transgenic mouse on a PPAR -/- background that has the complete human PPARα (hPPARα) gene on a PAC genomic clone, introduced onto the mouse PPARα-null background and expressed hPPARα not only in the liver but also in other tissues. Mice were administered WY-14,643 or Fenofibrate (0.1 or 0.2% [w/w]). The authors showed a figure representing expression of the hPPARα for two mice with the tissue used for the genotyping exhibiting great variation in expression between the two cloned mice as indicated by intensity of staining. The authors stated that in agreement with mRNA expression, hPPARα protein was highly expressed in the liver of hPPARα^{PAC} mice to an extent similar to the mPPARα in wild-type mice. They reported that following 2 weeks of Fenofibrate treatment, a robust induction of mRNA expression of genes encoding enzymes responsible for peroxisomal (Acox), mitochondrial (MCAD and LCAD), microsomal (CYP4A) and cytosolic (ACOT) fatty acid metabolism were found in liver, kidney, and heart of both wild-type and hPPARα^{PAC} mice, indicating that hPPARα functions in the same manner as mPPARα to regulate fatty acid metabolism and associated genes.

However, the authors did no measures in Fenofibrate-treated animals, only WY-14,643, raising the issue of whether there was a difference in the relative mRNA expression of genes for ACOX etc. and lipids between the two peroxisomal proliferator treatments. The expression of enzymes associated with PPARα induction was presented only for mice treated with Fenofibrate. However, the lipid results were presented only for mice treated with WY-14,643. Therefore, it cannot be established that these two agonists give the same response for both parameters. Also for the enzymes, the relative expressions compared to wild-type controls, the absolute expression, and variation between animals is not reported.

It appears that the peroxisomal enzyme induction by Fenofibrate is the same in the wild-type and transgenic mice. However, in Figure 4 of the paper, the mice treated with WY-14,643 instead of Fenofibrate were presented for the peroxisomal membrane protein 70 (PMP70) in total liver protein gel. There appears to be more PMP70 in the transgenic mice than wild-type mice as a baseline. The PMP70 appeared to be similar after WY-14,643 treatment. However, only one gel was given and no other quantitation was given by the authors.

The authors stated that "in addition WY-14,643 and Fenofibrate treatment produced similar effect to the liver specific humanized PPARα mouse line (Cheung et al., 2004)." However, the results were not the same between Fenofibrate and WY-14,643 and the mouse line used by Cheung et al. (Cheung et al., 2004) had background differences in response and pathology. In one figure in the paper, there appears to be a difference in background level of serum total triglyceride between the wild-type and hPPARα^{PAC} mice that the authors did not note. The power of using such few mice does not help discern any significant differences in background level of triglycerides.

The authors note that WY-14,643 treatment also resulted in decreased serum triglycerides levels in hPPARα^{PAC} mice, consistent with the induction of expression of genes encoding fatty acid metabolism, and that the hypolipidemic effects of fibrates are generally explained by increased expression of LPL and decreased expression of apolipoprotein C- III (Apo C-III) (Auwerx et al., 1996). However, the alteration of these genes by WY-14,643 treatment was only observed in wild-type mice and not in hPPARα^{PAC} mice, suggesting that the hypolipidemic effect observed in hPPARα^{PAC} mice are not through LPL and APO C-III. The authors do not note that there could be a difference in the regulation of these pathways by the transgene rather than how the normal gene is regulated and the pathways it affects. The rationale for examining this question with WY-14,643 treatment rather than with Fenofibrate treatment is not addressed by the authors, especially since the other "markers" of peroxisomal gene induction appear to be affected by Fenofibrate in the wild-type and hPPARα^{PAC} mice.

Hepatomegaly was reported to be observed in the hPPARα^{PAC} mice following 2 weeks of WY-14,643 treatment as revealed by the increase liver to body weight ratio compared to untreated hPPARα^{PAC} mice, but to be markedly lower when compared to wild-type mice under the same treatment.

Histologically, the livers of the wild-type mice treated with WY-14,643 were hypertrophic with clear eosinophilic regions. These phenotypic effects were observed in both wild-type and hPPARα^{PAC} mice. The percent liver/body weight was reported to increase from ~4% in wild-type mice to ~9% after WY-14,643 treatment and from ~4% in hPPARα^{PAC} to little less that 6% after treatment with WY-14,643.

In wild-type mice treated with WY-14,643, the labeling index was 21.8% compared with 1.1% in untreated wild-type controls. In hPPARα^{PAC} mice, WY-14,643 treatment was reported to give an average labeling index of 1.0% compared with 0.8% in the untreated control hPPARα^{PAC} mice. Treatment with WY-14,643 treatment was reported to result in a marked induction in the expression of CDK4 and cyclin D1 in the livers of wild-type mice but to be unaffected hPPARα^{PAC} mice treated with WY-14,643. These data were reported to be in agreement with the liver-specific PPARα-humanized mice that showed no increase in incorporation of BrdU into hepatocytes upon treatment with WY-14,643 (Cheung et al., 2004) and further confirmed that activation of hPPARα does not induce hepatocyte proliferation.

However, the authors present a figure as an example with one liver each with no quantitation given by the authors for BrdU incorporation. It is not clear whether the pictures were taken from the same area of the liver or how representative they are. The numbers of mice were never reported for the labeling index. The data presented do suggest that there was hypertrophy and hepatomegaly in the humanized mice, but not proliferation in this particular WY-14,643 model. Of interest would be investigation of proliferation by other peroxisome proliferators besides WY-14,643 at this necrogenic dose, as it is WY-14,643 that is the anomaly to continue to induce proliferation or DNA synthesis at 2 weeks. The photomicrographs presented by the authors are so small and at such low magnification that little detail can be discerned from them. There are no portal triads or central veins to orient the reader as to what region of the liver has been affected and where, if any, there would be hepatocellular vacuolization.

To determine whether peroxisome proliferation occurred in the hPPARα^{PAC} mice upon administration of peroxisome proliferators, Yang et al. (2007) examined by Western Blot analysis the protein levels of the major PMP70 a marker of peroxisome proliferation). After 2 weeks of treatment with 1,000 ppm WY-14,643, induction of PMP70 was reported to be observed in the wild-type mice as well as in hPPARα^{PAC} mice. The authors suggested that this result indicates that peroxisomal proliferator treatment induced peroxisomal proliferation in hPPARα^{PAC} mice. The results of this study indicate that hepatomegaly and peroxisome proliferation occur in this humanized mouse model when treated with large concentrations of WY-14,643. Thus, these results are inconsistent with claims that peroxisome proliferators cannot cause hepatomegaly or peroxisome proliferation in humans or that humans are refractory to these effects. Like the lipid effects, they suggest that a broader spectrum of effects may occur in humans and decreases the specificity of these effects as species specific. However, due to the

model compound being WY-14,643 at a necrogenic dose of 1,000 ppm, the effect may not be seen in humans using the lower potency peroxisome proliferators. It would have been useful for this study to include an examination of these effects with Fenofibrate rather than WY-14,643 and then attempting to extrapolate such effects to other peroxisome proliferators. The authors often attributed the effects of peroxisome proliferators to those reactions induced by WY-14,643 and did not acknowledge that the changes induced by WY-14,643 may be different. This is especially true in regards to hepatocellular DNA synthesis in which other peroxisome proliferators can cause liver tumors without the sustained proliferation that WY-14,643 induces, especially at a necrogenic dose.

Yang et al. (2007) reported the results of induction of various genes by WY-14,643 in wild-type and hPPARα^{PAC} mice by microarray analysis followed by confirmation and quantitation by qPCR and report that more genes were induced by WY-14,643 in wild-type mice than in hPPARα^{PAC} mice. They reported that:

> importantly, the oncogene c-myc was not induced in hPPARα^{PAC} mice. Moreover, genes encoding cell surface proteins such as Anxa2, CD39, CD63, Ly6D, and CD24a, and several other genes such as *Cidea, Cidec, Dhrs8* and *Hsd11b* were also not induced in hPPARα^{PAC} mice. Interestingly, Sult2a1 was only induced in hPPARα^{PAC} mice and not in WT mice; this gene is also induced in human hepatocytes by PP (Fang et al., 2005). The regulation of several of these genes has previously been demonstrated through a PPARα-dependent mechanism. Additional studies will be necessary to fully explore the molecular regulatory mechanism and the functional implication associated with these differently regulated genes.

The authors did not indicate the context of how the mice were treated, whether these were pooled results, and when the samples were taken. It is assumed to be whole liver. There are several limitations for interpretations of the results such as those presented by Yang et al. (2007), which include the lack of phenotypic anchoring for the results. The authors have shown changes from whole liver and have listed changes in genes between wild-type and humanized mice on a PPAR -/- background that, in itself, will bring about changes in gene expression. The authors acknowledge difficulties in determining what their reported gene changes mean.

Yang et al. (2007) reported that "activation of PPARα alters hepatic miRNA expression (Shah et al., 2007)." They report that let-7C, a miRNA critical in cell growth and shown to target c-Myc, was inhibited by WY-14,643 treatment in wild-type mice and that the expression levels of both pri-let-7C and mature let-7C were significantly higher in hPPARα^{PAC} mice compared to wild-type mice. Treatment with WY-14,643 was reported to decrease the expression of Pri-let-7C and mature let-7C in wild-type mice but in hPPARα^{PAC} mice. The authors noted that:

in addition, the induction of *c-myc* by WY-14,643 treatment in wild type mice did not occur in WY-14,643 treated hPPARα^{PAC} mice. This is in agreement with the previous observation in liver-specific humanized PPARα (Shah et al., 2007) and further indicates the activation of human PPARα does not cause a change in hepatic miRNA and c-*myc* gene expression.

A qPCR analysis of pri-let-7C following 2 weeks of WY-14,632 treatment was reported for wild-type and hPPARα^{PAC} mice (n = 3–4). There appeared to be ~20 times more let-7C expression in hPPARα^{PAC} mice than control wild mice as a baseline. The gel given by the authors showed a very small difference in wild-type mice in let-7C northern blot analysis between a control wild-type and a WY-14,643-treated wild-type mouse. There appeared to be no difference in the hPPARα^{PAC} mice between control and WY-14,643 treatment and a larger stained area than the control wild-type mice. The relative c-Muc expression between the hPPARα^{PAC} mice and wild-type control mice did not correlate with changes in let-7C expression.

Thus, the amount of decrease by treatment with WY-14,632 in wild-type mice appeared to be extremely small compared to the much greater baseline expression in the hPPARα^{PAC} mice. The change brought by WY-14,632 treatment in wild-type mice was a small change compared to the 20-fold greater baseline expression in the hPPARα^{PAC} mice. The authors stated that the expression of the c-Myc regulator was higher in the hPPARα^{PAC} mice, indicating overregulation of cell division and an inability for hepatocytes to proliferate. However, their results showed that there was a greater difference in regulatory baseline function of the PPAR using this paradigm and this construct. Are these differences due to human PPAR or to the way PPAR was put back into PPAR -/- mouse and expected to function? If the experiment included mouse PPAR put back in this way on a null background, what would such an experiment show? Are these results representative of the PPAR or how it is now controlled and expressed? In addition, what would the study of other peroxisome proliferators besides WY-14,643 show in regard to changes in miRNA. Are these results reflective of a just the transient effect that is prolonged in a special case?

As discussed in Section E.3.1.2, there are issues with microarray data in addition to the newly emerging field of miRNA arrays, which include phenotypic anchoring and whether they are from whole liver or pooled samples. The results given in this report are for relative let-7C expression given and not absolute values. The changes in baseline let-7C expression between the wild-type and the hPPARα^{PAC} mice did not correlate with the magnitude of difference in northern blot analysis and did not correlate at all with c-Myc expression reported in this study. Thus, a direct correlation between the effect of let-7C expression and function and effects from WY-14,643 was not supported. The relative expression was reported, but the variation of baseline expression of the "PPAR controlled genes" was not. Given that one of the first figures reported a large difference between animals in expression of the human PPAR gene in the

transgenic animals, how did this difference affect the results given here as relative changes downstream?

Yang et al. (2007) concluded that the hPPARα^{PAC} mice represent the most relevant model for humans since, the tissue distribution of PPARα is similar to that observed in wild-type mice and the hPPARα in hPPARα^{PAC} mice is underregulation of its native promoter. Indeed upregulation of hepatic mPPARα in wild-type mice by fasting was mirrored by the hPPARα in hPPARα^{PAC} mice. However, there was no demonstration that the artificial chromosome that is replicating along with other DNA is controlled sterically by the same control since it is not on the mouse genome in the same place as the native PPAR. There is also not a demonstration of how stable the baseline of PPAR DNA expression is in this mouse model—does it vary as much or more than native PPAR between mice? The authors stated that:

> induction of PPARα target genes for fatty acid metabolism and a decrease in serum triglycerides by PP in hPPARα^{PAC} mice indicates that hPPARα is functional in the mouse environment with respects to regulation of fatty acid metabolism. This is in agreement with the liver-specific PPARα humanized mice that also exhibit these responses (Cheung et al., 2004). Indeed the DNA binding domain of hPPARα is 100% homologous with that of the mouse suggesting that both bind to the same PPRE binding site in the promoter region of target genes. Transfection of hPPAR into murine hepatocytes increased PPs induced peroxisome proliferation related effects (Macdonald et al., 1999). These results suggest that hPPARα and mPPARα do not differ in induction of target genes with known PPRE.

However, replacement with human PPAR in the Cheung et al. (Cheung et al., 2004) model is not sufficient to prevent the same types of toxicity as seen with PPAR knockouts on the hepatocytes such as steatosis.

Yang et al. (2007) note that:

> the increased LPL and decreased expression of apo C-III are proposed to explain the hypolipidemic effects of PPS (Auwerx et al., 1996). However, hPPARα^{PAC} mice treated with PP exhibit lowered serum triglycerides without alteration of the expression of LPL and apo C-III. This indicates the hypolipidemic effects in rodents are mediated via other molecular regulatory mechanisms. It is also suggested that the activation of PPARα by PPs stimulates hepatic fatty acid oxidation and thereby diminishing their incorporation into triglycerides and secretion of VLDL (Frøyland et al., 1997). Consistent with this idea, a robust induction of the genes encoding enzymes for fatty acid oxidation by PP in hPPARα^{PAC} mice were observed. Thus, the exact mechanism by which PPs exert their hypolipidemic effects needs reexamination.

However, the use of two different peroxisome proliferators (i.e., WY-14,643 and Fenofibrate) for two types of effects (peroxisomal and lipid) may be the cause of some paradoxes

here in terms of the mode of action for lipid effects. The baseline differences in the hPPARα^{PAC} mice for serum total triglycerides was not explored by these authors and the small number of animals used make conclusions difficult about the magnitude of difference. The differences in baseline expression for LPL are not discernable in the graphic representation of the results.

Yang et al. (2007) noted that:

> on the other hand, the difference in the affinity of ligands for the human and mouse PPARα receptor was proposed to account for the species difference. The ligand binding domain of hPPARα is 94% homologous with that of the mouse. *In vitro* transactivation assays have previously shown that WY has a higher affinity for rodent PPARα than human PPARα, while Fenofibrate has similar affinity for rodent and human PPARα (Shearer and Hoekstra, 2003; Sher et al., 1993). In the present study WY and Fenofibrate exhibit the same capacity to induce known PPARα target genes in the liver, kidney and heart in both wild-type and hPPARα^{PAC} mice.

The statement by the authors that Fenofibrate and WY-14,643 had the same affinity "as shown by this study" is not correct. The two treatments were not studied for the same enzymes or genes in the data reported in the study. Both WY-14,643 and Fenofibrate can induce PPARα targets, but it was not shown to the same extent. Yang et al. (2007) stated that:

> This is in agreement with the liver-specific PPARα humanized mice that also exhibit a similar capacity to induce PPARα target genes in liver by WY and Fenofibrate (Cheung et al., 2004). Thus, the ligand affinity difference between mouse and human PPARα may not be critical under the conditions of these studies.

Alternatively, these results could reflect that these studies were conducted with two different agonists with different affinities and responses due to receptor activation.

Finally, a useful comparison to make are the differences between wild-type mice, PPARα -/- mice that serve as the background for the transgenic human mouse models, and both transgenic models. The small and variable number of animals examined in these studies is readily apparent. The results of the Cheung et al. (2004) humanized mouse model and those reported for Yang et al. (2007) show differences in the study designs including PPARα agonists studied for particular effects and results reported for similar treatments (see Table E-18).

As shown above, the effect on the PPARα -/- by the knockout included decreased triglyceride levels and slightly increased liver weight. Although treatment with WY-14,643 and Fenofibrate were reported to decrease triglyceride levels in wild-type mice, paradoxically, so did knocking out the receptor. Exposures to WY-14,643 appeared to induce a slight increase and exposures to Fenofibrate induced a slight decrease in triglyceride levels in PPARα -/- mice, but the variability of response and small number of animals in the experiments limited the ability to discern a quantitative difference in the treatments.

In the study by Cheung et al. (2004), it appears that the insertion of humanized PPARα restored the baseline and treatment responses for triglyceride levels. Of note is that use of the same humanized mode in Ramdhan et al. (2010) showed accumulation of triglycerides in the liver of untreated mice. Overall, the results reported by Yang et al. (2007) appeared to show a lower level of triglycerides in control wild-type mice that was similar in magnitude to the treatment effect reported by Fenofibrate by Cheung et al. (2004). However, there also appeared to be restoration of this effect in the humanized mouse model of Yang et al. (2007).

In regard to DNA synthesis, both Cheung et al. (2004) and Yang et al. (2007) only gave results for WY-14,643 and for different durations of exposure, so they were not comparable. It appeared that ~60% of hepatocytes were labeled by 8 weeks of WY-14,643 treatment (Cheung et al., 2004) compared to ~20% after 2 weeks of exposure. Again, this highlights the difference between using WY-14,643 as a model for the PPARα as a class at times when almost all other PPARα agonists have ceased to increase DNA synthesis or have reductions in this parameter. The background changes due to the PPARα -/- knockout were not reported so that the effects of the knockout could not be ascertained. It appeared that insertion of humanized PPARα did not result in restoration of WY-14,643-induced DNA synthesis. The correlation with this parameter and any focal areas of necrosis were not discussed by the authors of the study.

Table E-18. Comparison between results for Yang et al. (2007) and Cheung et al. (2004)[a]

Effect	Wild type mice	PPAR -/- knockout mice	Humanized mice (liver only)	Humanized PAC mice
Triglycerides	**Cheung** **(n = 6–9)** Control 145 mg/mL 0.1% WY-14,643 (2 wks) 60 mg/mL 0.2% Fenofibrate (2 wks) 85 mg/mL **Yang** **(n = 4–6)** Control 95 mg/mL 0.1 % WY-14,643 (2wks) 55 mg/mL	**Cheung** **(n = 6–9)** Control 100 mg/mL 0.1% WY-14,643 (2 wks) 115 mg/mL 0.2% Fenofibrate (2 wks) 85 mg/mL	**Cheung** **(n = 6–9)** Control 175 mg/mL 0.1%WY-14,643 (2 wks) 60 mg/mL 0.2% Fenofibrate (2 wks) 85 mg/mL	**Yang** **(n = 4–6)** Control 120 mg/mL 0.1%WY-14,643 (2 wks) 75 mg/mL
BrdU incorporation	**Cheung** **(n = 5)** Control 1.6% 0.1% WY-14,643 (8 wks) 57.9% **Yang** **(n = 4–6)** Control 1.1% 0.1% WY-14,643 (2 wks) 21.8%	Not done	**Cheung** **(n = 5)** Control 1.6% 0.1% WY-14,643 (8 wks) 2.8%	**Yang** **(n = 4–6)** Control 0.8% 0.1% WY-14,643 (2 wks) 1.0%

Table E-18. Comparison between results for Yang et al. (2007) and Cheung et al. (2004) (continued)

Effect	Wild type mice	PPAR -/- knockout mice	Humanized mice (liver only)	Humanized PAC mice
Hepatomegaly[b] (% liver body weight ratio)	**Cheung (n = 5–9)** Control 4% 0.1% WY-14,643 (2 wks) 11% 0.2% Fenofibrate (2 wks) 8.5% **Yang (n = 4–6)** Control 4% 0.1% WY-14,643 (2 wks) 9%	**Cheung (n = 5–9)** Control 5% 0.1% WY-14,643 (2 wks) 5% 0.2% Fenofibrate (2 wks) 5.5%	**Cheung (n = 5–9)** Control 4.5% 0.1% WY-14,643 (2 wks) 7% 0.2% Fenofibrate (2 wks) 7%	**Yang (n = 4–6)** Control 4% 0.1% WY (2 wks) 6%

[a]The ages of the humanized knockout mice are not given for Cheung et al. (2004) but are 8–10 weeks for Yang et al. (2007).
[b]Percentages are approximate values extrapolated from figures for hepatomegaly.

In regard to hepatomegaly, Fenofibrate and WY-14,643 appeared to both give an increase in liver weight in the humanized mouse model of Cheung et al. (2004) with little effect in the knockout mouse. For Fenofibrate, there was little difference in liver weight gain in the wild-type mouse and that of the humanized mouse model of Cheung et al. (Cheung et al., 2004). However, Fenofibrate was not tested in the humanized mouse model of Yang et al. (2007). In that model, only WY-14,643 was used, but there was still an increase in liver weight. Thus, in terms of effects on liver weight gain and triglyceride levels, both models gave comparable results and appeared to indicate that insertion of humanized PPARα would restore some of the effects of the knockout. However, the results from both experiments highlight the need for adequate numbers of animals and other PPARα agonists to be tested besides WY-14,463 at such a high dose and certainly for longer periods of time to ascertain whether such manipulations will affect carcinogenicity.

The study by Ramdhan et al. (2010) is more definitive in regard to characterization of the effects of the knockout and insertion of human PPARα (see Section E.2.1.15). From this study, dysregulation by the knockout and by reinsertion of human PPARα at levels of >10-fold protein expression can be observed and include alterations in a number of gene and protein expression levels and an underlying background level of hepatic steatosis and triglyceride accumulation.

E.3.4.1.4. NF-κB Activation

NF-κB activation has also been proposed as a key event in the induction of liver cancer through PPARα activation. As discussed in Sections E.3.1.6 and E.3.3.3.3, activation of the NF-κB pathway is implicated in carcinogenesis, nonspecific for a particular mode of action for liver cancer, and is context-dependent on its effects. Its specific actions depend on the cell type and type of agent or signal that activates translocation of the complex. NF-κB is not only involved in biological processes other than tumor induction, but also exhibits some apparently contradictory behaviors (Perkins and Gilmore, 2006). Although many studies point to a tumor-promoting function of NF-κB subunits, evidence also exists for tumor suppressor functions. NF-κB actions are associated with TNF and JNK, among many other cell signaling systems and molecules, and have functions that alter proliferation and apoptosis. NF-κB activation reported in some studies may be associated with early Kupffer cell responses and be associative but not key events in the carcinogenic process. However, most assays look at total NF-κB expression in the whole liver and at the early periods of proliferation and apoptosis. The origin of the NF-κB is crucial as to its effect in the liver. For instance, hepatocyte specific deletion of IKKβ increased DEN-induced hepatocarcinogenesis, but a deletion of IKKβ in both hepatocytes and Kupffer cells, however, was reported to have the opposite effect (Maeda et al., 2005).

E.3.4.1.5. Phenotype as an Indicator of a PPARα Mode of Action

As discussed previously (see Sections E.3.1.5, and E.3.1.8), FAH precede both hepatocellular adenomas and carcinomas in rodents and in humans with chronic liver diseases that predispose them to HCCs. Striking similarities in specific changes of the cellular phenotype of preneoplastic FAH are emerging in experimental and human hepatocarcinogenesis, irrespective of whether this was elicited by chemicals, hormones, radiation, or viruses, or in animal models, by transgenic oncogenes or *Helicobacter hepaticus*. Several authors have noted that the detection of phenotypically similar FAH in various animal models and in humans prone to developing or bearing HCCs favors the extrapolation from data obtained in animals to humans (Bannasch et al., 2003; Su and Bannasch, 2003; Bannasch et al., 2001). In regard to phenotype by tincture, Caldwell and Keshava (2006) stated:

> In addition, the term "basophilic" in describing preneoplastic foci or tumors can be misleading. The different types of FAH have been related to three main preneoplastic hepatocellular lineages: 1) the glycogenotic-basophilic cell lineage, 2) its xenomorphic-tigroid cell variant, and 3) the amphophilic-basophilic cell lineage. Specific changes of the cellular phenotype of the first two lineages of FAHs are similar in experimental and human hepatocarcinogenesis, irrespective of whether they were elicited by DNA-reactive chemicals, hormones, radiation, viruses, transgenic oncogenes and local hyperinsulinism as described by the first two FAHs and this similarity favors extrapolation from data obtained in animals to humans (Bannasch et al., 2003; Su and Bannasch, 2003; Bannasch et al., 2001). In contrast, the amphophilic cell lineage of hepatocarcinogenesis has been observed mainly after exposure of rodents to peroxisome proliferators or to hepadnaviridae (Bannasch et al., 2001).
>
> Bannasch (1996) describes "amphophilic" FAH and tumors induced by peroxisome proliferators to maintain the phenotype as the foci progress to tumors. They are glycogen poor from the start with increased numbers of mitochondria, peroxisomes and ribosomes. The author further states that the "homogenous basophilic" descriptions by others of foci induced by WY are really amphophilic. Agents other than peroxisome proliferators can induce "acidophilic" or "eosinophilic" (due to increased smooth endoplasmic reticulum) or glycognotic foci which tend to progress to basophilic stages (due to increased ribosomes).
>
> Tumors and foci induced by peroxisome proliferators have been suggested to have a phenotype of increased mitochondrial proliferation and mitochondrial enzymes (thyromimetic rather than insulinomimetic) (2006).

Tumors from peroxisome proliferators in Kraupp-Grasl et al. (1990) and Grasl-Kraupp et al. (1993) for rat liver tumors were characterized as weakly basophilic with some eosinophilia and as similar to the description given by Bannasch et al. as amphophilic. However, a number of recent studies indicate that other "classic" peroxisome proliferators may

have a different phenotype than has been attributed to the class through studies of WY-14,643. A recent study of DEHP, another peroxisome proliferator assumed to induce liver tumors through activation of the PPARα receptor, reported the majority of liver FAH to be of the first two types after a lifetime of exposure to DEHP with a dose-related tendency for increased numbers of amphophilic FAHs in rats (Voss et al., 2005). As stated previously, the mode of action of DEHP-induced liver tumors in mice also appears not to be dependent on PPARα activation.

Michel et al. (2007) reported the phenotype of tumors and foci in rats treated with clofibric acid at a very large dose (5,000 ppm for 20 months) and noted that in controls, the first type of foci to appear was tigroid on day 264 and their incidence increased with time representing the most abundant type in this group. They reported no adenomas or carcinomas at up to 607 days after giving saline injection in the control animals.

DEN treatment was examined up to 377 days only, with tigroid, eosinophilic, and clear cell foci observed at that time. Clofibric acid was examined up to 607 days, with tigroid and clear cell foci reported to be the first to appear on day 264, but no other foci class. By day 377, there were tigroid, eosinophilic, and clear cell foci, but no basophilic foci reported with clofibric acid treatment and, although only a few animals were examined, 2/5 had adenomas but not carcinomas. By day 524, all types of foci were seen (including basophilic for the first time) and there were adenomas and carcinomas in 2/5 animals. By 607 days, a similar pattern was observed without adenomas, but 3/6 animals had carcinomas.

Although the number of animals examined was very small, these results indicate that clofibric acid was not inducing primarily "basophilic foci" as reported for peroxisome proliferators, but that the first foci are tigroid and clear cell foci. Basophilic foci did not appear until day 524 as similar to control values for foci development and distribution. However, unlike controls, clofibric acid induced eosinophilic and clear cell foci earlier. This is inconsistent with the phenotype ascribed to peroxisome proliferators as exemplified by WY-14,643.

In regard to GST-π and γ-transpeptidase (GGT), Rao et al. (1986) fed two male F344 rats a diet of 0.1% WY-14,643 for 19 months or three F344 rats 0.025% Ciprofibrate for 15–19 months and reported "altered areas,"(AA) "neoplastic nodules" (NN), and HCCs (HCC). For WY-14,643 treatment, 107 AA, 75 NN, and 5 HCC were noted, and for Ciprofibrate treatment, 107 AA, 27 NN, and 16 HCC were identified. In the WY-14,643-treated rats, HCC, and NN were both GGT and GST-π negative (96–100%) with 87% of AA was negative for both. In Ciprofibrate-treated rats, NN and HCC were negative for both markers (95%) but only 46% of AA were negative for both markers. Thus, a different pattern for tumor phenotype was reported for WY-14,643 and another peroxisome proliferator, Ciprofibrate, in this study as well.

In addition, GGT phenotype is reported not to be specific to weakly basophilic foci. GGT staining was reported to be negative in eosinophilic tumors after initiation and promotion.

Kraupp-Grasl et al. (1990) noted differences among PPARα agonists in their ability to promote tumors and suggested they not necessarily be considered a uniform group. Caldwell and Keshava (2006) suggested that the reports of a simple designation of "basophilic" is not enough to associate a foci as caused by peroxisome proliferators (Bannasch, 1996; Grasl-Kraupp et al., 1993; Kraupp-Grasl et al., 1990). Increased basophilia of tumors and increased numbers of carcinomas is consistent with the progressive basophilia described by Bannasch (1996), as many adenomas progress to carcinomas.

It should be noted that the amphophilic foci and tumors described by Bannasch et al. were primarily studied in rats. Morimura et al. (2006) noted that WY-14,643 induced diffusely basophilic tumors in mice and therefore, identified the WY-14,643 tumors in a way consistent with the descriptions of amphophilic tumors by Bannasch et al. The tumor induced by WY-14,643 in their humanized mouse was reported to be similar to those arising spontaneously in the mouse. However, the mouse response could differ from the rat, especially for PPARα agonists other than WY-14,643.

H-ras activation and mutation studies have attempted to assign a pattern to peroxisome proliferator-induced tumors as noted in Section E.2.4.4. However, also as noted in Section E.2.4.4, the genetic background of the mice used, the dose of carcinogen, and the stage of progression of "lesions" (i.e., foci vs. adenomas vs. carcinomas) may affect the number of activated H-ras containing tumors that develop. Fox et al. (1990) noted that tumors induced by Ciprofibrate (0.0125% diet, 2 years) had a much lower frequency of H-ras gene activation than those that arose spontaneously (2-year bioassays of control animals) or induced with the "genotoxic" carcinogen benzidine-2 HCl (120 ppm, drinking H_2O, 1 year) and that the Ciprofibrate-induced tumors were reported to be more eosinophilic as were the surrounding normal hepatocytes than spontaneously occurring tumors. Anna et al. (1994) also stated that mice treated with Ciprofibrate had a markedly lower frequency of tumors with activated H-ras but that the spectrum of mutations in tumors was similar those in "spontaneous tumors." Hegi et al. (1993) tested Ciprofibrate-induced tumors from Fox et al. (1990) in the NIH3T3 cotransfection-nude mouse tumorigenicity assay and concluded that ras protooncogene activation was not a frequent event in Ciprofibrate-induced tumors and that spontaneous tumors were not promoted with it.

Stanley et al. (1994) studied the effect of MCP, a peroxisome proliferator, in $B6C3F_1$ (relatively sensitive) and C57BL/10J (relatively resistant) mice for H-ras codon 61-point mutations in MCP-induced liver tumors (hepatocellular adenomas and carcinomas). In the $B6C3F_1$ mice, ~24% of MCP-induced tumors had codon 61 mutations, and for C57BL/10J mice, ~13%. The findings of an increased frequency of H-ras mutation in carcinomas compared to adenomas in both strains of mice is suggestive that these mutations were related to stage of progression. Thus, in mice, the phenotype of tumors did not appear to be readily distinguishable

from spontaneous tumors based on tincture for peroxisome proliferators other than WY-14,643, but did have more of a signature in terms of H-ras mutation and activation.

The expression of c-Jun has been used to discern TCE tumors from those of its metabolites. However, as pointed out by Caldwell and Keshava (2006), although Bull et al. (2004) have suggested that the negative expression of c-Jun in TCA-induced tumors may be consistent with a characteristic phenotype shown in general by peroxisome proliferators as a class, there is no supporting evidence of this. While increased mitochondrial proliferation and mitochondrial enzymes (thyromimetic rather than insulinomimetic) properties have been ascribed to peroxisome proliferator-induced tumors, the studies cited in Bull et al. (2004) have not examined TCA-induced tumors for these properties.

E.3.4.1.6. Human Relevance

In its framework for making conclusions about human relevance, the U.S. EPA Cancer Guidelines (U.S. EPA, 2005b) asks that critical similarities and differences between test animals and humans be identified. Humans possess PPARα at sufficient levels to mediate the human hypolipidemic response to peroxisome-proliferating fibrate drugs. Fenofibrate and Ciprofibrate induce treatment-related increases in liver weight, hypertrophy, numbers of peroxisomes, numbers of mitochondria, and smooth endoplasmic reticulum in cynomologous monkeys at 15 days of exposure (Hoivik et al., 2004). Given the species difference in the ability to respond to a mitogenic stimulus such as partial hepatectomy (see Section E.3.3), lack of hepatocellular DNA synthesis at this time point is not unexpected, and as Rusyn (2006) noted, examination at differing time point may produce differing results. It is therefore, generally acknowledged that "a point in the rat and mouse key events cascade where the pathway is biologically precluded in humans in principle cannot be identified" (Klaunig et al., 2003); NRC, 2006). Thus, from a qualitative standpoint, the effects described above are plausible in humans.

As for quantitative differences, there are two key issues. First, as stated in the Cancer Guidelines, when considering human relevance, "Any information suggesting quantitative differences between animals and humans is flagged for consideration in the dose-response assessment." Therefore, while Klaunig et al. (2003) and NRC (2006) go on to suggest that "this mode of action is not likely to occur in humans based on differences in several key steps when taking into consideration kinetic and dynamic factors," under the Cancer Guidelines, such "kinetic and dynamic factors" need to be made explicit in the dose-response assessment, and should not be part of the qualitative characterization of hazard. Second, the discussion above points to the lack of evidence supporting associations between the postulated events and carcinogenic potency. Thus, because interspecies differences in carcinogenicity do not appear to be associated with interspecies differences in postulated events, they do not provide reliable metrics with which to make inferences about relative human sensitivity.

E.3.4.2. Other TCE Metabolite Effects That May Contribute to its Hepatocarcinogenicity

While the focus of most studies of TCA has been its effects on peroxisomal proliferation, DCA has been investigated for a variety of effects that are also observed either in early stages of oncogenesis (glycogen deposition) or conditions that predispose patients to liver cancer. Some studies have examined microarray profiles in attempt to study the mode of action of TCE (see Section E.3.1.2 for caveats regarding such approaches). Caldwell and Keshava (2006) have provided a review of these studies, which is provided below.

E.3.4.2.1. DCA Effects and Glycogen Accumulation Correlations with Cancer

As noted previously, DCA administration has been reported to increase the observable amount of glycogen in mouse liver via light microscopy and, although to not be primarily responsible for DCA-induced liver mass increases, to increase whole-liver glycogen as much by 50% (Kato-Weinstein et al., 2001). Given that TCE and DCA tumor phenotypes indicate a role for DCA in TCE hepatocarcinogenicity (see Section E.2.4.4), Caldwell and Keshava (2006) described the correlations with effects induced by DCA that have been associated with hepatocarcinogenicity.

> A number of studies suggest DCA-induced liver cancer may be linked to its effects on the cytosolic enzyme glutathione (GST)-S-transferase-zeta. GST-zeta is also known as maleylacetoacetate isomerase and is part of the tyrosine catabolism pathway whose disruption in type 1 hereditary tyrosinemia has been linked to increased liver cancer risk in humans. GST-zeta metabolizes maleylacetoacetate (MAA) to fumarylacetoacetate (FAA) which displays apoptogenic, mutagenic, aneugenic, and mitogenic activities (Bergeron et al., 2003; Jorquera and Tanguay, 2001; Kim et al., 2000). Increased cancer risk has been suggested to result from FAA and MAA accumulation (Tanguay et al., 1996). Cornett et al. (1999) reported DCA exposure in rats increased accumulation of maleylacetone (a spontaneous decarboxylation product of MAA), suggesting MAA accumulation. Ammini et al. (2003) report depletion of the GST-zeta to be exclusively a post-transcriptional event with genetic ablation of GST-zeta causing FAA and MAA accumulation in mice. Schultz et al. (2002) report that elimination of DCA is controlled by liver metabolism via GST-zeta in mice, and that DCA also inhibits the enzyme (and thus its own elimination) with young mice being the most sensitive to this inhibition. On the other hand, older mice (60 weeks) had a decreased capacity to excrete and metabolize DCA in comparison with younger ones. The authors suggest that exogenous factors that deplete or reduce GST-zeta will decrease DCA elimination and may increase its carcinogenic potency. They also suggest that, due to suicide inactivation of GST-zeta, an assumption of linear kinetics can lead to an underestimation of the internal dose of DCA at high exposure rates. In humans, GST-zeta has been reported to be inhibited by DCA and to be polymorphic (Blackburn et al., 2001; Blackburn et al., 2000; Tzeng et al., 2000). Board et al. (2001) report one variant

to have significantly higher activity with DCA as a substrate than other GST zeta isoforms, which could affect DCA susceptibility.

Individuals with glycogen storage disease or with poorly controlled diabetes have excessive storage of glycogen in their livers (glycogenosis) and increased risk of liver cancer (Rake et al., 2002; Wideroff et al., 1997; Adami et al., 1996; La Vecchia et al., 1994). In an animal model where hepatocytes are exposed to a local hyperinsulinemia from transplanted islets of Langerhans and the remaining tissue is hypoinsulinemic, insulin induces alterations that resemble preneoplastic foci of altered hepatocytes (FAH) and develop into hepatocellular tumors in later stages of carcinogenesis (Evert et al., 2003). A number of studies have reported suppression of apoptosis, decreases in insulin, and glycogenosis in mice liver by DCA at levels that also induce liver tumors (Bull, 2004b; Bull et al., 2004; Lingohr et al., 2001). In isolated murine hepatocytes, Lingohr et al. (2002) reported DCA-induced glycogenosis was dose related, occurred at very low doses (10 μM), occurred without the presence of insulin, was not affected by insulin addition, was dependent on phosphatidylinositol 3-kinase (P13K) activity, and was not a result of decreased glycogen breakdown. The authors noted that PI3K is also known to regulate cell proliferation and apoptosis in hepatocytes, and that understanding these mechanisms may be important to understanding DCA-induced carcinogenesis. They also report insulin receptor (IR) protein levels decreased to 30% of controls in mice liver after up to 52 weeks of DCA treatment. Activation of the IR is also the principal pathway by which insulin stimulates glycogen synthetase (the rate limiting enzyme of glycogen biosynthesis). However, in DCA-induced liver tumors IR protein was elevated as well as mitogen-activated protein kinase (a downstream target protein of the IR) phosphorylation. DCA-induced tumors were glycogen poor (Lingohr et al., 2001). The authors suggest that normal hepatocytes down-regulate insulin-signaling proteins in response to the accumulation of liver glycogen caused by DCA and that the initiated cell population, which does not accumulate glycogen and is promoted by DCA treatment, responds differently from normal hepatocytes to the insulin-like effects of DCA.

Gene expression studies of DCA show a number of genes identified with cell growth, tissue remodeling, apoptosis, cancer progression, and xenobiotic metabolism to be altered in mice liver at high doses (2 g/L DCA) in drinking water (Thai et al., 2003, 2001). After 4 weeks, RNA expression was altered in 4 known genes (alpha-1 protease inhibitor, cytochrome B5, stearoyl-CoA desaturase and caboxylesterase) in two mice (Thai et al., 2001). Except for Co-A desaturase, a similar pattern of gene change was reported in DCA-induced tumors (10 tumors from 10 different mice) after 93 weeks. Using cDNA microarray in the same mice, Thai et al. (2003) identified 24 genes with altered expression, of which 15 were confirmed by Northern blot analysis after 4 weeks of exposure. Of the 15 genes, 14 revealed expression suppressed two- to fivefold and included: MHR 23A, cytochrome P450 (CYP), 2C29, CYP 3A11, serum paraoxonase/arylesterase 1, liver carboxylesterase, alpha-1 antitrypsin, ER p72, GST-pi 1, angiogenin, vitronectin precursor, cathepsin D, plasminogen precursor (contains angiostatin), prothrombin precursor and integrin alpha 3 precursor. An additional gene, CYP 2A4/5, had a twofold elevation in expression. After 93

weeks of treatment with 3.5 g/L DCA, Northern blot analyses of total RNA isolated from DCA-induced hepatocellular carcinomas showed similar alteration of expression (11 of 15). It was noted that peroxisome proliferator-activated receptor (PPAR)α and IR gene expression were not changed by DCA treatment. Genes involved in glycogen or lipid metabolism were not tested.

Although it has not been possible to determine directly whether DCA is produced from TCE at carcinogenic levels, there is indirect evidence that DCA is formed from TCE *in vivo* and contributes to liver tumor development. Pretreatment with either DCA or TCE inhibits GST-zeta while TCA pretreatment does not (Bull et al., 2004; Schultz et al., 2002). TCE treatment decreased V_{max} for DCA metabolism to 49% of control levels with a 1 g/kg TCE dose resembling effects those of 0.05 g/L DCA (Schultz et al., 2002).

E.3.4.2.2. Genetic Profiling Data for TCE: Gene Expression and Methylation Status Studies

Caldwell and Keshava (2006) and Keshava and Caldwell (2006) reported on both genetic expression studies and studies of changes in methylation status induced by TCE and its metabolites (see Sections E.4.1.3 and E.3.3.5) as well as differences and difficulties in the patterns of gene expression between differing PPARα agonists. In Section E.4.3, the effects of co-exposures of DCA, TCA, and chloroform on methylation status are discussed. In particular are concerns for the interpretation of studies that employ pooling of data as well as interpretation of "snapshots in time of multiple gene changes."

For the Laughter et al. (2004) study in particular, it is not clear whether transcription arrays were performed on pooled data (no data on variability between individual animals were provided and the methodology section of the report is not transparently written in this regard). The issue of phenotypic anchoring also arises as data on percent liver/body weight indicates significant variability within TCE treatment groups, especially in PPARα-null mice. For studies of gene expression using microarrays, Bartosiewicz et al. (2001) used a screening analysis of 148 genes for xenobiotic-metabolizing enzymes, DNA repair enzymes, heat shock proteins, cytokines, and housekeeping gene expression patterns in the liver in response TCE. The TCE-induced gene induction was reported to be highly selective; only Hsp 25 and 86 and Cyp2a were upregulated at the highest dose tested. Collier et al. (2003) reported differentially expressed mRNA transcripts in embryonic hearts from Sprague-Dawley rats exposed to TCE, with sequences downregulated with TCE exposure appearing to be those associated with cellular housekeeping, cell adhesion, and developmental processes. TCE was reported to induce upregulated expression of numerous stress-response and homeostatic genes.

Laughter et al. (2004) reported transcription profiles using macroarrays containing approximately 1,200 genes in response to TCE exposure. Forty-three genes were reported to be significantly altered in the TCE-treated wild-type mice and 67 genes were significantly altered in the TCE-treated PPARα knockout mice. Out of the 43 genes expressed in wild-type mice

upon TCE exposure, 40 genes were reported by the authors to be dependent on PPARα and included genes for CYP4a12, epidermal growth factor receptor, and additional genes involved in cell growth. However, the interpretation of this information is difficult because in general, PPARα knockout mice have been reported to be more sensitive to a number of hepatotoxins, partly because of defects in the ability to effectively repair tissue damage in the liver (Shankar et al., 2003; Mehendale, 2000) and because a comparison of gene expression profiles between controls (wild-type and PPARα knockout) were not reported.

As stated previously, knockout mice in this study also responded to TCE exposure with increased liver weight, had increased background liver weights, and had higher baseline levels of hepatocyte proliferation than wild-type mice. Nakajima et al. (2000) reported that the number of peroxisomes in hepatocytes increased by twofold in wild-type mice but not in PPARα knockout mice. However, TCE induced increased liver weight in both male and female wild-type and knockout mice, suggesting hepatic effects independent of PPARα activation. Ramdhan et al. (2010) also reported increased liver weight after TCE exposure in male wild type, PPARα-null, and PPARα humanized mice to a similar extent.

In regards to toxicity, after 3 weeks of TCE treatment (0–1,500 mg/kg via gavage), Laughter et al. (2004) reported toxicity at the 1,500 mg/kg level in the knockout mice that was not observed in the wild-type mice—all knockout mice were moribund and had to be removed from the study. Differences in experimental protocol made comparisons between TCE effects and those of its metabolites difficult in this study (see Section E.2.1.13). After short-term inhalation exposure, Ramdhan et al. (2010) reported increased TCE induction of toxicity in PPARα-null and humanized mice in terms of hepatic steatosis and minimal levels of necrosis.

As reported by Voss et al. (2006), dose-, time course-, species-, and strain-related differences should be considered in interpreting gene array data. The comparison of differing PPARα agonists presented in Keshava and Caldwell (2006) illustrate the pleiotropic and varying liver responses of the PPARα receptor to various agonists, but did imply that these responses were responsible for carcinogenesis.

As discussed in Section E.3.3.5 and in Caldwell and Keshava (2006),

> Aberrant DNA methylation has emerged in recent years as a common hallmark of all types of cancers, with hypermethylation of the promoter region of specific tumor suppressor genes and DNA repair genes leading to their silencing (an effect similar to their mutation) and genomic hypomethylation (Pereira et al., 2004a; Ballestar and Esteller, 2002; Berger and Daxenbichler, 2002; Rhee et al., 2002; Herman et al., 1998). Whether DNA methylation is a consequence or cause of cancer is a long-standing issue (Ballestar and Esteller, 2002). Fraga et al. (2005; 2004) reported global loss of monoacetylation and trimethylation of histone H4 as a common hallmark of human tumor cells; they suggested, however, that genomewide loss of 5-methylcytosine (associated with the acquisition of a transformed phenotype) exists not as a static predefined value throughout the

process of carcinogenesis but rather as a dynamic parameter (i.e., decreases are seen early and become more marked in later stages).

Although little is known about how it occurs, a hypothesis has also been proposed that that the toxicity of TCE and its metabolites may arise from its effects on DNA methylation status. In regard to methylation studies, many are co-exposure studies as they have been conducted in initiated animals, and as stated above, some are very limited in regard to the reporting and conduct of the study.

Caldwell and Keshava (2006) reviewed the body of work regarding TCE, DCA, and TCA for this issue. Methionine status has been noted to affect the emergence of liver tumors. As noted by Counts et al. (1996), a choline/methionine-deficient diet for 12 months did not increase liver tumor formation in C3H/HeN mice, but was tumorigenic to $B6C3F_1$ mice. Tao et al. (2000) and Pereira et al. (2004a) have studied the effects of excess methionine in the diet to see if it has the opposite effects as a deficiency (i.e., and reduction in a carcinogenic response rather than enhancement). As noted above for Tao et al. (2000), the administration of excess methionine in the diet is not without effect. The data of Tao et al. (2000) suggested that percent liver/body weight ratios are affected by short-term methionine exposure (300 mg/kg) in female $B6C3F_1$ mice.

Pereira et al. (2004a) reported that very high levels of methionine supplementation to an AIN-760A diet affected the number of foci and adenomas after 44 weeks of co-exposure to 3.2.g/L DCA. While the highest concentration of methionine (8.0 g/kg) was reported to decrease both the number of DCA-induce foci and adenomas, the lower level of methionine co-exposure (4.0 g/kg) increased the incidence of foci. Co-exposure of methionine (4.0 or 8.0 g/kg) with 3.2 g/L DCA was reported to decrease by ~25% DCA-induced glycogen accumulation and increase mortality, but not to have much of an effect on peroxisome enzyme activity (which was not elevated by >33% over control for DCA exposure alone).

Methionine treatment alone at the 8 g/kg level was reported to increase liver weight, decrease lauroyl-CoA activity, and increase DNA methylation. The authors suggested that their data indicate that methionine treatment slowed the progression of foci to tumors. Given that increasing hypomethylation is associated with tumor progression, decreased hypomethylation from large doses of methionine are consistent with a slowing of progression. Whether these results would be similar for lower concentrations of DCA and lower concentrations of methionine that were administered to mice for longer durations of exposure cannot be ascertained from these data. It is possible that in a longer-term study, the number of tumors would be similar. Whether methionine treatment co-exposure had an effect on the phenotype of foci and tumors was not presented by the authors in this study. Such data would have been valuable to discern if methionine co-exposure at the 4.0 mg/kg level that resulted in an increase in DCA-induced foci also resulted in foci of a differing phenotype or a more heterogeneous

composition than DCA treatment alone. Finally, a decrease in tumor progression by methionine supplementation is not shown to be a specific event for the mode of action for DCA-induced liver carcinogenicity.

Tao et al. (2000) reported that 7 days of gavage dosing of TCE (1,000 mg/kg in corn oil), TCA (500 mg/kg, neutralized aqueous solution), and DCA (500 mg/kg, neutralized aqueous solution) in 8-week-old female B6C3F$_1$ mice resulted in not only increased liver weight but also increased hypomethylation of the promoter regions of c-Jun and c-Myc genes in whole-liver DNA (data shown for 1–2 mice per treatment). Treatment with methionine was reported to abrogate this response only at a 300 mg/kg i.p. dose with 0–100 mg/kg doses of methionine having no effect. Ge et al. (2001b) reported DCA- and TCA-induced DNA hypomethylation and cell proliferation in the liver of female mice at 500 mg/kg and decreased methylation of the c-Myc promoter region in liver, kidney, and urinary bladder. However, increased "cell proliferation" preceded hypomethylation. Ge et al. (2002) also reported hypomethylation of the c-Myc gene in the liver after exposure to the peroxisome proliferators 2,4-dichlorophenoxyacetic acid (2,4-D)(1,680 ppm), dibutyl phthalate (20,000 ppm), Gemfibrozil (8,000 ppm), and WY-14,643 (50–500 ppm, with no effect at 5 or 10 ppm) after 6 days in the diet. Caldwell and Keshava (2006) concluded that hypomethylation did not appear to be a chemical-specific effect at these concentrations. As noted in Section E.3.3.5, chemical exposure to a number of differing carcinogens have been reported to lead to progressive loss of DNA methylation.

Caldwell and Keshava (2006) also noted similar changes in methylation after initiation and treatment with DCA or TCA.

> After initiation by N-methyl-N-nitrosourea (25 mg/kg) and exposure to 20 mmL/L DCA or TCA (46 weeks), Tao et al. (2004a) report similar hypomethylation of total mouse liver DNA by DCA and TCA with tumor DNA showing greater hypomethylation. A similar effect was noted for region-2 (DMR-2) of the insulin-like growth factor-II (IGF-II) gene. The authors suggest that hypomethylation of total liver DNA and the IGF-II gene found in non-tumorous liver tissue would appear to be the result of a more prolonged activity and not cell proliferation, while hypomethylation of tumors could be an intrinsic property of the tumors. Over expression of IGF-II gene in liver tumors and preneoplastic foci has been shown in both animal models of hepatocarcinogenesis and humans, and may enhance tumor growth, acting via the over-expressed IGF-I receptor (Scharf et al., 2001; Werner and Le Roith, 2000). IGF-I is the major mediator of the effects of the growth hormone; it thus has a strong influence on cell proliferation and differentiation and is a potent inhibitor of apoptosis (Fürstenberger and Senn, 2002). Normally, expression of IGF-II in liver is greater during the fetal period than the adult, but is over-expressed in human hepatocarcinomas due to activation of fetal promoters (Scharf et al., 2001) and loss of imprinting (Khandwala et al., 2000). Takeda et al. (1996) report IGF-II expression in the liver is monoallelic (maternally imprinted) in the fetal period is relaxed during the postnatal period,

(resulting in biallelic expression), and is imbalanced in human hepatocarcinomas (leading to restoration of monoallelic IG-II expression).

However, Bull (2004b) and Bull et al. (2004) have recently suggested that hypomethylation and peroxisome proliferation occur at higher exposure levels than those that induce liver tumors for TCE and its metabolites. They reported that a direct comparison in the no-effect level or low-effect level for induction of liver tumors in the mouse and several other endpoints shows that, for TCA, liver tumors occur at lower concentrations than peroxisome proliferation in vivo, but that PPARα activation occurs at a lower dose than either tumor formation or peroxisome proliferation. A similar comparison for DCA shows that liver tumor formation occurs at a much lower exposure level than peroxisome proliferation, PPARα activation, or hypomethylation. In addition, they reported that these chemicals are effective as carcinogens at doses that do not produce cytotoxicity.

E.3.4.2.3. Oxidative Stress

Several studies have attempted to study the possible effects of "oxidative stress" and DNA damage resulting from TCE exposures. The effects of induction of metabolism by TCE, as well as through co-exposure to ethanol, have been hypothesized in itself to increase levels of "oxidative stress" as a common effect for both exposures (see Section E.4.3.4). Oxidative stress has been hypothesized to be the mode of action for peroxisome proliferators as well, but has been found to be correlated with neither cell proliferation nor carcinogenic potency of peroxisome proliferators (see Section E.3.4.1.1). As a mode of action, it is not defined or specific, as the term "oxidative stress" is implicated as part of the pathophysiologic events in a multitude of disease processes and is part of the normal physiologic function of the cell and cell signaling.

In regard to measures of oxidative stress, Rusyn (2006) noted that although an overwhelming number of studies draw a conclusion between chemical exposure, DNA damage, and cancer based on detection of 8-OHdG, a highly mutagenic lesion, in DNA isolated from organs of in vivo treated animals, a concern exists as to whether increases in 8-OHdG represent damage to genomic DNA, a confounding contamination with mitochondrial DNA, or an experimental artifact. As described in Section E.2.2.8, the study by Channel et al. (1998) demonstrated that corn oil as vehicle had significant effects on measures of "oxidative stress" such as TBARS. Also as noted previously (see Sections E.2.1.1 and E.2.2.11), studies of TCE that employ the i.p. route of administration can be affected by inflammatory reactions resulting from routes of administration and subsequent toxicity that can involve oxygen radical formation from inflammatory cells.

The issues with interpretation of the Channel et al. (1998) study of TCE administered via corn oil gavage to mice have already been discussed in Section E.2.2.8. The TBARS results

indicated suppression of TBARS with increasing time of exposure to corn oil alone with data presented in such a way for 8-OHdG and total free radical changes that the pattern of corn oil administration was obscured. It was not apparent from that study that TCE exposure induced oxidative damage in the liver.

Toraason et al. (1999) measured 8-OHdG and a "free radical-catalyzed isomer of arachidonic acid and marker of oxidative damage to cell membranes, 8-Epi-prostaglandin F2α (8epiPGF)," excretion in the urine and TBARS (as an assessment of malondialdehyde and marker of lipid peroxidation) in the liver and kidney of male Fischer rats (150–200 g) exposed to single 0, 100, 500, or 1,000 mg/kg TCE i.p. injections in Alkamuls vehicle (n = 6/group). Two sequential urine samples were collected 12 hours after injection and animals were sacrificed at 24 hours with DNA collected from liver tissues and TBARS measured in liver homogenates. The mean body weights of the rats were reported to vary by 13%, but the liver weights varied by 44% after the single treatments of TCE. In contrast to the large volume of the literature that reports TCE-induced increases in liver weight, the 500 and 1,000 mg/kg exposed rats were reported to have reduced liver weight by 44% in comparison to the control values.

Using this paradigm, 500 mg/kg TCE was reported to induce stage II anesthesia and a 1,000 mg/kg TCE to induce Level III or IV (absence of reflex response) anesthesia and burgundy colored urine with 2/6 rats at 24 hours comatose and hypothermic. The animals were sacrificed before they could die and the authors suggested that they would not have survived another 24 hours. Thus, using this paradigm, there was significant toxicity and additional issues related to route of exposure. Urine volume declined significantly during the first 12 hours of treatment and while water consumption was not measured, it was suggested by the authors to be decreased due to the moribundity of the rats. Given that this study examined urinary markers of "oxidative stress," the effects on urine volume and water consumption, as well as the profound toxicity induced by this exposure paradigm, limit the interpretation of the study.

The authors noted that because both using volume and creatinine excretion were affected by experimental treatment, urinary excretion of 8-OHdG changed significantly based on the mode of data expression. Excretion of 8epiPGF was reported to be no different from controls at 12–24 hours and was decreased 24 hours after TCE exposure at the two highest levels. Excretion of 8-OHdG was reported to not be affected by any exposure level of TCE and, if expressed on the basis of 24-hours, decreased. TBARS concentration per g of liver was reported to be increased at the 500 and 1,000 mg/kg TCE exposure levels (~2–3–fold). The effects of decreased liver size in the treated animals for this measure in comparison to control animals, was not discussed by the authors. For 8-OHdG measures in the liver and lymphocytes, the authors reported that "cost prohibited analysis of all of the tissues samples" so that a subset of animals was examined exhibiting the highest TBARS levels. The number of animals used for this determination was not given nor were the data reported, except for 500 mg/kg TCE exposure level. TCE was reported to increase 8-OHdG/dG in liver DNA relative to controls to about the same extent in

lymphocytes from blood and liver (approximately twofold) with the results for liver reported to be significant. The issues of bias in selection of the data for this analysis, as well as the issues already stated for this paradigm limit interpretation of these data, while the authors suggest that evidence of oxidative damage was equivocal.

DCA and TCA have also been investigated using similar measures. Larson and Bull (1992b) exposed male B6C3F$_1$ mice (26 ± 3 g [SD]) to a single dose of 0, 100, 300, 1,000, or 2,000 mg/kg-day TCA or 0, 100, 300, or 1,000 mg/kg-day DCA in distilled water by gavage (n = 4). F344 rats (237 ± 4 g) received a single oral dose of 0, 100, or 1,000 mg/kg DCA or TCA (n = 4 or 5) TBARS was measured from liver homogenates and assumed to be malondialdehyde. The authors stated that a preliminary experiment had shown that maximal TBARS was increased 6 hours after a dose of DCA and 9 hours after a dose of TCA in mice (data shown) and that by 24 hours, TBARS concentrations had declined to control values (data not shown). However, time-course information in rats was not presented and the same times used for both species (i.e., 6- and 9-hour time periods after administration of DCA and TCA) for examination of TBARS activity. A dose of 100 mg/kg DCA (rats or mice) or TCA (mice) did not elevate TBARS concentrations over that of control liver, with this concentration of TCA not examined in rats.

For TCA, there was a slight dose-related increase in TBARS over control values starting at 300 mg/kg in mice (i.e., 1.68-, 2.02-, and 2.70-fold of control for 300, 1,000, and 2,000 mg/kg TCA). For DCA, there were similar increases over control for both the 300 and 1,000 mg/kg dose levels in mice (i.e., 3.22- and 3.45-fold of control, respectively).

For rats, the 1,000 and 2,000 mg/kg levels of TCA were reported to show a statistically significant increase in TBARS over control (i.e., 1.67- and 2.50-fold, respectively) with the 300 and 1,000 mg/kg level of DCA showing similar increases, but with only the 300 mg/kg-induced change statistically significant different than control values (i.e., 3- and 2-fold of control, respectively). Of note is the report that the induction of TBARS in mice is transient and had subsided within 24 hours of a single dose of DCA or TCA, that the response in mice appeared to be slightly greater with DCA than TCA at similar doses, and that for DCA, there was similar TBARS induction between rats and mice at similar dose levels.

A study by Austin et al. (1996) appears to a follow-up publication of the preliminary experiment cited in Larson and Bull (1992b). Male B6C3F$_1$ mice (8 weeks old) were treated with single doses of DCA or TCA in buffered solution (300 mg/kg) with liver examined for 8-OHdG. The authors stated that in order to conserve animals, controls were not employed at each time point. For DCA, the time course of 8-OHdG was studied at 0, 4, 6, and 8 hours after administration, and for TCA, at 0, 6, 8, and 10 hours after of a 300 mg/kg dose (n = 6). There was a statistically significant increase over controls in 8-OHdG for the 4- and 6-hour time points for DCA (~1.4- and 1.5-fold of control, respectively) but not at 8 hours in mice. For TCA, there was a statistically significant increase in 8-OHdG at 8 and 10 hours for TCA (~1.4- and 1.3-fold of control, respectively).

The results for PCO and liver weight for Parrish et al. (1996) are discussed in Section E.2.3.2.3 for male B6C3F$_1$ mice exposed to TCA or DCA (0, 0.01, 0.5, and 2.0 g/L) for 3 or 10 weeks (n = 6). The study focused on an examination of the relationship with measures of peroxisome proliferation and oxidative stress. The dose-related increase in PCO activity at 21 days (~1.5-, 2.2-, and ~4.1-fold of control, for 0.1, 0.5, and 2.g/L TCA) was reported not to be increased similarly for DCA. Only the 2.0 g/L dose of DCA was reported to induce a statistically significant increase at 21 days of exposure of PCO activity over control (~1.8-fold of control). After 71 days of treatment, TCA induced dose-related increases in PCO activities that were approximately twice the magnitude as that reported at 21 days (i.e., ~9-fold greater at 2.0 g/L level). Treatments with DCA at the 0.1 and 0.5 g/L exposure levels produced statistically significant increase in PCO activity of ~1.5- and 2.5-fold of control, respectively. The administration of 1.25 g/L clofibric acid in drinking water, used as a positive control, gave ~6–7-fold of control PCO activity at 21 and 71 days exposure.

Parrish et al. (1996) reported that laurate hydroxylase activity was reported to be elevated significantly only by TCA at 21 days and to approximately the same extent (~1.4–1.6-fold of control) increased at all doses tested. At 71 days, both the 0.5 and 2.0 g/L TCA exposures induced a statistically significant increase in laurate hydroxylase activity (i.e., 1.6- and 2.5-fold of control, respectively) with no change reported after DCA exposure. The actual data rather than percent of control values were reported for laurate hydroxylase activity with the control values varying 1.7-fold between 21- and 71-day experiments. Levels of 8-OHdG in isolated liver nuclei were reported to not be altered from 0.1, 0.5, or 2.0 g/L TCA or DCA after 21 days of exposure and this negative result was reported to remain even when treatments were extended to 71 days of treatment.

The authors noted that the level of 8-OHdG increased in control mice with age (i.e., approximately twofold increase between 71- and 21-day control mice). Clofibric acid was also reported not to induce a statistically significant increase of 8-OHdG at 21 days, but to produce an increase (~1.4-fold of control) at 71 days. Thus, the increases in PCO activity noted for DCA and TCA were not associated with 8-OHdG levels (which were unchanged) and, also, not with changes in laurate hydrolase activity observed after either DCA or TCA exposure. Of note is the variability in both baseline levels of PCO and laurate hydrolase activity. Also of note is that the authors report taking steps to minimize artifactual responses for their 8-OHdG determinations. The authors concluded that their data do not support an increase in steady-state oxidative damage to be associated with TCA initiation of cancer and that extension of treatment to time periods sufficient to insure peroxisome proliferation failed to elevate 8-OHdG in hepatic DNA. The increased 8-OHdG at 10 weeks after Clofibrate administration but lack of 8-OHdG elevation at similar levels of PCO induction were also noted by the authors to suggest that peroxisome proliferative properties of TCA were not linked to oxidative stress or carcinogenic response.

As noted above for the study of Leakey et al. (2003b) (see Section E.2.5), hepatic malondialdehyde concentration in ad-libitum-fed and dietary-controlled mice did not change with CH exposure at 15 months, but the dietary-controlled groups were all approximately half that of the ad-libitum-fed mice. Thus, while overall increased tumors observed in the ad libitum diet correlated with increased malondialdehyde concentration, there was no association between CH dose and malondialdehyde induction for either diet.

E.4. EFFECTS OF CO-EXPOSURES ON MODE OF ACTION—INTERNAL AND EXTERNAL EXPOSURES TO MIXTURES INCLUDING ALCOHOL

Caldwell et al. (2008b) published a review of the issues and studies involved with the effects of co-exposures to TCE metabolites that could be considered internal (i.e., an internal co-exposure for the liver) and co-exposures to metabolites and other commonly occurring chemicals that are present in the environment. As they stated:

> Human exposure to a pollutant rarely occurs in isolation. EPA's Cumulative Exposure project and subsequent National Air Toxics Assessment have demonstrated that environmental exposure to a number of pollutants, classified as potential human carcinogens, is widespread [U.S. EPA, 2006;(Woodruff et al., 1998)]. Interactions between carcinogens in chemical mixtures found in the environment have been a concern for several decades. Furthermore, how these interactions affect the mode of action (MOA) by which these chemicals operate and how such effects may modulate carcinogenic risk is of concern as well. Thus, an understanding of the MOA(s) of a pollutant can help elucidate its potential carcinogenic risk to humans, and can also help identify susceptible subpopulations through their intrinsic factors (e.g., age, gender, and genetic polymorphisms of key metabolic and clearance pathways) and extrinsic factors (e.g. co-exposures to environmental contaminants, ethanol consumption, and pharmaceutical use). Trichloroethylene (TCE) can be a useful example for detailing the difficulties and opportunities for investigating such issues because, for TCE, there is both internal exposure to a "chemical mixture" of multiple carcinogenic metabolites (Chiu et al., 2006a; Chiu et al., 2006b) and co-exposures from environmental contamination of TCE metabolites, and from pollutants that share common metabolites, metabolic pathways, MOAs, and targets of toxicity with TCE.
>
> Typically, ground water or contaminated waste sites can have a large number of pollutants that vary in regard to information available to support the characterization of their potential hazard, and that have differing MOAs and targets. For example, Veeramachaneni et al. (2001) reported reproductive effects in male rabbits, resulting from exposure to drinking water containing concentrations of chemicals typical of ground water near hazardous waste sites. The drinking water exposure mixture contained arsenic, chromium, lead, benzene, chloroform, phenol, and TCE. Even at 45 weeks after the last exposure, mating desire/ability, sperm quality, and Leydig cell function were subnormal. However, while the exposure levels are relevant to human

environmental exposures, design of this study precludes a conclusion as to which individual toxicant, or combination of the seven toxicants, caused the effects. Thus, this study exemplifies he problems associated with studying a multi-mixture milieu. Studies of the interactions of TCE metabolites or common co-exposures that report the interactions of 2 or 3 chemicals at one time are easier to interpret.

Since EPA published its 2001 draft assessment, several approaches have been reported that include examination of tumor phenotype, gene expression, and development of physiologically-based pharmacokinetic (PBPK) models to assess possible effects of co-exposure. They attempt to predict whether such co-exposures would produce additivity of response or if co-exposure would change the nature of responses induced by TCE or its metabolites. In addition, new studies on co-exposure to DBA may help identify a co-exposure of concern. These studies may give potential insights into possible MOAs and modulators of TCE toxicity. More recent information on the toxicity of individual metabolites of TCE (Caldwell and Keshava, 2006) may be helpful in trying to identify which are responsible for TCE toxicity, but may also identify the effects of environmental co-exposures.

Recently, EPA sought advice from the National Academy of Sciences (NAS) (Chiu et al., 2006a) with the NAS charge questions including the following. (1) What TCE metabolites, or combinations of metabolites, may be plausibly involved in the toxicity of TCE? (2) What chemical co-exposures may plausibly modulate TCE toxicity? (3) What can be concluded about the potential for common drinking water contaminants such as other solvents and/or haloacetates to modulate TCE toxicity? (4) What can be concluded about the potential for ethanol consumption to modulate TCE toxicity? Thus, the understanding of the effects of co-exposure, in the context of MOA, is an important element in understanding the risk of a potential human carcinogen.

U.S. EPA's draft TCE risk assessment (U.S. EPA, 2001) identified several factors involving co-exposure to TCE metabolites, environmental contaminants, and ethanol that could lead to differential sensitivity to TCE toxicity. Research needs identified there, as well as in previous reviews (Bull, 2000; Pastino et al., 2000), included further elucidation of the interaction of TCA and DCA in TCE-induced liver tumors and a better understanding of the functional relationships among risk factors. The complexity of TCE's potential interactions with chemical co-exposures from either common environmental co-contaminants or common behaviors such as alcohol consumption mirrors the complexity of the metabolism and the actions of TCE metabolites. Thus, TCE presents a good case study for further exploration of the effects of co-exposure on MOA.

The following sections first reiterate the findings of Bull et al. (2002) in regard to simple co-exposures of DCA and TCA that can be experienced as an internal co-exposure after TCE exposure. A number of studies have examined the effects of TCE or its metabolites after previous exposure to presumably genotoxic carcinogen to not only determine the effect of the co-exposure on liver carcinogenicity but also to use such paradigms to distinguish between the

effects of TCA and DCA. Finally, not only is TCE a common co-exposure with its own metabolites, but is also a common co-exposure with other solvents, and the brominated analogues of TCA and DCA. The available literature is examined for potential similarities in target and effects that may cause additional concern. The effects of ethanol on TCE toxicity is examined as well as the potential pharmacokinetic modulation of risk using recently published reports of PBPK models that may be useful in predicting co-exposure effects.

E.4.1. Internal Co-exposures to TCE Metabolites: Modulation of Toxicity and Implications for TCE Mode of Action

Exposure to TCE will produce oxidative metabolites in the liver as an internal co-exposure. As stated above, the phenotypic analysis of TCE-induced tumors have similarities to combinations of DCA and TCA and in some reports to resemble more closely DCA-induced tumors in the mouse. Results from Bull et al. (2002) are presented in Section E.2.2.22 for the treatment of mice to differing concentrations of DCA and TCA in combination and the resemblance of tumor phenotype to that of TCE. In regard to cancer dose-response, the most consistent treatment-related increase in response occurred with combinations of exposure to DCA and TCA that appeared to increase lesion multiplicity when compared to effects from individual chemicals separately. Bull et al. (2002) presented results for "selected" lesions examined for pathology characterization that suggest co-exposure of 0.5 g/L DCA with either 0.5 or 2 g/L TCA had a greater-than-additive effect on the total number of hyperplastic nodules. In addition, co-exposure to 0.1 g/L DCA and 2 g/L TCA was reported to have a greater-than-additive effect on the total number of adenomas, but not carcinomas, induced. The random selection of lesions for the determination of potential treatment-related effects on incidence and multiplicity, rather than characterization of all lesions, increases the uncertainty in this finding.

E.4.2. Initiation Studies as Co-exposures

There is a body of literature that has focused on the effects of TCE and its metabolites after rats or mice have been exposed to "mutagenic" agents to "initiate" hepatocarcinogenesis. Given that most of these "initiating agents" have many effects that are not only mutagenic but also epigenetic, that the dose and exposure paradigm modify these effects, that "initiators" can increased tumor responses alone, and that the tumors that arise from these protocols are reflective of simultaneous actions of both "initiator" and "promoter," paradigms that first expose rats or mice to a "mutagen" and then to other carcinogenic agents can be described as a co-exposure protocols.

As stated previously, DEN and *N*-nitrosomorpholine have been reported to increase differing populations of mature hepatocytes with DEN not only being a mutagen but also being able to induce concurrent hepatocyte regeneration at a high dose. Thus, the effects of the TCE

or its metabolites are hard to discern from the effects of the "initiating" agent in terms of mode of action.

As demonstrated in the studies of Pereira et al. (1997) below, the gender also determines the nature of the tumor response using these protocols. In addition, when the endpoint for examination is tumor phenotype the consequences of tumor progression are hard to discern from the mode of action of the agents using paradigms of differing concentrations, different durations of exposure, lesions counted as "tumors" to include different stages of tumor progression (foci to carcinoma), and highly variable and low numbers of animals examined. However, differences in phenotype of tumors resulting from such co-exposures, like the co-exposure studies cited above for just TCE metabolites, can help determine that exposure to TCE metabolites results in differing actions as demonstrated by differing effects in the presence of cocarcinogens. As stated above, Kraupp-Grasl et al. (1990) use the same approach and note differences among PPARα agonists in their ability to promote tumors suggest that they should not necessarily be considered a uniform group.

E.4.2.1. Herren-Freund et al. (1987)

The results of TCE exposure alone were reported previously (Section E.2.2.17) for this study. This study's focus was on the effect of TCE, TCA, DCA, and phenobarbital on hepatocarcinogenicity in male $B6C3F_1$ mice after "initiation" at 15 days with 2.5 or 10 μg/g body weight of ethylnitrosourea (ENU) and then subsequent exposure to TCE and other chemicals in drinking water begging at 4 weeks of age (an age when the liver is already undergoing rapid growth). DCA and TCA were given in buffered solutions and sodium chloride was given in the water of control animals. The experiment was reported to be terminated at 61 weeks because the "mice started to exhibit evidence of tumors." Concentrations of TCE were 0, 3 and 40 mg/L, of DCA and TCA 0, 2 and 5 g/L, and of phenobarbital 0 and 500 mg/L. The number of animals examined in each group ranged from 16 to 32. ENU alone in this paradigm was reported to induce statistically significant increases in adenomas and HCCs (39% incidence of adenomas and 39% incidence of carcinomas vs. 9 and 0% for controls) at the 10 μg/g dose (n = 23), but not at 2.5 μg/g dose (n = 22).

The effects of high doses of DCA and TCA alone have already been discussed for other studies, as well as the lack of statistical power using a paradigm with so few and variable numbers of animals, the limitations of an abbreviated duration of exposure that does not allow for full expression of a carcinogenic response, and problems of volatilization of TCE in drinking water. DCA and TCA treatments at these levels (5 g/L) were reported to increase adenomas and carcinomas irrespective of ENU pretreatment and to approximately the same extent with and without ENU. TCE at the highest dose was reported to increase the number of animals with adenomas (37 vs. 9% in control) and carcinomas (37 vs. 0% in controls) but only the number of adenomas/animal was statistically significant as the number of animals examined was only 19 in

the TCE group. Phenobarbital was reported to have no effect on ENU tumor induction using this paradigm.

E.4.2.2. Parnell et al. (1986)

This study used a rat liver foci bioassay (GGT) for hepatic foci after at 3 and 6 month using protocols that included partial hepatectomy, DEN (10 mg/kg) or TCA (1,500 ppm in drinking water) treatment, and then promotion with 5,000 ppm TCA (i.e., 5 g/L) for 10, 20, or 30 days and phenobarbital (500 ppm) in male Sprague-Dawley rats (5–6 weeks old at partial hepatectomy). The number of animals per group ranged from 4 to 6. PCO activities were given for various protocols involving partial hepatectomy, DEN, TCA, and phenobarbital treatments, but there were no control values given that did not have a least one of these treatments.

Overall, it appeared that there was a slight decrease of PCO activity in rats treated with partial hepatectomy/DEN/phenobarbital treatments and a slight increase over other treatments for rats treated with partial hepatectomy/DEN/5,000 ppm TCA or just TCA from 2 weeks to 6 months of sampling. In regard to GGT-positive foci, the partial hepatectomy/DEN/ phenobarbital group (n = 6) was reported to have more positive foci at 3 or 6 months than rats "initiated" with TCA and phenobarbitol after partial hepatectomy or partial hepatectomy/ phenobarbital treatment alone (2.05 foci/cm^2 vs. ~0.05–0.10 foci/cm^2 for all other groups). The number of GGT-positive foci in rats without any treatment were not studied or presented by the authors. For "promotion" protocols, the number of GGT-positive foci induced by the partial hepatectomy/DEN/phenobarbital protocol at 3 and 6 months, appeared to be reduced when phenobarbital exposure was replaced by TCA co-exposure, but there was no dose-response between the 50, 500, and 5,000 ppm. However, TCA treatment along with partial hepatectomy and DEN treatment did increase the levels of foci (means of 0.71–0.39 foci/cm^2 at 3 months and 1.83–2.45 foci/cm^2 at 6 months) over treatment of just partial hepatectomy and DEN (0.05 ± 0.20 foci/cm^2 at 3 months and 0.30 ± 0.39 foci/cm^2 at 6 months).

For the TCA animals treated only with 5,000 ppm TCA, the number of GGT-positive foci at 3 months was 0.23 ± 0.16 foci/cm^2 and at 6 months 0.03 ± 0.32 foci/cm^2 with no values for untreated animals presented. For the positive control (partial hepatectomy/DEN/ phenobarbital), the number of GGT-positive foci increased from 3 to 6 months (1.65 ± 0.23 foci/cm^2 and at 6 months 7.61 ± 0.72 foci/cm^2). The authors concluded that:

> although TCA is reported to cause hepatic peroxisomal stimulation in rats and mice, the results of this study indicate that it is unlikely TCA's effects are related to the promoting ability seen here. The minimal stimulation of, 10 to 20% over controls of peroxisomal associated, PCO activity in TCA exposed rats was seen only at the 5000 ppm level and only within the promotion protocol. This finding is in contrast to the promoting activity seen at all three concentrations of TCA.

E.4.2.3. Pereira and Phelps (1996)

The results for mice that were not "initiated" by exposure to MNU, but exposed to DCA or TCA, are discussed in Section E.2.3.2. However, differences in responses after initiation are useful for showing differences between single and co-exposures as well as differences between DCA and TCA effects. On day 15 of age, female $B6C3F_1$ mice received an i.p. injection of MNU (25 mg/kg) and at 7 weeks of age, received DCA (2.0, 6.67, or 20 mmol/L), TCA (2.0, 6.67 mmol, or 20 mmol/L) or sodium chloride continuously for 31 or 51 weeks of exposure. The number of animals studied ranged from 6 to 10 in 31-week groups and from 6 to 39 in the 52-week groups. There was a "recovery group" in which mice received either 20 mmol/L DCA (2.58 g/L DCA) (n = 12) or TCA (3.27 g/L TCA) (n = 11) for 31 weeks and then switched to saline for 21 weeks until sacrifice at 52 weeks. Strengths of the study included the reporting of hepatocellular lesions as either foci, adenomas, or carcinomas and the presentation of incidence and multiplicity of each separately reported for the treatment paradigms. Limitations included the low and variable number of animals in the treatment groups.

MNU was reported to not "significantly" induce foci or altered hepatocytes, adenomas, or carcinomas at 31 (n = 10) or 51 weeks (n = 39). However, MNU did increase the incidence and number/mouse of foci, adenomas, and carcinomas at the 52-week sacrifice time in comparison to saline controls, albeit at lower levels than observed in DCA or TCA cotreatments groups (e.g., 10 vs. 0% foci, 17.5 vs. 2.5% adenomas, and 10 vs. 0% incidence of carcinomas at 52 weeks for MNU-treated mice vs. saline control). Co-exposure of DCA (20.0 mmol/L) for 52 weeks in MNU-treated mice increased the number of foci and hepatocellular adenomas with the authors reporting "the yield of total lesions/mouse increased as a second order function of the concentration of DCA (correlation coefficients $\geq$0.998)." TCA co-exposure in MNU-treated mice was reported not to result in a significant difference in yield of foci or altered hepatocytes with either continuous 52- or 31-week exposure, but exposures to 20.0 or 6.67 mmol/L TCA did result in increased yield of liver tumors with both exposure protocols (see below).

For TCA treatment in MNU-treated mice, the incidences of foci were similar (12.5 vs. 18.2%), but the number of foci/mouse was ~3-fold greater in the cessation protocol than with continuous exposure. The incidence of adenomas was reported to be the same (~66%) as well as the number of adenomas/animal between continuous and cessation exposures. For carcinomas, there was a greater incidence for mice with continuous TCA exposure (83 vs. 36%) as well as a greater number of carcinomas/mouse (~4-fold) than for those initiated mice with cessation of TCA exposure. As noted above, the number of animals treated with TCA was low and variable (e.g., 23 mice studied at 52 weeks 20.0 mmol/L TCA, and 6 mice at 52 weeks 6.67 mmol/L TCA), limiting the ability to discern a statistically significant effect in regard to dose-response. The concentration-response relationship for tumors/mouse after 31 and 51 weeks was reported to be best represented by linear progression.

A comparison of results for animals treated with MNU and 20.0 mmol/L DCA or TCA for 31 weeks and sacrificed at 31 weeks and those that were treated with MNU and DCA or TCA for 31 weeks and then sacrificed at 52 weeks is limited by the number of animals exposed (n = 10 for 31-week sacrifice DCA or TCA, n = 12 for DCA recovery group, and n = 11 for TCA recovery group). No carcinoma data were reported for animals exposed at 31 weeks and sacrificed at 31 weeks, making comparisons with recovery groups impossible for this parameter and thus, determinations about progression from adenomas to carcinomas. For the MNU- and DCA-treated animals, the incidence or number of animals reported to have foci at 31 weeks was reported to be 80% but 38.5% for in the recovery group. For adenomas, the incidence was reported to be 50% for DCA-treated animals at 31 weeks and 46.2% for the recovery group. For MNU- and TCA-treated animals, the incidence of foci at 31 weeks was reported to the 20 and 18.2% for the recovery group. For adenomas, the incidence was reported to be 60% for the TCA-treated animals at 31 weeks and 63.6% for the recovery group. Thus, this limited data set shows a decrease in incidence of foci for the MNU and DCA-treated recovery group but no change in incidence of foci for TCA or for adenomas for DCA or TCA treatment between those sacrificed at 31 weeks and those sacrificed 21 weeks later.

In regard to multiplicity, the number of foci/mouse was reported to be 2.80 ± 0.20 for the 31-week DCA group and 0.46 ± 0.18 for the recovery group (mean ± SEM). The number of adenomas/mouse was reported to be 1.80 ± 0.83 for the 31-week group and 0.69 ± 0.26 for the recovery group. Thus, both the number of foci and adenomas per mouse was reported to be decreased after the recovery period for MNU- and DCA-treated mice. Given that the number of animals with foci was decreased by half, the concurrent decrease in foci/mouse is not surprising. For TCA treatments, the numbers of foci/mouse were reported to be 0.20 ± 0.13 for the 31-week group and 0.45 ± 0.31 for the recovery group. The number of adenomas/mouse for TCA-treatment groups was reported to be 1.30 ± 0.45 for the 31-week group and 0.91 ± 0.28 for the recovery group. For the MNU- and TCA-treated mice, the numbers of foci/mouse were reported to be increased and the number of adenomas/mouse reported to be slightly lower. Because carcinoma data are not presented for the 31-week group, it is impossible to determine whether the TCA adenomas regressed to foci or the TCA adenomas progressed to carcinomas and more foci apparent with increased time.

For the comparison of the numbers of foci, adenomas, or carcinomas per mouse that were reported for the mice exposed at 31 weeks and sacrificed and those exposed for 52 weeks, issues arise as to the impact of such few animals studied at 31 weeks, and the differing incidences of lesions reported for these mice on tumor multiplicity estimates. The number of animals studied who treated with MNU and 20.0 mmol/L DCA or TCA for 31 weeks and then sacrificed was n = 10, while the number of animals exposed to 20.0 mmol/L DCA or TCA for 52 weeks was 24 for the DCA group and 23 for the TCA group. The number of animals treated at lower concentrations of DCA or TCA were even lower at the 31-week sacrifice (e.g., n = 6

for MNU and 6.67 mmol/L DCA at 31 weeks) and also for the 52-week durations of exposure (e.g., n = 6 for MNU and 6.6.7 mmol/L TCA).

At 31 weeks, 80% of the animals were reported to have foci and 50% to have foci after 52 weeks of exposure to 20.0 mmol/L DCA and MNU treatment. Thus, similar to the "recovery" experiment, the number of animals with foci decreased even with continuous exposure between 31 and 52 weeks. For adenomas, 20.0 mmol DCA exposure for 31 weeks was reported to induce adenomas in 50% of mice and after 52 weeks of exposure to induce adenomas in 73% of mice. For TCA, the number of animals with foci was reported to be 20% at 31 weeks and 12% at 52 weeks after exposure to 20.0 mmol/L TCA after MNU treatment and similar to the incidence of foci reported for the TCA-recovery group. For 20.0 mmol TCA, adenomas reported in 60% of mice after 31 weeks and in 67% of mice after 52 weeks of exposure and also similar to the incidence of adenomas reported for the TCA-recovery group.

In regard to multiplicity, the number of foci/mouse was decreased from 2.80 ± 0.20 to 1.46 ± 0.48 between 31 and 52 weeks of 20.0 mmol DCA in MNU exposed mice. The number of adenomas/mouse was reported to be increased from 1.80 ± 0.83 to 3.62 ± 0.70 between 31 and 52 weeks of 20.0 mmol DCA and MNU exposed mice. For 20.0 mmol/L TCA, the number of foci/mouse was 0.20 ± 0.13 and 0.13 ± 0.7 for 31- and 52-week exposures. The number of adenomas/mouse was reported to be 1.30 ± 0.45 and 1.29 ± 0.24 for 31- and 52-week exposures. Thus, by only looking at foci and adenoma multiplicity data, there would not appear to be a change between 31 and 52 weeks.

However, during progression, a shift may occur such that foci become adenomas with time and adenomas become carcinomas with time. For carcinomas, there were no data reported for 31-week exposure in MNU and DCA- or TCA-treated mice. However, at 52 weeks, 20.0 mmol DCA was reported to induce carcinomas in 19.2% of mice and 20.0 mmol TCA to induce carcinomas in 83% of mice. The corresponding numbers of carcinomas/mouse was 0.23 ± 0.10 for 20.0 mmol/L DCA treatment and 2.79 ± 0.48 for 20.0 mmol/L TCA treatment at 52 weeks in MNU treated mice. Thus, although fewer than 20% of MNU-treated mice were reported to have foci at 20.0 mmol TCA, by 52 weeks, almost all had carcinomas with ~67% also having adenomas. For DCA, many more mice had foci at 31 weeks (80%) than for TCA and by 52 weeks ~70% had adenoma with only ~20% reported to have carcinomas. The incidence data are suggestive that as these high doses of DCA and TCA, TCA was more efficient inducing progression of a carcinogenic response than DCA in MNU-treated mice.

The authors interpreted the decrease in foci and adenomas between animals treated with MNU and 20.0 mmol/L DCA for 31 weeks and sacrificed and those sacrificed 21 weeks later to indicate that these lesions were dependent on continued exposure. However, the total number of lesions cannot be ascertained because carcinoma data were not reported for 31-week exposures. Carcinomas were reported in the recovery group at 52 weeks (0.15 ± 0.10 carcinomas/mouse in 15.4% of animals). Of note is that not only did the number of foci/mouse

and incidence decrease between the 31-week group and the recovery group, but also between 31 and 52 weeks of continuous exposure for the MNU and 20.0 mmol/L DCA treated groups. Although derived from very few animals, the 6.67 mmol/L DCA group reported no change for foci/mouse but a decrease in the incidence of foci between 31- and 52 weeks of exposure in MNU treated mice (i.e., 0.67 ± 0.18 foci/mouse in 50% of the animals at 31 weeks and 0.50 ± 0.34 foci/mouse in 20% of mice treated for 52 weeks). The numbers of foci/mouse for both MNU-treated and untreated control mice were reported to be decreased between 31 and 51 weeks as well.

As noted in Section E.3.1.8, the number of "nodules" in humans, which may be analogous to foci and adenomas, can spontaneously regress with time rather than becoming HCCs. Also, as tumors get larger with progression, the number of tumors/mouse can decrease due to coalescence of tumors and difficulty distinguishing between them. While data are suggestive of a decrease in the number of adenomas/mouse after cessation of DCA exposure, the incidence data are similar between the 31-week exposure and recovery groups.

Of note is that the number of carcinomas/mouse and the incidence of carcinomas was reported to be similar between the MNU-treated mice exposed continuously to 20.0 mmol/L DCA for 52 weeks and those that were treated for 31 weeks and then sacrificed at 52 weeks. Also of note is that, although incidences and multiplicities of foci and adenomas were reported to be relatively low in the 2.0 mmol/L DCA exposure groups, at 52 weeks, 40% of the mice tested had carcinomas with 0.70 ± 0.40 carcinomas/mouse. This was a greater percentage of animals with carcinomas and multiplicity than that reported for the highest dose of DCA. This result suggests that the effects in regard to tumor progression, and specifically for carcinoma induction, differ between the lowest and highest doses used in this experiment. However, the low numbers of animals examined for the lower doses, 31-week exposures, and in the recovery group decrease the confidence in the results of this study in regard to the effects of cessation of exposure on tumor progression.

In regard to tumor phenotype, in MNU-treated female mice that were not also exposed to either DCA or TCA, all four foci and 86.7% of 15 adenomas were reported to be basophilic and 13.3% eosinophilic at the end of the 52-week study. However, when MNU-treated female mice were also exposed to DCA, the number eosinophilic foci and tumors increased with increasing dose after 52 weeks of continuous exposure. At the 20.0 mmol/L level, all 38 foci examined were eosinophilic and 99% of the tumors (almost all adenomas) were eosinophilic. At the 2.0 mmol/L DCA exposure, there were no foci examined but about five of nine tumors examined (~2:1 carcinoma:adenoma ratio) were basophilic and the other four were eosinophilic.

For TCA co-exposure in MNU-treated mice, the 20 mmol/L TCA treatment was reported to give results of one of the three foci examined to be basophilic and two that were eosinophilic. For the 98 tumors examined (~2:1 carcinoma/adenoma ratio), 71.4% were

reported to be basophilic and 28.6% were eosinophilic. At the 2.0 mmol/L TCA exposure level, the two foci examined were reported to be basophilic, while the six tumors (all adenomas) were reported to be 50% eosinophilic and 50% basophilic. Thus, after 52 weeks, female mice treated with MNU and a high dose of DCA had eosinophilic foci and adenomas and those treated with the high dose of TCA had a mixture of basophilic and eosinophilic foci and tumors with a 3:1 ratio of tumors (mostly carcinomas) being basophilic. At the lower doses of either DCA or TCA, the tumors tended to be mostly carcinomas for DCA and adenomas for TCA, but both were ~50% basophilic and 50% eosinophilic. The tumors observed from MNU treatment alone were all adenomas and mostly 87% basophilic. Thus, not only did treatment concentrations of DCA and TCA give a different result for tumor multiplicity and incidence, but also for tumor phenotype in MNU treated female mice. Eosinophilic foci and tumors were reported to be consistently GST-π positive while basophilic lesions "did not contain GST-π, except for a few scattered cells or very small area comprising less than 5% of the tumor."

Thus, exposure to either DCA or TCA increased the incidence and number of animals with lesions (foci, adenomas, or carcinomas) in MNU-treated vs. nontreated mice (see Section E.2.3.2). These results suggest that the pattern of foci, adenoma and carcinoma incidence, multiplicity, and progression appeared to differ between TCA and DCA in MNU-treated female mice. However, the low and variable number of animals used in this study, make quantitative inferences between DCA and TCA exposures in "initiated" animals, problematic.

E.4.2.4. Tao et al. (2000)

The source of liver tumors for this analysis was reported to be the study of Pereira and Phelps (1996). Samples of liver "tumors" and "noninvolved" liver were homogenized for protein expression for c-Jun and c-Myc and therefore, contained homogeneous cell types for study. The term "liver tumors" was not defined, so it cannot be ascertained as to whether the lesions studied were altered foci, hepatocellular adenomas, or carcinomas. Liver tissues were reported to be frozen prior to study which raises issues of m-RNA quality. Although this study reports that there were no MNU-induced "tumors," the original paper of Pereira and Phelps (1996) reports that there were 4 foci and 15 adenomas in MNU-only treated mice. The authors reported no difference in c-Jun and c-Myc m-RNA from DCA or TCA-induced tumors from mice "initiated" with MNU. DNA methyltransferase was reported to be decreased in noninvolved liver in MNU-only treated mice in comparison to that from TCA- and DCA-treated mice. For a comparison between noninvolved liver and tumors, tumors were reported to have a greater level than did noninvolved liver.

E.4.2.5. Latendresse and Pereira (1997)

This study used the tumors from Pereira and Phelps (1996), except for the MNU-treated only groups and those groups treated with either DCA or TCA but not MNU initiation, to further study various biomarkers. The omissions were cited as to be due to insufficient tissue. For immunohistochemical evaluation of the molecular biomarkers other than GST-π, liver specimens from seven MNU/20.0 mmol DCA- (i.e., 2.58 g/L DCA) treated and six MNU/20.0 mmol TCA- (i.e., 3.27 g/L TCA) treated female mice randomly selected. For GST-π, the number of animals from which lesion specimens were derived, was 24 MNU/DCA-treated and 23 MNU/TCA-treated mice.

The DCA-treated mice were reported to have 1–9 lesions/mouse and TCA-treated mice had 1–3 lesions/mouse. The number of lesions examined for each biomarker varied greatly. For TCA-induced foci, no foci were examined for any biomarker except 3 lesions for GST-π, while for DCA, 12–15 foci were examined for each biomarker and 38 lesions were examined for GST-π. Similarly for TCA-induced adenomas, there were 8–10 lesions examined for all biomarkers with 32 lesions examined GST-π, while for DCA, there were 12 lesions for all biomarkers with 94 lesions examined for GST-π. Finally, for TCA-induced carcinomas, there were 3–4 lesions examined per group with 64 lesions examined for GST-π, while for DCA-induced carcinomas, there were no lesions examined for any biomarker except 3 examined for GST-π. The biomarkers used were: GST-π, TGF-α, TGF-β, c-Jun, c-Fos, c-Myc, cytochrome oxidase CYP2E1, and cytochrome oxidase CYP4A1.

MNU/DCA treatment was reported to produce "predominantly eosinophilic lesions" with:

> in general, the hepatocytes of DCA-promoted foci and tumors were less pleomorphic and uniformly larger and had more distinctive cell borders than the hepatocytes in lesions caused by TCA. Parenchymal hepatocytes of DCA-promoted mice were uniformly hypertrophied, with prominent cell borders, and the cytoplasm was markedly vacuolated, which was morphologically consistent with the previous description of glycogen deposition in these lesions. In contrast, TCA-promoted proliferative lesions tended to be basophilic, as previously reported, and were composed of hepatocytes with less distinct cell borders, slight cytoplasmic vacuolization, and greater variability in nuclear size and cellular size.

The hepatocytes of altered foci and hepatocellular adenomas from MNU-treated female mice also treated with DCA were reported to stain positively for TGF-α, c-Jun, c-Myc, CYP2E1, CYP4A1, and GST-π. The authors do not present the data for foci and adenomas separately, but as an aggregate, and as the number of lesions with <50% cells stained or the number of lesions with >50% cells stained either "minimally to mildly" or "moderately to densely" stained.

Because no carcinomas for DCA were examined and especially because no foci for TCA analyses were included in the aggregates, it is difficult to compare the profile between TCA and DCA exposure in initiated animals and to separate these results from the effects of differences in tumor progression. Thus, any differences seen in these biomarkers due to progression from foci to adenoma in DCA-induced lesions or from progression of adenoma to carcinoma in TCA-induce lesions, was lost. If the results for adenomas had been reported separately, there would have been a common stage of progression from which to compare the DCA and TCA effects on initiated female mice liver tumors. For DCA-induced "lesions" (~50% foci and ~50% adenomas), most lesions had >50% cells staining with moderate to dense levels for TGF-α, and CYP2E1, CYP4A1, and GST-π and most lesions had <50% cells staining for even minimally to mild staining for TGF-β and c-Fos. For c-Jun and c-Myc, the aggregate DCA-induced "lesions" were heterogeneous in the amount of cells and the intensity of cell staining for these biomarkers in MNU-treated female mice.

For the TCA "lesions" (~60% adenomas and ~30% carcinomas) the authors note that:

> in general, the hepatocytes of tumors promoted by TCA demonstrated variable immunostaining. With the exception of c-Jun, greater than 50% of the hepatocytes in TCA lesions were essentially negative or stained only minimally to mildly for the protein biomarkers studies. In some instances, particularly in TCA-promoted tumors, there was regional staining variability within the lesions, including immunoreactivity for c-Jun and c-Myc proteins, consistent with clonal expansion or tumor progression.

As stated above, the term "lesion" refers to foci and adenomas for DCA, but for adenomas and carcinomas for TCA, making inferences as to differences in the actions of the two compounds through the comparisons of biomarkers confounded by the effects of tumor progression. The largest differences in patterns between TCA induced "lesions" and those by DCA appeared to be TGF-α (with no lesions having >50% cells stained mildly or moderately/densely for TCA-induced lesions), CYP2E1 (with few lesions having >50% stained moderately/densely for TCA-induced lesions), CYP4A1 (with no lesions having >50% stained mildly or moderately/densely for TCA-induced lesions), and GST-π (with all lesions having <50% cells stained even mildly for TCA-induced lesions). However, as shown by these data, while the "lesions" induced by TCA and DCA had some commonalities within each treatment, there was heterogeneity of lesions produced by both treatments in female mice already exposed to MNU. Overall, the tumor biomarker pattern suggests differences in the effects of DCA and TCA through differences in tumor phenotype they induce as co-exposures with MNU treated female mice.

The authors noted that nonlesion parenchymal hepatocytes in DCA-treated initiated mice stained mostly negative for CYP2E1 and CYP4A1, while in TCA-treated mice, staining patterns

in parenchymal nonlesions hepatocytes were centrilobular for CYP2E1 and panlobular for CYP4A1 (a pattern for CYP4A1 that is opposite of that found in the TCA-induced lesions).

E.4.2.6. Pereira et al. (1997)

This study used a similar paradigm as that of Pereira and Phelps (1996) to study co-exposures of TCA and DCA to female B6C3F$_1$ mice already exposed to MNU. At 15 days, the mice received 25 mg/kg MNU and starting at 6 weeks of age neutralized solutions of either 0, 7.8, 15.6, or 25.0 mmol/L DCA (n = 30 for control and 25 mmol/L DCA and n = 20 for 7.8 and 15.6 mmol/L DCA), 6.0 or 25.0 mmol/L TCA (n = 30 for 25.0 mmol/L TCA and n = 20 for 6.0 TCA), or combinations of DCA and TCA that included 25.0 mmol/L TCA + 15.6 mmol/L DCA (n = 20), 7.8 mmol/L DCA + 6.0 mmol/L TCA (n = 25), 15.6 mmol/L DCA + 6.0 mmol/L TCA (45), and 25.0 mmol/L DCA + 6.mmol/L TCA (n = 25). The corresponding concentrations of DCA and TCA in g/L are 25 mmol = 3.23 g/L, 15.6 mmol = 2.01 g/L and 7.8 mmol = 1.01 g/L DCA and 25 mmol = 4.09 g/L, and 6.0 mmol = 0.98 g/L TCA. Accordingly, the number of animals at the beginning of the study varied between 20 and 45. At terminal sacrifice (after 44 weeks of exposure), the numbers of animals examined were less with the lowest number examined to be 17 mice in the 7.8 mmol/L DCA group and the largest to be 42 mice in the 15.6 mmol/L DCA + 6.0 mmol/L TCA exposed group.

The authors reported that only a total of eight HCCs were found in the study (i.e., 25.0 mmol/L DCA induced three carcinomas, 7.8 mmol DCA + 6.0 mmol TCA induced one carcinoma, and 25.0 mmol/L TCA induced four carcinomas). Thus, they presented data for foci/mouse, adenomas/mouse, and their sum of both as "total lesions." The incidences of lesions (i.e., how many mice in the groups had lesions) were not reported. The shortened duration of exposure (i.e., 44 weeks), the omission of carcinomas from total "lesion" counts (precluding consideration of progression of adenomas to carcinomas), the lack of reporting of tumor incidences between groups, and the variable and low numbers of animals examined in each group make quantitative inferences regarding additivity of these treatments difficult. MNU-treated mice did have a neoplastic response, albeit low using this paradigm.

For mice that were only exposed to MNU (n = 30 at terminal sacrifice), the mean numbers of foci, adenomas, and "lesions" per mouse were 0.21, 0.07, and 0.28, respectively. No data were given for mice without MNU treatment but few lesions would be expected in controls. Pereira and Phelps (1996) reported that saline-only treatment in 40 female mice for 51 weeks resulted in 0% foci, 0.03 adenomas/mouse in 2.5% of mice, and 0% carcinomas. In general, it appeared that the numbers of foci, adenomas, and the combination of both reported as "lesions" per mouse that would have been predicted by the addition of multiplicities given for DCA, TCA, and MNU treatments alone, were similar to those observed as co-exposure treatments. The largest numbers of foci and adenomas/mouse were reported for the 25.0 mmol/L DCA and 6.0 mmol/L TCA treatments in MNU-treated mice (mean of

6.57 “lesions”/mouse) with the lowest number reported for 7.8 mmol/L DCA and 6 mmol/L TCA (mean of 1.16 “lesions”/mouse).

The authors reported that the foci of altered hepatocytes were predominantly eosinophilic in DCA-treated female mice initiated with MNU, while those observed after MNU and TCA treatment were basophilic. MNU treatment alone induced four basophilic and two eosinophilic foci, and two basophilic adenomas. MNU and DCA treatment was reported to produce only eosinophilic foci and adenomas at the 25.0 mmol/L DCA exposure level. At the 7.8 mmol/L DCA level of treatment in MNU-treated mice, two foci were basophilic, four were eosinophilic, and the one adenoma observed was reported to be eosinophilic. Thus, the concentration of exposure appeared to alter the tincture of the foci observed after MNU and DCA exposure using this paradigm. In this study, MNU and TCA treatment was reported to induce foci and adenomas that were all basophilic at both 25.0 mmol/L TCA and 6.0 mmol/L TCA exposures. After 7.8 mmol/L DCA + 6.0 mmol/L TCA exposure, 2/23 foci were basophilic and 21/23 foci were reported to be eosinophilic, while all four adenomas reported for this group were eosinophilic.

Irrespective of treatment, eosinophilic foci for were reported to be GST-π positive and basophilic foci to be GST-π negative. An exception was the four carcinomas in the group treated with 25 mmol/L TCA, which were reported to be predominantly basophilic but contained small areas of GST-π positive hepatocytes.

It should be noted that the increased dose (up to 3.23 g/L DCA and 4/09 g/L TCA) raises issues of toxicity and effects on water consumption, as other studies have noted toxicity at highly doses of DCA and TCA. The use of an abbreviated duration of exposure in the study raises issues of sensitivity of the bioassay at the lower doses used in the experiment. In particular, was enough time provided to observe the full development of a tumor response? Finally, a question arises as to what can be concluded from the low numbers of foci examined in the study and the effect of such low numbers on the ability to discern differences in these foci by treatment. As with Pereira and Phelps (1996), there appeared to be a difference the nature of the response induced by co-exposure of MNU to relatively high vs. low DCA concentrations. Of note is that while this experiment reported no HCCs at the lowest dose of DCA at 44 weeks (7.8 mmol DCA), Pereira and Phelps (1996) reported that in nine mice treated with MNU and 2.0 mmol DCA for 52 weeks, there were no foci, but 20% of mice had adenomas (0.20 adenomas/mouse) and 40% of mice had carcinomas (0.70 carcinomas/mouse).

These results suggest that DCA co-exposure affects TCA-induced lesions. The authors concluded that mixtures of DCA and TCA appear to be at least additive and likely synergistic and similar to the pathogenesis of DCA.

E.4.2.7. Tao et al. (1998)

The focus of this study was an examination of tumors resulting from MNU and DCA or TCA exposure in mice with the source of tumors was reported to be the study of Pereira et al. (1997). Thus, similar concerns discussed above for that study paradigm are applicable to the results of this study. The authors stated that there were also two recovery groups in which exposure was terminated 1 week prior to euthanization at week 44. The Pereira et al. (1997) study does not report a cessation group in the study. In this study, the number of animals treated in the cessation group, the incidences of tumors in the mice, and the number of tumors examined were not reported. Another group of female $B6C3F_1$ mice (7–8 weeks old) were reported to not be administered MNU but given 25 mmol/L DCA (3.23 g/L DCA), 25 mmol TCA (4.09 g/L TCA), or control drinking water for 11 days (n = 7).

Hepatocellular adenomas in DCA-treated mice and adenomas and carcinomas in TCA-treated mice were reported to be analyzed for percent-5-methylcytosine in the DNA of tumor tissues. The levels of 5-methylcytosine in liver DNA of mice administered DCA or TCA for 11 days were reported to be reduced in comparison to control tissues (reduced to ~36% of control for DCA and ~41% of control for TCA with the control value reported to be ~3.5% of DNA methylated). The number of animals examined was reported to be 7–10 animals per group.

For control liver from mice that had received MNU but not DCA or TCA, and noninvolved liver after 44 weeks of exposure to either, the levels of 5-methylcytosine were similar and not different from the ~3.5% of DNA methylated in untreated mice in the 11-day experiment. Thus, initial decreases in methylated DNA shown by exposure to DCA or TCA alone for 11 days, were not observed in "noninvolved" liver of animals exposed to either DCA or TCA and MNU.

In regard to tumor tissues, the level of 5-methylcytosine in DNA of hepatocellular adenomas receiving DCA and MNU was reported to be decreased by 36% in comparison to noninvolved liver from the same animals. When exposure to DCA was terminated for 1 week prior to sacrifice, the level of 5-methylcytosine in the adenomas was reported to be higher and no longer differed statistically from the noninvolved liver from the same animal or liver from control animals only administered MNU. The number of samples was reported to be 9–16 samples without identification as to how many samples were used for each tumor analysis or how many animals provided the samples (i.e., were most of the adenomas from on animal?)

For TCA, the 5-methylcytosine level was reported to be reduced by 40% in hepatocellular adenomas and 51% reduction in HCCs in comparison to noninvolved liver from the same animals. These levels were also reported to be less than that the control animals administered only MNU.

Termination of exposure to TCA 1 week prior to sacrifice was reported to not produce a statistically significant change in the level of 5-methylcytosine in either adenomas or

carcinomas. The levels of 5-methylcytosine were reported to be lower in carcinomas than adenomas (~20% reduction) and to be lower in the "recovery" carcinomas than continuous carcinomas (~25%), but were not reported as statistically significant. The results are reported to have been derived from 8 to 16 "samples each." Again, information on the number of animals with tumors, whether the tumors were from primarily from one animal, and which DNA results are from 8 vs. 16 samples, was not provided by the authors.

Given that Pereira et al. (1997), the source for material of this study, reported that treatment of MNU and 25.0 mmol/L TCA treatment for 44 weeks induced only four carcinomas, a question arises as to how many carcinomas were used for the 44-week 5-methylcytosine results in this study for carcinomas (i.e., how can 8–16 samples arise from four carcinomas?). In addition, a question arises as to whether there was a difference in tumor-response in those animals with and without 1 week of cessation of exposure, which cannot be discerned from this report. The use of highly variable number of samples between analysis groups and lack of information as to how many tumors were analyzed adds uncertainty to the validity of these findings. There did not appear to be a difference in methylation activity from short-term exposure to either DCA or TCA alone in whole-liver DNA extracts. However, the authors conclude that the difference in methylation status between tumors resulting from MNU and DCA or TCA exposures supports differences in the action between DCA and TCA.

E.4.2.8. Stauber et al. (1998)

In this study, 5–8-week-old male B6C3F$_1$ mice were used for isolation of primary hepatocytes, which were subsequently isolated and cultured in DCA or TCA. In a separate experiment, 0.5 g/L DCA was given to mice as pretreatment for 2 weeks prior to isolation. The authors note that an indication of an "initiated cell" is anchorage-independent growth. DCA and TCA solutions were neutralized before use. The primary hepatocytes from three mice per concentration were cultured for 10 days with DCA or TCA colonies (eight cells or more) determined in quadruplicate. The levels of DCA used were 0, 0.2, 0.5, and 2.0 mM DCA or TCA. At concentrations of ≥0.5 mM, DCA and TCA both induced an increase in the number of colonies that was statistically significant and increased with dose, with DCA giving a slightly greater effect. The authors noted that concentrations >2.0 mM were cytotoxic, but did not show data on toxicity for this study.

Of great interest is the time-course experiment from this study in which the number of colonies from DCA treatment in vitro peaked by 10 days and did not change through days 15–25 at the highest dose. For the lower concentrations of DCA, increased time in culture induced similar peak levels of colony formation by days 20–25 as that reached by 10 days at the higher dose. Therefore, the number of colonies formed was independent of dose if the cells were treated long enough in vitro. The number of colonies that formed in control hepatocyte cultures also increased with time but at a lower rate than those treated with DCA (2.0 mM DCA gave

approximately twofold of control by 25 days of exposure to hepatocytes in culture). However, the level reached by cells untreated in tissue culture alone by 20 days was similar to the level induced by 0.5 mM DCA by 10 days of exposure. This finding raises the issue of what these "colonies" represent, as tissue culture conditions alone transform these cells to what the authors suggest is an "initiated" state. TCA exposure was not tested with time to see if it had a similar effect to DCA.

At 10 days, colonies were tested for c-Jun expression with the authors noting that "colonies promoted by DCA were primarily c-Jun positive in contrast to TCA promoted colonies that were predominantly c-Jun negative." For colonies that arose spontaneously from tissue culture conditions, 10/13 (76.9%) were reported to be c-Jun+, those treated with DCA 28/34 (82.3%) were c-Jun+, and those treated with TCA 5/22 (22.7%) were c-Jun+. These data show heterogeneity in cell in colonies, although more were c-Jun+ with DCA than TCA. The number of colonies reported in the c-Jun labeling results represent sums between experiments and thus, present total numbers of the control and the of colonies derived from doses of DCA and TCA at 0.2–2.0 mM at 10 days. Thus, changes in colony c-Jun+ labeling due to increasing dose cannot be determined.

The authors reported that with time (24, 48, 72, and 96 hours) of culture conditioning, the number of c-Jun+ colonies was increased in untreated controls. DCA treatment was reported to delay the increase in c-Jun+ expression induced by tissue culture conditions alone in untreated controls. TCA treatment was reported to not affect the increasing c-Jun+ expression that increased with time in tissue culture. In this instance, tissue culture environment alone was shown to transform cells and can be viewed as a "co-exposure." DCA pretreatment in vivo was reported to increase the number of colonies after plating, which reached a plateau at 0.10 mM and gave changes as at low a concentration of 0.02mM DCA administered in vitro. The background level of colony formation varied between controls (i.e., twofold different in pretreatment experiments and nonpretreatment experiments). Therefore, although the number of colonies was greater for pretreatment with DCA, the magnitude of difference over the control level was the same after DCA treatment in vitro with and without pretreatment.

The authors presented a comparison of "tumors" from Stauber and Bull (1997) and state that DCA tumors were analyzed after 38 weeks of treatment but that TCA tumors were analyzed after 52 weeks. They note that 97.5% of DCA-induced "tumors" were c-Jun+, while none of the TCA-induced "tumors" were c-Jun+. The concentrations used to give tumors in vivo for comparison with in vitro results were not reported. What was considered to be "tumors" from the earlier report for this analysis was also not noted. Stauber and Bull (1997) reported results for combination of foci and tumors raising issues as to what was examined in this report. The authors stated that because of such short time, no control tumors results were given. The short and variable time of duration of exposure increases the possibility of differences between the in

vivo data resulting from differences in tumor progression as well as a decreased ability by the shortened time of observation for full expression of the tumor response.

E.4.3. Co-exposures of Haloacetates and Other Solvents

As noted by Caldwell et al. (2008b), drinking water exposure data suggest that co-exposure of TCE and its haloacetic acid metabolites, TCA and DCA, is not an uncommon event, as DCA and TCA are the two most abundant haloacetates in most water supplies (Boorman, 1999; Weisel et al., 1999). Dibromoacetic acid (DBA) concentrations have also been reported to range up to approximately 20 μg/L in finished water and distribution systems (U.S. EPA, 2002a). Caldwell et al. (2008b) have also noted that co-exposure in different media also occurs with solvents like perchloroethylene (PERC) that may share some modes of action, targets of toxicity, and common metabolites that can, therefore, potentially affect TCE health risk (Wu and Schaum, 2000). Some of the information contained in the following sections has been excerpted from the discussions by Caldwell et al. (2008b) regarding the implications for the risk of TCE exposure as modulated by co-exposures to haloacetates and other solvents that have been studied and reported in the literature.

E.4.3.1. Carbon tetrachloride, DCA, TCA: Implications for Mode of Action from Co-exposures

Studies of specific combinations of TCE and chemicals colocated in contaminated areas have been reported by Caldwell et al. (2008b). For carbon tetrachloride:

> Pretreatment with TCE in drinking water at levels as low as 15 mM for three days has been reported to increase susceptibility to liver damage to subsequent exposure to a single IP injection of 1 mM/kg carbon tetrachloride (CCl_4) in Fischer 344 rats (Steup et al., 1991). Potential mechanistic explanations for this observation included altered metabolism, decreased hepatic repair capability, decreased detoxification ability, or combination of one or more of the above activities. Simultaneous administration of an oral dose of TCE (0.5ml/kg) has also been reported to increase the liver injury induced by an oral dose of 0.05 ml/kg CCl_4 (Steup et al., 1993). The authors suggested that TCE appeared to impair the regenerative activity in the liver, thus leading to increased damage when CCl_4 is given in combination with TCE.

As discussed in Section E.4.2, initiation studies are in themselves a co-exposure. The study of Bull et al. (2004) is included here as it not only used a co-exposure of vinyl carbamate with TCE metabolites, but also used carbon tetrachloride as a co-exposure. The rationale for this approach was that co-exposure of TCE (and therefore, to its metabolites) and carbon tetrachloride are likely to occur as they are commonly found together at contaminated sites.

Bull et al. (2004) hypothesized that modification of tumor growth rates is an indication of promotion rather than effects on tumor number, and that by studying tumor growth rates, they could classify carcinogens by their modes of action. $B6C3F_1$ male mice were initiated with vinyl carbamate (3 mg/kg) at 2 weeks of age and then treated with DCA, TCA, or carbon tetrachloride (0.1, 0.5, or 2.0 g/L for DCA and TCA; 50, 100 or 500 mg/kg carbon tetrachloride in 5% Alkamuls via gavage) in pair-wise combinations of the three for 18–36 weeks. The exposure level of carbon tetrachloride to 5, 20, and 50 mg/kg was reported to be reduced at week 24 due to toxicity for carbon tetrachloride. The number of mice in each group was reported to be 10 with the study divided into five segments. There were evidently differences between treatment segments as the authors state that "because of some significant quantitative differences in results that were obtained with replicate experiments treated in different time frames, the simultaneous controls have been used in the analysis and presentation of these data."

As with Bull et al. (2002), the interpretation of the results of the study is limited by a low number of animals per group, short duration time of exposure, and limited examination and reporting of results. For example, a sample of 100/8,000 lesions identified in the study was examined to verify that the general descriptor of neoplastic and nonneoplastic lesion was correctly labeled with "tumors" describing a combination of hyperplastic nodules, adenomas, and carcinomas. No incidence data were reported by the authors, but general lesion growth information included mean lesion volume and multiplicity of lesions (numbers of lesions/mouse). Using these reported indices, there appeared to be differences in treatment-related effects.

As discussed in Caldwell et al. (2008b):

> Each treatment was examined alone and then in differing combinations with each other. Mice initiated with vinyl-carbamate, but without further exposure to the other toxicants, were reported to have a few lesions that were of small size during the examination period (20–36 weeks). At 30 weeks of CCl4 exposure, there was a dose-related response reported for multiplicity but mean lesion size was smaller at the highest dose in initiated animals. At 36 weeks, DCA exposure was reported to increase multiplicity at the two highest exposure levels and increased lesion size at all levels compared to initiated-only animals. However, at a similar level of induction, multiplicity and mean size of those lesions resulting from DCA treatment were reported to be much smaller in comparison with CCl4 treatment (i.e., a 20-fold difference for lesion volume). At 36 weeks, treatments with the same concentration of TCA or DCA induced similar multiplicity, but the mean lesion volume was reported to be approximately 4-fold greater in tumors induced by DCA as compared to TCA, and in animals treated with DCA multiplicity had reached a plateau by 24 weeks rather than 36 for those treated with TCA.

Thus, using multiplicity of lesions and lesion volume as indicators of differences in mode of action, exposure to carbon tetrachloride, DCA, and TCA appeared to produce distinct differences in results in animals previously treated with vinyl carbamate.

As discussed in Caldwell et al. (2008b):

> Simultaneous coexposure of differing combinations of CCl_4, DCA, and TCA were reported to give more complex results between 24 and 36 weeks of observation but to show that coexposure had effects on lesion multiplicity and volume in initiated animals. At 36 weeks, TCA coexposure appeared to reduce the lesion volume of either DCA- or CCl_4-induced lesions after vinyl carbamate treatment. Similarly, DCA coexposure was reported to reduce the lesion volume of either TCA- or CCl_4-induced lesions when each was given alone after vinyl carbamate treatment. With regard to multiplicity, TCA coexposure was reported to reduce DCA-induced multiplicity only at the lowest dose of TCA while coexposure with DCA increased multiplicity of CCl_4-induced lesions at all exposure levels. At 24 weeks, there appeared to be little effect on mean lesion volume by any of the coexposures but DCA coexposure decreased multiplicity of TCA-induced lesions (up to 3-fold) while TCA treatment slightly increased the number of CCl_4-induced multiplicity (1.6-fold). This study confirms that short duration of exposure to all three of these chemicals can cause lesions in already exposed to vinyl carbamate, and suggests that combinations of these agents differentially influence lesion number and growth rates. The authors have interpreted their results to indicate differences in MOA between such treatments. However, the limitations of the study limit conclusions regarding how such coexposure may be able to affect toxicity and tumor induction and what the MOA is for each of these agents. This is especially true at lower and more environmentally relevant concentrations given for longer durations to uninitiated animals.

E.4.3.2. Chloroform, DCA, and TCA Coexposures: Changes in Methylation Status

In Section E.3.4.2.2, information on the effects of TCE and its metabolites was presented in regard to effects on methylation status. After 7 days of gavage dosing, TCE, TCA, and DCA were reported to increased hypomethylation of the promoter regions of c-Jun and c-Myc genes in mouse whole-liver DNA; however, Caldwell and Keshava (2006) concluded that hypomethylation did not appear to be a chemical-specific effect at the concentration used. Bull et al. (2004) suggested that hypomethylation occurs at higher exposure levels than those that induce liver tumors for TCE and its metabolites. Along with studies of methylation changes induced by a exposure to a single agent, Pereira et al. (2001) have attempted to examine the effects on methylation changes from co-exposures. This study was also reviewed by Caldwell et al. (2008b).

Pereira et al. (2001) hypothesized that changes in the methylation status of DNA can be a key event for the mode of action for DCA- and TCA-induced liver carcinogenicity through changes in gene regulation, and that chloroform ($CHCl_3$) co-exposure may result in modification of DNA methylation. As discussed in Caldwell et al. (2008b),

> After 17 days of exposure of exposure to $CHCl_3$ (0, 400, 800, 1,600 mg/L, n = 6 mice per treatment group) female $B6C3F_1$ mice were coexposed to DCA or TCA (500 mg/kg) during the last 5 days of exposure to chloroform. As noted by

> Caldwell et al. (2008b), Pereira et al. (2001) reported the effects of hypomethylation of the promoter region of *c-Myc* gene and on expression of its mRNA in the whole livers of female $B6C3F_1$ mice and thus, these results represent composite changes in DNA methylation status from a variety of cell types and for hepatocytes lumped from differing parts of the liver lobule. When given alone, DCA, TCA, and to a lesser extent, the highest concentration of $CHCl_3$ (1,600 mg/L), was reported to decrease methylation of the *c-myc* promoter region. Coadministration of $CHCl_3$ (at 800 and 1,600 mg/L) was reported to decrease DCA-induced hypomethylation while exposure to $CHCl_3$ had no effect on TCA-induced hypomethylation. Treatment with DCA, TCA, and, to a lesser extent $CHCl_3$, was reported to increase levels of *c-myc* mRNA. While expression of *c-myc* mRNA was increased by DCA or TCA treatment, increasing coexposures to $CHCl_3$ were reported to attenuate the actions of DCA but not TCA. Thus, differences in methylation status and expression of the *c-myc* gene induced by DCA or TCA exposure was reported to be differentially modulated by coexposure to $CHCl_3$. The authors suggest these differences support differing actions by DCA and TCA. However, whether these changes represent key events in the induction of liver cancer is a matter of debate, especially as a "snapshot in time" approach for such a nonspecific endpoint.

In a co-exposure study in which an "initiating agent" was used as a co-exposure along with other co-exposure, Pereira et al. (2001) treated male and female 15-day-old $B6C3F_1$ mice with MNU (a cause of liver and kidney tumors) and then, starting at 5 weeks of age, treated them with DCA (3.2 g/L) or TCA (4.0 g/L) along with co-exposure to $CHCl_3$ (0, 800, or 1,600 mg/L) for 36 weeks. Mice were reported to be examined for evidence of promotion of liver and kidney tumors. The numbers of animals in the exposure groups were highly variable, ranging from 25 (female-initiated mice exposed to DCA) to 6 (female-initiated mice exposed to DCA and 1,600 mg/L $CHCl_3$), thus limiting the power of the study to ascertain treatment-related changes. However, unlike Bull et al. (2004), all liver tissues were examined with incidences of foci, adenomas, carcinomas, and both adenoma and carcinoma reported separately for treatment groups. Multiplicity for a combination of adenomas and carcinomas were reported as well as the tincture of foci and tumors.

Although as noted by Caldwell et al. (2008b):

> [T]he statistical power of the study to detect change was very low, an examination of the pattern of tumors induced by coexposure to MNU and TCE metabolites in female mice suggested that: (1) DCA exposure increased the incidence of adenomas but not carcinomas; (2) TCA increased incidence of carcinomas with little change in adenoma incidence; (3) coexposure to 800 and 1,600 mg/L of $CHCl_3$ decreased adenoma incidence by DCA treatment but not TCA; and (4) $CHCl_3$ coexposure decreased multiplicity of TCA-induced tumors and foci, but not for DCA.

Caldwell et al. (2008b) also note that this study suggests:

> [A] gender-related effect on tumor induction from this study with: (1) adenoma incidences similar in male and female mice treated with DCA, but carcinoma incidence greater in males; (2) adenoma and carcinoma incidences greater in males than females treated with TCA; (3) tumor multiplicity similar in both genders for DCA treatments, but lower in females mice for TCA; and (4) less of an inhibitory effect by $CHCl_3$ on adenoma incidence from DCA exposure in male mice.

Pereira et al. (2001) also described the tinctural characteristics of the specific lesions induced by their co-exposure treatments. Both foci and tumors induced by DCA exposure in "initiated" mice were reported to be over 95% eosinophilic in females, while in males, 89% of the foci were eosinophilic and 91% of tumors were basophilic. Thus, not only was there a gender-related difference in the incidences of tumors and foci but also foci and tumor phenotype. $CHCl_3$ co-exposure was reported to change the DCA-induced foci from primarily eosinophilic to basophilic (i.e., 11 vs. 75% basophilic) in male mice coexposed to MNU. In male and female mice, TCA-induced tumors and foci were basophilic with no effect of $CHCl_3$ on phenotype in MNU treated mice.

E.4.3.3. Co-exposures to Brominated Haloacetates: Implications for Common Modes of Action and Background Additivity to Toxicity

As noted by Caldwell et al. (2008b), along with chlorinated haloacetates and other solvents, "co-exposures with TCE and brominated haloacetates may occur through drinking water. These compounds may affect TCE toxicity in a similar fashion to their chlorinated counterparts. As bromide concentrations increase, brominated haloacetates increase in the water supply."

Kato-Weinstein et al. (2001) administered dibromoacetate (DBA), bromochloroacetate (BCA), bromodichloroacetate (BDCA), TCA, and DCA in drinking water at concentrations of 0.2–3 g/L for 12 weeks to $B6C3F_1$ male mice. The focus of the study was to determine the similarity in action between the brominated and chlorinated haloacetates. Each of the haloacetates, given individually, were reported to increase liver/body weight ratios in a dose-dependent manner.

The dihaloactates, DCA, BCA, and DBA, caused liver glycogen accumulation both by chemical measurements in liver homogenates and in ethanol-fixed liver sections (to preserved glycogen) stained with PAS. For DCA, a maximal level of glycogen increase was observed at 4 weeks of exposure at a 2 g/L exposure concentration. They report a 1.60-fold of control percent liver/body weight and 1.50-fold of control glycogen content after 8 weeks of exposure to 2 g/L DCA in male $B6C3F_1$ mice. The baseline level of glycogen content (~60 mg/g) and the increase in glycogen after DCA exposure was consistent with the results reported by Pereira et al. (2004a). The percent liver/body weight data for control mice was for animals sacrifice at

20 weeks of age. The 4–12-week exposure to DCA were staggered so that all animals would be 20 weeks of age at sacrifice. Thus, the animals were at differing ages at the beginning of DCA treatments between the groups.

However, as with Pereira et al. (2004a), the ~10% increase in liver mass that the glycogen increases represent are lower than the total increase in liver mass reported for DCA exposure. The authors noted possible contamination of BCA with small percentages of DCA and DBA in their studies (i.e., 84% BCA, 6% DCA and 8% DBA). The trihaloacetates (TCA and low concentrations of BDCA) were reported to produce slight decreases in liver glycogen content, especially in the central lobular region in cells that tended to accumulate glycogen in control animals. These effects on liver glycogen were reported at the lowest dose examined (i.e., 0.3 g/L). At the highest concentration, BDCA was reported to induce a pattern of glycogen distribution similar to that of DCA in mice.

All dihaloacetates were reported to reduce serum insulin levels at high concentrations. Conversely, trihaloacetates were reported to have no significant effects on serum insulin levels. For the study of peroxisome proliferation and DNA synthesis, mice were treated with BCA, DBA, and BDCA for 2, 4, or 26 weeks. The effects on DNA synthesis were small for all brominated haloacetates with only DBA reported to show a significant increase in DNA synthesis at 2 and 4 weeks but not at 26 weeks (the increase in DNA synthesis was threefold of the highest control level). Of note is the highly variable level of DNA synthesis reported for control values that varied to a much higher degree (~3–6-fold variation within control groups at the same time points) than did treatment-related changes. DBA was the only brominated haloacetate that was reported to consistently increased PCO activity as a percentage of control values (actual values and variability between controls were not reported) with a 2–3-fold increase in PCO activity at 0.3–3.0 g/L DBA. DBA-induced PCO activity increases were reported to be limited to 2–4 weeks of treatment in contrast to TCA, which the authors reported to increase PCO activity consistently over time.

Tao et al. (2004a) reported DNA methylation, glycogen accumulation, and peroxisome proliferation after exposure of female $B6C3F_1$ mice and male F344 rats exposed to 1 or 2 g/L DBA in drinking water for 2–28 days. DBA was reported to induce dose-dependent DNA hypomethylation in whole mouse and rat liver after 7 days of exposure with suppression sustained for the 28-day exposure period. The expression of mRNA for c-Myc in mice and rats and mRNA expression of the IGF-II gene in female mice were reported to be increased during the same period. Both rats and mice were reported to exhibit increased glycogen with mice having increased levels at 2 day and rats at 4 days. DBA was reported to cause an increase in lauroyl-CoA oxidase activity (a marker of peroxisome proliferation) in both mice (after 7 days) and rats (after 4 days) that was sustained for 28 days.

Methylation changes reported here for DBA exposure in rats and mice are consistent with those reported for TCA and DCA by Pereira et al. (2001) in mice. The pattern of glycogen

accumulation was also similar to that reported for DCA by Kato-Weinstein et al. (2001) and suggests that the brominated analogues of TCE metabolites exhibited similar actions as their chlorinated counterparts. In regard to peroxisomal enzyme activities, Kato-Weinstein et al. (2001) reported PCO activity to be limited to 2–4 weeks with Tao et al. (2004b) reporting lauroyl-CoA oxidase activity to be sustained for the lengths of the study (28 days) for DBA.

As noted by Caldwell et al. (2008b):

> "given the similarity of DCA and DBA effects, it is plausible that DBA exposure also induces liver cancer. Melnick (2008) reported the results of DBA exposure to F344/N rats and B6C3F$_1$ mice exposed to DBA for 3 months or 2 years in drinking water (0, 0.05, 0.5, or 1.0 g/L DBA for 2 years). Neoplasms at multiple sites were reported in both species exposed to DBA for 2 years with no effects on survival and little effect on mean body weight in either species. Similar to TCE, DCA and TCA, the liver was reported to be a target of DBA exposure. After 3-months of exposure, there were dose-related increases in hepatocellular vacuolization and liver weight reported in rats and mice described as 'glycogen-like.'"

The authors report that the major neoplastic effects of DBA in rats were induction of malignant mesotheliomas in males and increased incidence of mononuclear cell leukemia in males and females. For mice, the major neoplastic effect of DBA exposure was reported to be the increased incidence of hepatocellular adenomas and carcinomas at all exposure levels.

In addition to these liver tumors, hepatoblastomas were also reported to be increased in all exposure groups of male mice and exceeded historical control rates. The incidence of alveolar/bronchiolar adenoma and carcinoma was reported to be increased in the 0.5 g/L group of male mice along with marginal increases in alveolar hyperplasia in DBA-treated groups. The authors reported that the increases in hepatocellular neoplasms were not associated with hepatocellular necrosis or regenerative hyperplasia and concluded that an early increase in hepatocyte proliferation was not likely involved in the mode of action for DBA because no increases in hepatocyte DNA labeling index were observed in mice exposed for 26 days and the slight increase that occurred in male F344 rats was not accompanied by an increase in liver tumor response.

As noted by Caldwell et al. (2008b),

> [T]he results of Kato-Weinstein et al. (2001), Tao et al. (2004b), and Melnick et al. (2008) are generally consistent for DBA and show a number of activities that may be common to TCE metabolites (i.e., brominated and chlorinated haloacetate analogues generally have similar effects on liver glycogen accumulation, serum insulin levels, peroxisome proliferation, hepatocyte DNA synthesis, DNA methylation status, and hepatocarcinogenicity). It is therefore, plausible that these effects may be additive in situations of coexposure. However, as noted by (Melnick et al., 2008), methylation status, events associated with PPARα

agonism, hepatocellular necrosis, and regenerative hyperplasia are not established as key events in the MOA of these agents, and the MOAs for DCA- and DBA-induced liver tumors are unknown.

E.4.3.4. Co-exposures to Ethanol: Common Targets and Modes of Action

As noted in the U.S. EPA's draft TCE assessment (U.S. EPA, 2001), alcohol consumption is a common co-exposure that has been noted to affect TCE toxicity with TCE exposure cited as potentially increasing the toxicity of methanol and ethanol, not only by altering their metabolism to aldehydes, but also by altering their detoxification (e.g., similar to the "alcohol flush" reported for those who have an inactive aldehyde dehydrogenase allele). As noted by Caldwell et al. (2008b) "chemical co-exposures from both the environment and behaviors such as alcohol consumption may have effects that overlap with TCE in terms of active agents, pharmacokinetics, pharmacodynamics, and/or target tissue toxicity." Caldwell et al. (2008b) also noted:

> In their review of solvent risk (including TCE), Brautbar and Williams (2002) suggest that laboratory testing that is commonly used by clinicians to detect liver toxicity may not be sensitive enough to detect early liver hepatotoxicity from industrial solvents and that the final clinical assessment of hepatotoxicity and industrial solvents must take into account synergism with medications, drugs of use and abuse, alcohol, age-dependent toxicity, and nutrition. Although many of these factors may be important, the focus in this section is on the effects of ethanol. Contemporary literature reports effects similar to those of TCE's and previous reports indicate ethanol consumption impacts TCE toxicity in humans, affects the pharmacokinetics and toxicity of TCE in rats, and is also a risk factor for cancer.
>
> The association between malignant tumors of the upper gastrointestinal tract and liver and ethanol consumption is based largely on epidemiological evidence, and thought to be causally related (Bradford et al., 2005; Badger et al., 2003). Studies of the mechanisms of ethanol carcinogenicity have suggested the importance of its metabolism, including induction of CYP2E1 associated increases in production of reactive oxygen species and enhanced activation of a variety of pro-carcinogens, alteration of retinol and retinoic acid metabolism, and the actions of the metabolite acetaldehyde (Badger et al., 2003). While ethanol is primarily metabolized by alcohol dehydrogenase, it undergoes simultaneous oxidation to acetate by hepatic P450s, primarily CYP2E1. Both chronic ethanol consumption as well as TCE treatment induces CYP2E1 (U.S. EPA, 2001). Oneta et al. (2002) report that even at moderate chronic ethanol consumption, hepatic CYP2E1 is induced in humans, which they suggest, may be of importance in the pathogenesis of alcoholic liver disease; of ethanol, drug, and vitamin A interactions; and in alcohol-associated carcinogenesis. Induction of CYP2E1 can cause oxidative stress to the liver from nicotinamide dinucleotide phosphate (NADPH)-dependent reduction of dioxygen to reactive products even in the absence of substrate, and subsequent apoptosis (Badger et al., 2003).

Bradford et al. (2005) suggest that CYP2E1, and not NADPH oxidase, is required for ethanol-induced oxidative DNA damage to rodent liver but that NADPH oxidase-derived oxidants are critical for the development of ethanol-induced liver injury.

There is increasing evidence that acetaldehyde, which is toxic, mutagenic, and carcinogenic, rather than alcohol is responsible for its carcinogenicity (Badger et al., 2003). Mitochondrial aldehyde dehydrogenase (ALDH2) disposes of acetaldehyde generated by the oxidation of ethanol, and ALDH2 inactivity through mutation or polymorphism has been linked to esophageal cancer in humans (everyday drinkers and alcoholics) (Badger et al., 2003). For instance, increased esophageal cancer risk was reported for patients with the ALDH3*1 polymorphism as well as increased acetaldehyde in their saliva. TCE exposure has also been reported to induce a similar alcohol flush in humans which may be linked to its ability to decrease ALDH activities at relatively low concentrations and thus conferring a similar status to individuals with inactive ALDH2 allele (Wang et al., 1999). Whether the MOA for the buildup of acetaldehyde after ethanol and TCE co-exposure is: (1) the induction of CYP2E1 by TCE resulting in increased metabolism to acetaldehyde; (2) inhibition of ALDH and thus reduced clearance of acetaldehyde, or (3) a number of other actions are unknown. Crabb et al. (2001) reported 20–30% reductions in ALDH2 protein level by PPARα agonists (Clofibrate treatment in rats and WY treatment in both wild and PPARα-null mice). This could be another pathway for TCE-induced effects on ethanol metabolism. It is an intriguing possibility that the reported association between the increased risk of human esophageal cancer and TCE exposure (Scott and Chiu, 2006) could be related to TCE effects on mitochondrial ALDH, given a similar association of this endpoint with ethanol consumption or ALDH polymorphism.

Finally, ethanol ingestion may have significant effects on TCE pharmacokinetics. Baraona et al. (2002a; 2002b) reported that chronic, but not acute, ethanol administration increased the hepatotoxicity of peroxynitrite, a potent oxidant and nitrating agent, by enhancing concomitant production of nitric oxide and superoxide. They also reported that nitric oxide mediated the stimulatory effects of ethanol on blood flow. Ethanol markedly enhanced portal blood flow (2-fold increase), with no changes in the hepatic, splenic, or pancreatic arterial blood flows in rats.

E.4.3.5. Co-exposure Effects on Pharmacokinetics: Predictions Using PBPK Models

Along with experimental evidence that has focused on chronic and acute experiments using rodents, the potential pharmacokinetic modulation of risk has also been recently published reports using PBPK models that may be useful in predicting co-exposure effects. Caldwell et al. (2008b) also examined and discussed these approaches and noted:

An important issue for prediction of the effects and relationship on MOAs by co-exposure is the degree to which modulation of TCE toxicity by other agents can be quantified. Pharmacokinetics or the absorption, distribution, metabolism, and

elimination of an agent, can be affected by internal and external co-exposure. Such information can help to identify the chemical species that may be causally associated with observed toxic responses, the MOA, and ultimately identify potentially sensitive subpopulations for an effect such as carcinogenicity.

Physiologically based pharmacokinetic (PBPK) models are often used to estimate and predict the toxicologically relevant dose of foreign compounds in the body and have been developed to predict effects on pharmacokinetics that are additive or less or greater than additive. One of the first such models was developed for TCE (Andersen et al., 1987b). Given that TCE, PERC, and methyl chloroform (MC) are often found together in contaminated groundwater, Dobrev et al. (2001) attempted to investigate the pharmacokinetic interactions among the three solvents to calculate defined "interaction thresholds" for effects on metabolism and expected toxicity. Their null hypothesis was defined as competitive metabolic inhibition being the predominant result for TCE given in combination with other solvents. Gas uptake inhalation studies were used to test different inhibition mechanisms. A PBPK model was developed using the gas uptake data to test multiple mechanisms of inhibitory interactions (i.e., competitive, noncompetitive, or uncompetitive) with the authors reporting competitive inhibition of TCE metabolism by MC and PERC in simulations of pharmacokinetics in the rat. Occupational exposures to chemical mixtures of the three solvents within their Threshold Limit Value (TLV)/TWA limits were predicted to result in a significant increase (22%) in TCE blood levels compared with single exposures.

Dobrev et al. (2002) extended this work to humans by developing an interactive human PBPK model to explore the general pharmacokinetic profile of two common biomarkers of exposure, peak TCE blood levels, and total amount of TCE metabolites generated in rats and humans. Increases in the TCE blood levels were predicted to lead to higher availability of the parent compound for GSH conjugation, a metabolic pathway that may be associated with kidney toxicity/carcinogenicity. A fractional change in TCE blood concentration of 15% for combined TLV exposures of the three chemicals (25/50/350 ppm of PERC/TCE/MC) resulted in a predicted 27% increase of the S-(1, 2-dichlorovinyl)-L-cysteine (DCVC) metabolites, indicating a nonlinear risk increase due to combined exposures to TCE. Binary combinations of the solvents produced GST-mediated metabolite levels almost twice as high as the expected rates of increase in peak blood levels of the parent compound. The authors suggested that using parent compound peak blood levels (a less sensitive biomarker) would result in two to three times higher (i.e., less conservative) estimates of potentially safe exposure levels. In regard to the detection of metabolic inhibition by PERC and MC, the simulations showed TCE blood concentrations to be the more sensitive dose-metric in rats, but the total of TCE metabolites to be the more sensitive dose measure in humans. Finally, interaction thresholds were predicted to be occurring at lower levels in humans than rats.

Thrall and Poet (2000) investigated the pharmacokinetic impact of low-dose co-exposures to toluene and TCE in male F344 rats *in vivo* using a real-time breath

analysis system coupled with PBPK modeling. The authors report that, using the binary mixture to compare the measured exhaled breath levels from high- and low-dose exposures with the predicted levels under various metabolic interaction simulations (competitive, noncompetitive, or uncompetitive inhibition), the optimized competitive metabolic interaction description yielded an interaction parameter Ki value closest to the Michaelis-Menten affinity parameter (K_M) of the inhibitor solvent. This result suggested that competitive inhibition is the most plausible type of metabolic interaction between these two solvents.

Isaacs et al. (2004) have reported gas uptake co-exposure data for $CHCl_3$ and TCE. The question as to whether it is possible to use inhalation data in combination with PBPK modeling to distinguish between different metabolic interactions was addressed using sensitivity analysis theory. Recommendations were made for design of optimal experiments aimed at determining the type of inhibition mechanisms resulting from a binary co-exposure protocol. This paper also examined the dual nature of inhibition of each chemical in the pair to each other, which is that TCE and $CHCl_3$ were predicted to interact in a competitive manner. Even though as stated by Dobrev et al. (2001), other solvents inhibit TCE metabolism, it is also possible to quantify the synergistic interaction that TCE has on other solvents, using techniques such as gas uptake inhalation exposures.

Haddad et al. (2000) has developed a theoretical approach to predict the maximum impact that a mixture consisting of co-exposure to dichloromethane, benzene, TCE, toluene, PERC, ethylbenzene, m-, p-, and o-xylene, and styrene would have on venous blood concentration due to metabolic interactions in Sprague-Dawley rats. Two sets of experimental co-exposures were conducted. The first study evaluated the change in venous blood concentration after a 4 hour constant inhalation exposure to the 10 chemical mixtures. This experiment was designed to examine metabolic inhibition for this complex mixture. The second study was designed to study the impact of possible enzyme induction by using the same inhalation co-exposure after a 3 day pretreatment with the same 10 chemical mixture. The resulting venous concentration measurements for TCE from the first study were consistent with metabolic inhibition theory. The 10-chemical mixture was the most complex co-exposure used in this study. The authors stated that as mixture complexity increased, the resulting parent compound concentration time courses changed less, an observation which is consistent with metabolic inhibition. For the pretreatment study, the authors found a systematic decrease in venous concentration (due to higher metabolic clearance) for all chemicals except PERC. Overall, these studies suggest a complex metabolic interaction between TCE and other solvents.

A PBPK model for TCE including all its metabolites and their interactions can be considered a mixtures model where all metabolites have a common starting point in the liver. An integrated approach taking into account TCE metabolites and their metabolic inhibition and interactions among each other is suggested in Chiu et al. (2006b).

E.5. POTENTIALLY SUSCEPTIBLE LIFE STAGES AND CONDITIONS THAT MAY ALTER RISK OF LIVER TOXICITY AND CANCER

As described in Sections E.1.2 and E.3.1, there are a number of conditions that are associated with increased risk of liver cancer and toxicity that include age, use of a number of prescription medications including fibrates and statins, disease state (e.g., diabetes, NALD, viral infections), and exposure to external environmental contaminants that have an effect on TCE toxicity and targets. Obviously, epigenetic and genetic factors play a role in determining the risk to the individual. In terms of liver cancer, there is general consensus that despite the associations that have been made with etiological factors and the risk of liver cancer, the mechanism is still unknown. The mode of action of TCE toxicity is also unknown, but exposure to TCE and its metabolites have shown in rodent models to induce liver cancer and in a fashion that is not consistent with only a hypothesized mode of action of PPARα receptor activation that is in need of revision. However, multiple TCE metabolites have been shown to also induce liver cancer with varying effects on the liver that have also been associated with early stages of neoplasia (glycogen storage) or other actions associated with risk of hepatocarcinogenicity. The growing epidemic of obesity has been suggested to increase the risk of liver cancer and may reasonably increase the target population for TCE effects on the liver.

Lifestyle factors such as ethanol ingestion have not only been shown to increase liver cancer risk in those who already have fatty liver, but also to increase the toxicity of TCE. However, as noted by Caldwell et al. (2008b), while there is evidence to suggest that TCE exposure may increase the risk of liver toxicity and cancer, there are no data to support a quantitative estimate of how co-exposures may modulate that risk.

> These findings can also serve to alert the risk manager to the possibility that multiple internal and external exposures to TCE that may act via differing MOAs for the production of liver effects. This information suggests a possible lack of "zero" background exposures and can help identify potential susceptible populations.
>
> Background levels of haloacetates in drinking water may add to the cumulative exposure an individual receives via the metabolism of TCE. The brominated haloacetates apparently share some common effects and pathways with their chlorinated counterparts. Thus, concurrent exposure of TCE, its metabolites, and other haloacetates may pose an additive response as well as an additive dose. However, personal exposures are difficult to ascertain and the effects of such co-exposures on toxicity are hard to quantify. EPA's guidance on cumulative risk assessments directs "each office to take into account cumulative risk issues in scoping and planning major risk assessments and to consider a broader scope that integrates multiple sources, effects, pathways, stressors, and populations for cumulative risk analyses in all cases for which relevant data are available" [U.S. EPA, 1997]. Widespread exposure to possible background levels of TCE metabolites or co-contaminants and other extrinsic factors have the potential to

affect TCE toxicity. However, the available data for co-exposures on TCE toxicity appears inadequate for quantifying these effects, particularly at environmental levels of contamination and exposure. Thus, the risk manager and assessor are going to be limited by not having information regarding either (1) the type of exposure data necessary to assess the magnitude of co-exposures that may affect toxicity, or (2) the potential quantitative impacts of these co-exposures that would enable specific adjustments to risk. Nonetheless, the risk manager should be aware that qualitatively a case can be made that extrinsic factors may affect TCE toxicity.

E.6. UNCERTAINTY AND VARIABILITY

Along with general conclusions about the coherence of data that enable conclusions about effects on the liver shown through experimental studies of TCE, there have also been extensive discussions throughout this report regarding the specific limitations of experimental studies whose design was limited by small and varying groups of animals and variability in control responses as well as reporting deficiencies. Section E.3.1.5 has brought forward the uncertainty in the mode of action for liver cancer in general. The consistency of different animal models with human HCC is described in Section E.3.3, with Section E.3.1.2 providing a discussion of the promise and limitations of emerging technologies to study the modes of action of liver cancer in general and for TCE specifically. Issues regarding the target cell for HCC and the complexities of studying the mode of action for a heterogeneous disease are described in Sections E.3.1.4 and E.3.1.8, respectively. Finally, the uncertainty regarding key events in how activation of the PPARα receptor my lead to hepatocarcinogenesis and the problems with extrapolation of results using the common paradigm to study them (exposure to high levels of WY-14,643 in abbreviated bioassays in knockout mice) are outlined in Section E.3.4.1. As such uncertainties are identified, future research can focus on resolving them.

F. NONCANCER DOSE-RESPONSE ANALYSES

F.1. DATA SOURCES

Data sources are cited in the body of this report in the section describing dose-response analyses (see Chapter 5).

F.2. DOSIMETRY

This section describes some of the more detailed dosimetry calculations and adjustments used in Section 5.1.

F.2.1. Estimates of TCE in Air From Urinary Metabolite Data Using Ikeda et al. (1972)

F.2.1.1. Results for Chia et al. (1996)

Chia et al. (1996) demonstrated a dose-related effect on hyperzoospermia in male workers exposed to TCE, lumping subjects into four groups based on range of TCA in urine (see Table F-1).

Table F-1. Dose-response data from Chia et al. (1996)

TCA, mg per g creatinine[a]	Number of subjects	Number with hyperzoospermia
0.8–<25	37	6
50–<75	18	8
75–<100	8	4
≥100–136.4	5	3

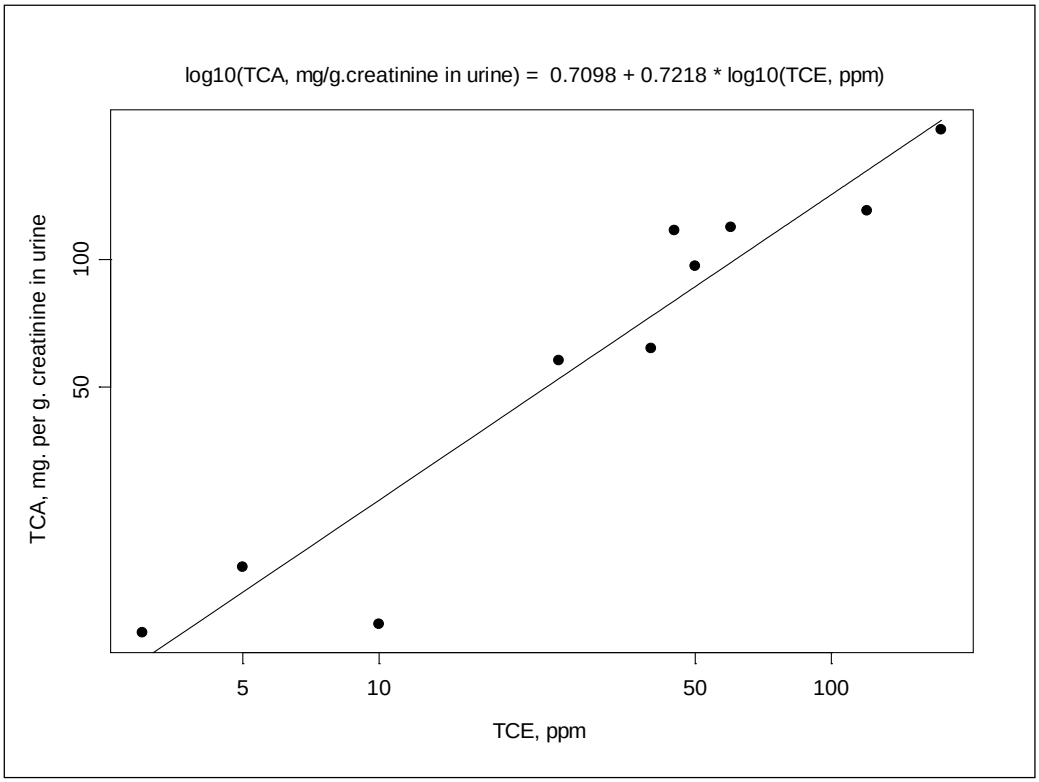
[a]Minimum and maximum TCA levels are reported in the text of Chia et al. (1996), the other data, in their Table 5.

Data from Ikeda et al. (1972) were used to estimate the TCE exposure concentrations corresponding to the urinary TCA levels reported by Chia et al. (1996). Ikeda et al. (1972) studied 10 workshops, in each of which TCE vapor concentration was "relatively constant." They measured atmospheric concentrations of TCE and concentrations in workers' urine of TTCs, TCA, and creatinine, and demonstrated a linear relation between TTC/creatinine (mg/g) in urine and TCE in the work atmosphere. Their data are tabulated as geometric means (the last column was calculated by U.S. EPA, as described in Table F-2).

Table F-2. Data on TCE in air (ppm) and urinary metabolite concentrations in workers reported by Ikeda et al. (1972)

n	TCE (ppm)	TTC (mg/L)	TCA (mg/L)	TTC (mg/g creatinine)	TCA (mg/g creatinine)
9	3	39.4	12.7	40.8	13.15127
5	5	45.6	20.2	42.4	18.78246
6	10	60.5	17.6	47.3	13.76
4	25	164.3	77.2	122.9	57.74729
4	40	324.9	90.6	221.2	61.68273
5	45	399	138.4	337.7	117.137
5	50	418.9	146.6	275.8	96.52012
5	60	468	155.4	359	119.2064
4	120	915.3	230.1	518.9	130.4478
4	175	1,210.9	235.8	1,040.1	202.5399

These data were used to construct the last column as follows: TCA (mg/g creatinine) = TCA (mg/L) × TTC (mg/g creatinine)/TTC (mg/L). The regression relation between TCE (ppm) and TCA (mg/g creatinine) was evaluated using these data. Ikeda et al. (1972) reported that the measured values are lognormally distributed and exhibit heterogeneity of variance, and that the reported data (above) are geometric means. Thus, the regression relation between log10(TCA [mg/g creatinine]) and log10(TCE [ppm]) was used, assuming constant variances and using number of subjects "*n*" as weights. Figure F-1 shows the results.




```
Coefficients:
                Value Std. Error t value Pr(>|t|)
   (Intercept) 0.7098 0.1132     6.2688  0.0002
log10(TCE.ppm) 0.7218 0.0771     9.3578  0.0000

Residual standard error: 0.3206 on 8 degrees of
freedom
Multiple R-Squared: 0.9163
F-statistic: 87.57 on 1 and 8 degrees of freedom,
the p-value is 0.0000139
```

Figure F-1. Regression of TCE in air (ppm) and TCA in urine (mg/g creatinine) based on data from Ikeda et al. (1972).

Next, a Berkson setting for linear calibration was assumed, in which one wants to predict *X* (TCE, ppm) from means for *Y* (TCA, mg/g creatinine), with substantial error in *Y* (Snedcor and Cochran, 1980). Thus, the inverse prediction for the data of Chia et al. (1996) was used to infer their mean TCE exposures. The relation based on data from Ikeda et al. (1972) is:

$$\log10(\text{TCA, mg/g creatinine}) = 0.7098 + 0.7218 \times \log10(\text{TCE, ppm}) \qquad \text{(Eq. F-1)}$$

and the inverse prediction is

$$\log10(\text{TCE}) = [\log10(\text{TCA}) - 0.7098]/0.7218 \quad \text{(Eq. F-2)}$$
$$\text{TCE, ppm} = 10\text{^}([\log10(\text{TCA}) - 0.7098]/0.7218)$$

Because of the lognormality of data reported by Ikeda et al. (1972), the means of the logarithms of the ranges for TCA (mg/g creatinine) in Chia et al. (1996), which are estimates of the median for the group, were used. The results are shown in Table F-3.

Table F-3. Estimated urinary metabolite and TCE air concentrations in dose groups from Chia et al. (1996)

TCA, mg per g Creatinine	Estimated TCA median[a]	Log10(TCA median)	Estimated ppm TCE[b]
0.8–<25	4.47	0.650515	0.827685
50–<75	61.2	1.787016	31.074370
75–<100	86.6	1.937531	50.226119
≥100–136.4	117	2.067407	76.008668

[a]10^(mean[log10(TCA limits in first column)]).
[b]10^([log10(TCA median)] – 0.7098)/0.7218.

Dose-response relations for the data of Chia et al. (1996) were modeled using both the estimated medians for TCA (mg/g creatinine) in urine and estimated TCE (ppm in air) as doses. The TCE-TCA-TTC relations are linear up to about 75 ppm TCE (Figure 1 of Ikeda et al. (1972)), and certainly in the range of the BMD. As noted (see Section F.2.2), the occupational exposure levels are further adjusted to equivalent continuous exposure for deriving the POD.

F.2.1.2. Results for Mhiri et al. (2004)

The LOAEL group for abnormal trigeminal nerve somatosensory evoked potential reported in Mhiri et al. (2004) had a urinary TCA concentration of 32.6 mg TCA/mg creatinine. Using Eq. F-2, above gives an occupational exposure level = 10^([log10(32.6) – 0.7098]/0.7218) = 12.97404 ppm. As noted below (see Section F.2.2), the occupational exposure levels are further adjusted to equivalent continuous exposure for deriving the POD.

F.2.2. Dose Adjustments to Applied Doses for Intermittent Exposure

The nominal applied dose was adjusted for exposure discontinuity (e.g., exposure for 5 days/week and 6 hours/day reduced the dose by the factor [5/7] × [6/24]). The PBPK dose-metrics took into account the daily and weekly discontinuity to produce an equivalent average dose for continuous exposure. No dose adjustments were made for duration of exposure or a less-than-lifetime study, as is typically done for cancer risk estimates, though in deriving the

candidate reference values, an UF for subchronic-to-chronic exposure was applied where appropriate.

For human occupational studies, inhalation exposures (air concentrations) were adjusted by the number of work (vs. nonwork) days and the amount of air intake during working hours as a fraction of the entire day (10 m^3 during work/20 m^3 for entire day). For the TCE ppm in air converted from urinary metabolite data using Ikeda et al. (1972), the work week was 6 days, so the adjustment for number of work days is 6/7.

F.2.3. Estimation of the Applied Doses for the Oral Exposures via Drinking Water and Feed

When oral doses were not reported in mg/kg/day and when study-specific data were not available for body weight and/or consumption rate, standard generic sex/strain-specific values from U.S. EPA (1988) were used to convert doses (e.g., in ppm in water) to doses in mg/kg/day.

For the feed study of George et al. (1986), study-specific data were used to estimate the applied dose. Female F334 rats were exposed for 19 weeks in their feed. Average body weights (W_t) are reported (Table A2, p. 53) for time periods having durations (d_t) of 1–4 weeks. Proportions of the 19 weeks of feeding were calculated for each time period as

$$Pt = dt/(\sum_t dt)$$

Average daily feed consumed (F_t) is reported (Table A3) for the same time periods as body weight. Concentration (%w/w) of TCE in feed (Table 1, p.31) is reported for weeks 1, 6, 12, and 18.[13] Two determinations are reported, which we averaged. The grouping of TCE feed concentrations into time periods (Table 1) differs from that used for body weight and feed consumption (Tables A2, A3). This was reconciled by linear interpolation of feed concentrations to produce concentrations (denoted C_t) for the time periods presented in Tables A2 and A3. We then calculated mg TCE consumed per kg-day, for each time period, as the product of:

$C_t/100$	feed concentration, %w/w, divided by 100 to give a fraction
F_t	feed consumed (grams)
1,000	1,000 (conversion of grams to mg)
$1/W_t$	1/[body weight, kg]

And found the TWA of these for each dose group:

[13]"Study Week 1" is repeated in the table, which is a typo for week 6, confirmed positively by the text on pages 19–20: "Analysis of Task 2 feed formulations at six week intervals … Similarly, during week 6 of Task 2, the 0.15%, 0.30%, and 0.60% TCE formulations assayed at 27%, 71% and 82% of the theoretical concentration, respectively (Table 1)".

$$\sum_t \{Pt \times ((Ct \times Ft \times 1000)/Wt)\}$$

The results were:

Nominal %w/w concentration in feed	Calculated mg/kg/day
0	0
0.15	72
0.30	186
0.60	389

F.2.4. PBPK Model-Based Internal Dose-Metrics

PBPK modeling was used to estimate levels of dose-metrics corresponding to different exposure scenarios in rodents and humans (see Section 3.5). The selection of dose-metrics for specific organs and endpoints is discussed under Section 5.1.

The PBPK model requires an average body weight. For most of the studies, averages specific to each species, strain, and sex were used. Where these were not reported in the text of an article, data were obtained by digitizing the body weight graphics (Maltoni et al., 1986) or by finding the median of weekly averages from graphs (NTP, 1990, 1988; NCI, 1976). Where necessary, default adult body weights specific to the strain were used (U.S. EPA, 1988).

F.3. DOSE-RESPONSE MODELING PROCEDURES

Where adequate dose-response data were available, models were fitted with the BMDS (http://www.epa.gov/ncea/bmds) using the applicable applied doses or PBPK model-based dose-metrics for each combination of study, species, strain, sex, endpoints, and BMR under consideration.

F.3.1. Models for Dichotomous Response Data

F.3.1.1. Quantal Models

For dichotomous responses, the log-logistic, multistage, and Weibull models were fitted. These models adequately describe the dose-response relationship for the great majority of data sets, specifically in past TCE studies (Filipsson and Victorin, 2003). If the slope parameter of the log-logistic model was <1, indicating a supralinear dose-response shape, then the model with the slope constrained to 1 was also fitted for comparison. For the multistage model, an order one less than the number of dose groups was used, in addition to the 2nd-order multistage model if it differed from the preceding model, and the first-order ('linear') multistage model (which is

identical to a Weibull model with power parameter equal to 1). The Weibull model with the power parameter unconstrained was also fitted t.

F.3.1.2. Nested Dichotomous Models

In addition, nested dichotomous models were used for developmental effects in rodent studies to account for possible litter effects, such maternal covariates or intralitter correlation. The available nested models in BMDS are the nested log-logistic model, the Rai-VanRyzin models, and the NCTR model. Candidates for litter-specific covariates (LSC) were identified from the studies and considered legitimate for analysis if they were not significantly dose-related (determined via regression, ANOVA). The need for a LSC was indicated by a difference of at least 3 in the AIC for models with and without a LSC. The need to estimate intralitter correlations (IC) was determined by presence of a high correlation coefficient for at least one dose group and by AIC. The fits for nested models were also compared with the results from quantal models.

F.3.2. Models for Continuous Response Data

For continuous responses, the distinct models available in BMDS were fitted: power model (power parameter unconstrained and constrained to ≥1), polynomial model, and Hill model. Both constant variance and modeled variance models were fit; but constant variance models were used for model parsimony unless the *p*-value for the test of homogenous variance was <0.10, in which case the modeled variance models were considered. For the polynomial model, model order was selected as follows. A model of order 1 was fitted first. The next higher order model (up to order $n-1$) was accepted if AIC decreased >3 units and the *p*-value for the mean did not decrease.

F.3.3. Model Selection

After fitting these models to the data sets, the recommendations for model selection set out in U.S. EPA's *Benchmark Dose Technical Guidance Document* (External Review Draft, (U.S. EPA, 2000b) were applied. First, models were generally rejected if the *p*-value for goodness of fit was <0.10. In a few cases in which none of the models fit the data with $p > 0.10$, linear models were selected on the basis of an adequate visual fit overall. Second, models were rejected if they did not appear to adequately fit the low-dose region of the dose-response relationship, based on an examination of graphical displays of the data and scaled residuals. If the BMDL estimates from the remaining models were "sufficiently close" (a criterion of within twofold for "sufficiently close" was used), then the model with the lowest AIC was selected. The AIC is a measure of information loss from a dose-response model that can be used to compare a set of models. Among a specified set of models, the model with the lowest AIC is considered the "best." If two or more models share the lowest AIC, the draft *Benchmark Dose*

Technical Guidance Document (U.S. EPA, 2000b) suggests that an average of the BMDLs could be used, but averaging was not used in this assessment (for the one occasion in which models shared the lowest AIC, a selection was made based on visual fit). If the BMDL estimates from the remaining models are not sufficiently close, some model dependence is assumed. With no clear biological or statistical basis to choose among them, the lowest BMDL was chosen as a reasonable conservative estimate, as suggested in the draft *Benchmark Dose Technical Guidance Document*, unless the lowest BMDL appeared to be an outlier, in which case further judgments were made.

F.3.4. Additional Adjustments for Selected Data Sets

In a few cases, the dose-response data necessitated further adjustments in order to improve model fits.

The behavioral/neurological endpoint "number of rears" from Moser et al. (1995) consisted of counts, measured at five doses and four measurement times (with eight observations each). The high dose for this endpoint was dropped because the mean was zero, and no monotone model could fit that well. Analysis of means and SDs for these counts suggested a Box-Cox power transform (Box et al., 1978) of 0.5 (i.e., square root) to stabilize variances (i.e., the slope of the regression of log[SD] on log[mean] was 0.46, and the relation was linear and highly significant). This information was helpful in selecting a suitable variance model with high confidence (i.e., variance constant, for square-root transformed data). Thus, the square root was taken of the original individual count data, and the mean and variance of the transformed count data were used in the BMD modeling.

The high-dose group was dropped due to supra-linear dose-response shapes in two cases: fetal cardiac malformations from Johnson et al. (2003) and decreased PFC response from Woolhiser et al. (2006). Johnson et al. (2003) is discussed in more detail below (see Section F.4.2.1). For Woolhiser et al. (2006), model fit near the BMD and the lower doses as well as the model fit to the variance were improved by dropping the highest dose, a procedure suggested in U.S. EPA (2000b).

In some cases, the supralinear dose-response shape could not be accommodated by these measures, and a LOAEL or NOAEL was used instead. These include NCI (1976) (toxic nephrosis, >90% response at lowest dose), Keil (2009) (autoimmune markers and decreased thymus weight, only two dose groups in addition to controls), and Peden-Adams et al. (2006) (developmental immunotoxicity, only two dose groups in addition to controls).

F.4. DOSE-RESPONSE MODELING RESULTS

F.4.1. Quantal Dichotomous and Continuous Modeling Results

Supplementary data files show the fitted model curves ("Supplementary data for TCE assessment: Non-cancer plots contin," 2011; "Supplementary data for TCE assessment: Non-

cancer plots dichot," 2011). The graphics include observations (group means or proportions), the estimated model curve (solid red line), and estimated BMD, with a BMDL. Vertical bars show 95% CIs for the observed means. Printed above each plot are some key statistics (necessarily rounded) for model goodness of fit and estimated parameters. Printed in the plots in the upper left are the BMD and BMDL for the rodent data, in the same units as the rodent dose.

More detailed results, including alternative BMRs, alternative dose-metrics, quantal analyses for endpoints for which nested analyses were performed, etc. are documented in the several spreadsheets. Input data for the analyses are in other supplementary data files ("Supplementary data for TCE assessment: Non-cancer input data contin," 2011; "Supplementary data for TCE assessment: Non-cancer input data dichot," 2011). Additional supplementary data files ("Supplementary data for TCE assessment: Non-cancer results contin," 2011; "Supplementary data for TCE assessment: Non-cancer results dichot," 2011) present the data and model summary statistics, including goodness-of-fit measures (χ^2 goodness-of-fit *p*-value, AIC), parameter estimates, BMD, and BMDL. The group numbers "GRP" are arbitrary and are the same as GRP in the plots. Finally, note that not all plots are shown in the documents above, since these spreadsheets include many "alternative" analyses.

F.4.2. Nested Dichotomous Modeling Results

F.4.2.1. Johnson et al. (2003) Fetal Cardiac Defects

F.4.2.1.1. Results using applied dose.

The biological endpoint was frequency of rat fetuses having cardiac defects, as shown in Table F-4. Individual animal data were kindly provided by Dr. Johnson (personal communication from Paula Johnson, University of Arizona, to Susan Makris, U.S. EPA, 26 August 2009). Cochran-Armitage trend tests using number of fetuses and number of litters indicated significant increases in response with dose (with or without including the highest dose).

One suitable candidate for a LSC was available: female weight gain during pregnancy. Based on goodness of fit, this covariate did not contribute to better fit and was not used. Some ICs were significant and these parameters were included in the model.

Table F-4. Data on fetuses and litters with abnormal hearts from Johnson et al. (2003)

Dose group (mg/kg/d):	0	0.00045	0.048	0.218	129
Fetuses					
Number of pups:	606	144	110	181	105
Abnormal heart:	13	0	5	9	11
Litters					
Number of litters:	55	12	9	13	9
Abnormal heart:	9	0	4	5	6

With the high dose included, the χ^2 goodness of fit was acceptable, but some residuals were large (1.5 to 2) for the control and two lower doses. Therefore, models were also fitted after dropping the highest dose. For these, goodness of fit was adequate, and scaled residuals were smaller for the low doses and control. Predicted expected response values were closer to observed when the high dose was dropped, as shown in Table F-5:

Table F-5. Comparison of observed and predicted numbers of fetuses with abnormal hearts from Johnson et al. (2003), with and without the high-dose group, using a nested model

	Abnormal hearts (pups)				
Dose group (mg/kg/d):	**0**	**0.00045**	**0.048**	**0.218**	**129**
Observed:	13	0	5	9	11
Predicted expected:					
With high dose	19.3	4.5	3.5	5.7	11
Without high dose	13.9	3.3	3.4	10	–

Accuracy in the low-dose range is especially important because the BMD is based upon the predicted responses at the control and the lower doses. Based on the foregoing measures of goodness of fit, the model based on dropping the high dose was used.

The nested log-logistic and Rai-VanRyzin models were fitted; these gave essentially the same predicted responses and POD. The former model was used as the basis for a POD; results are in Table F-6 and Figure F-2.

Table F-6. Results of nested log-logistic model for fetal cardiac anomalies from Johnson et al. (2003) without the high-dose group, on the basis of applied dose (mg/kg/day in drinking water)

Model	LSC?[a]	IC?	AIC	Pval	BMR	BMD	BMDL
NLOG	Y	Y	246.877	NA (df = 0)	0.01	0.252433	0.03776
NLOG	Y	N	251.203	0.0112	0.01	0.238776	0.039285
NLOG	N	N	248.853	0.0098	0.01	0.057807	0.028977
NLOG	N	Y	243.815	0.0128	0.1	0.71114	0.227675
NLOG	N	Y	243.815	0.0128	0.05	0.336856	0.107846
NLOG[b]	**N**	**Y**	**243.815**	**0.0128**	**0.01**	**0.064649**	**0.020698**

[a]LSC analyzed was female weight gain during pregnancy.
[b]Indicates model selected (Rai-VanRyzin model fits are essentially the same).

NLOG = "nested log-logistic" model

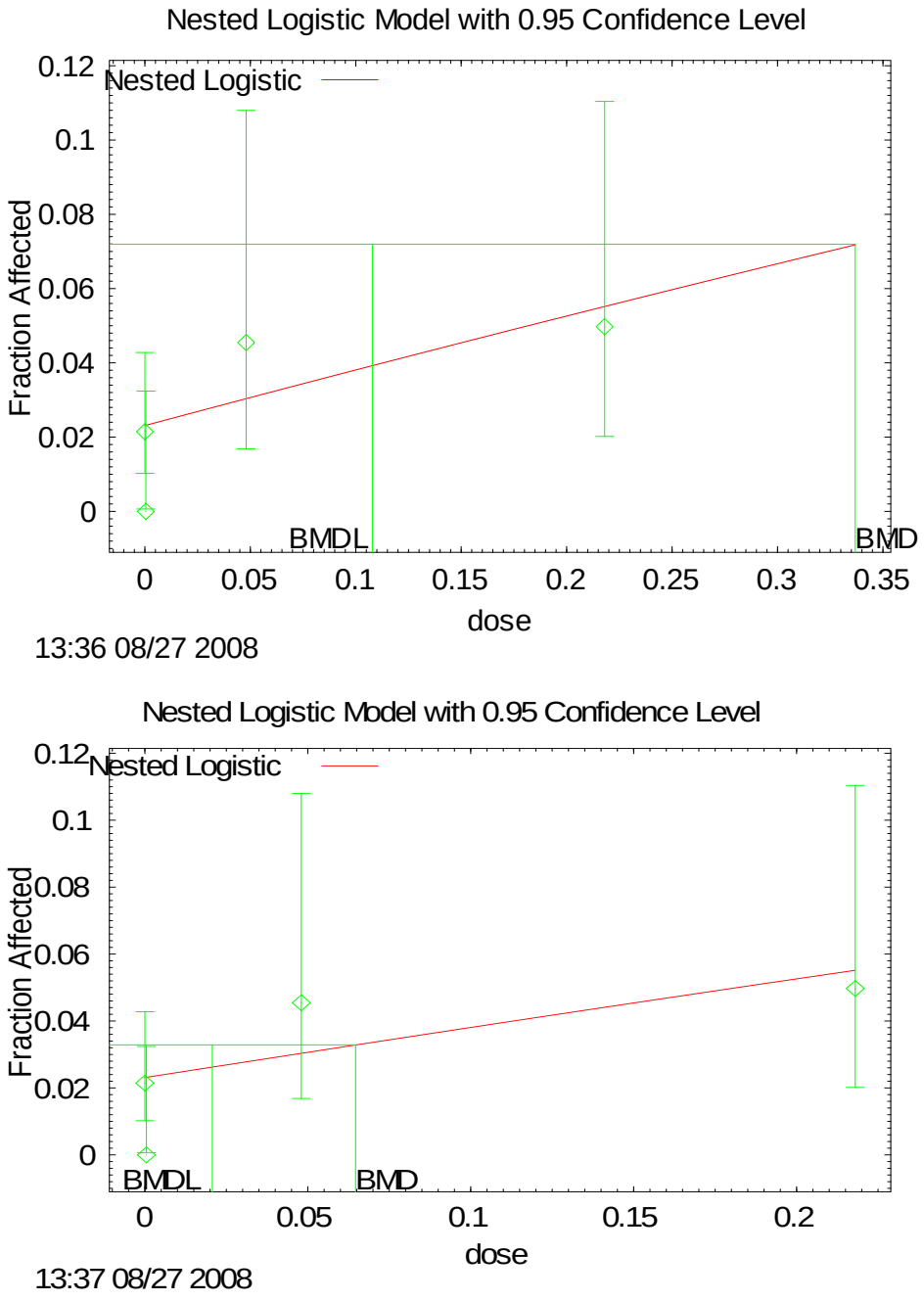


Figure F-2. BMD modeling of Johnson et al. (2003) using nested log-logistic model, with applied dose, without LSC, with IC, and without the high-dose group, using a BMR of 0.05 extra risk (top panel) or 0.01 extra risk (bottom panel).

F.4.2.1.2. χ^2 Goodness-of-Fit Test for nested log-logistic model.

The BMDS choice of subgroups did not seem appropriate given the data. The high-dose group of 13 litters was subdivided into three subgroups having sums of expected counts 3, 3, and 2. However, the control group of 55 litters could have been subdivided because expected

response rates for controls were relatively high. There was also concern that the goodness of fit might change with alternative choices of subgroupings.

An R program was written to read the BMDS output, reading parameters and the table of litter-specific results (dose, covariate, estimated probability of response, litter size, expected response count, observed response count, scaled χ^2 residual). The control group of 55 litters was subdivided into three subgroups of 18, 18, and 19 litters. Control litters were sampled randomly without replacement 100 times, each time creating 3 subgroups (i.e., 100 random assignments of the 55 control litters to three subgroups were made). For each of these, the goodness-of-fit calculation was made and the *p*-value saved. Within these 100 *p*-values, ≥75% were ≥0.05 and ≥50% had *p*-values ≥0.11; this indicated that the model is acceptable based on goodness-of-fit criteria.

F.4.2.1.3. **Results using PBPK model-based dose-metrics.**

The nested log-logistic model was also run using the dose-metrics in the dams of total oxidative metabolism scaled by body weight to the ¾-power (TotOxMetabBW34) and the AUC of TCE in blood (AUCCBld). As with the applied dose modeling, LSC (maternal weight gain) was not included, but IC was included, based on the criteria outlined previously (see Section F.3.1.2). The results are summarized in Table F-7 and Figure F-3 for TotOxMetabBW34 and Table F-8 and Figure F-4 for AUCCBld.

Table F-7. Results of nested log-logistic model for fetal cardiac anomalies from Johnson et al. (2003) without the high-dose group, using the TotOxMetabBW34 dose-metric

Model	LSC?[a]	IC?	AIC	Pval	BMR	BMD	BMDL
NLOG	Y	Y	246.877	NA (df = 0)	0.01	0.174253	0.0259884
NLOG	Y	N	251.203	0.0112	0.01	0.164902	0.0270378
NLOG	N	Y	243.815	0.0128	0.1	0.489442	0.156698
NLOG[b]	**N**	**Y**	**243.815**	**0.0128**	**0.01**	**0.0444948**	**0.0142453**
NLOG	N	N	248.853	0.0098	0.01	0.0397876	0.0199438

[a]LSC analyzed was female weight gain during pregnancy.
[b]Indicates model selected. BMDS failed with the Rai-VanRyzin and NCTR models.

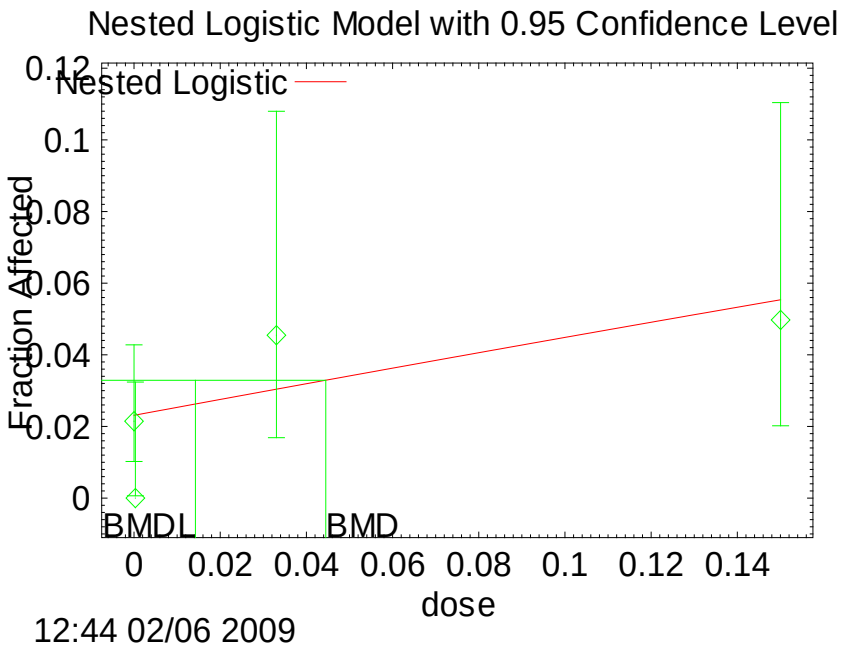


Figure F-3. BMD modeling of Johnson et al. (2003) using nested log-logistic model, with TotOxMetabBW34 dose-metric, without LSC, with IC, and without the high-dose group, using a BMR of 0.01 extra risk.

Table F-8. Results of nested log-logistic model for fetal cardiac anomalies from Johnson et al. (2003) without the high-dose group, using the AUCCBld dose-metric

Model	LSC?[a]	IC?	AIC	Pval	BMR	BMD	BMDL
NLOG	Y	Y	246.877	NA (df = 0)	0.01	0.00793783	0.00118286
NLOG	Y	N	251.203	0.0112	0.01	0.00750874	0.00123047
NLOG[b]	N	Y	243.816	0.0128	0.1	0.0222789	0.00712997
NLOG[b]	**N**	**Y**	**243.816**	**0.0128**	**0.01**	**0.00202535**	**0.000648179**
NLOG	N	N	248.853	0.0098	0.01	0.00181058	0.000907513

[a]LSC analyzed was female weight gain during pregnancy.
[b]Indicates model selected. BMDS failed with the Rai-VanRyzin and NCTR models.

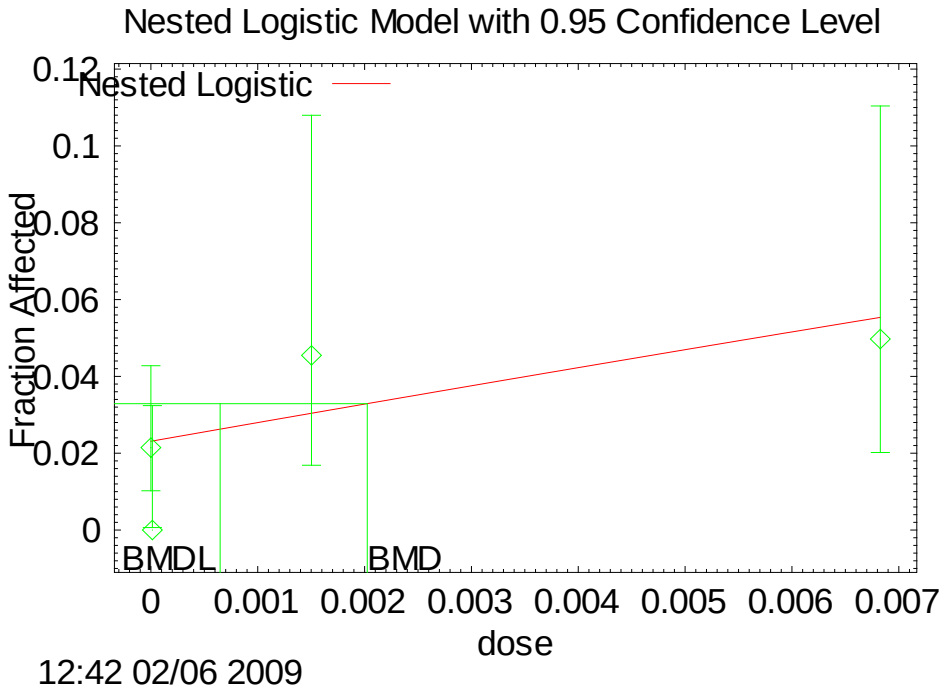


Figure F-4. BMD modeling of Johnson et al. (2003) using nested log-logistic model, with AUCCBld dose-metric, without LSC, with IC, and without the high-dose group, using a BMR of 0.01 extra risk.

F.4.2.2. Narotsky et al. (1995)

Data were combined for the high doses in the single-agent experiment and the lower doses in the 'five-cube' experiment. Individual animal data were kindly provided by Dr. Narotsky (personal communications from Michael Narotsky, U.S. EPA, to John Fox, U.S. EPA, 19 June 2008, and to Jennifer Jinot, U.S. EPA, 10 June 2008). Two endpoints were examined: frequency of eye defects in rat pups and prenatal loss (number of implantation sites minus number of live pups on PND 1).

Two LSCs were considered, with analyses summarized in Table F-9. The number of implants is unrelated to dose, as inferred from regression and ANOVA, and was considered as a LSC for eye defects. As number of implants is part of the definition for the endpoint of prenatal loss, it is not considered as a LSC for prenatal loss. A second LSC, the dam body weight on GD 6 (damBW6) was significantly related to dose and is unsuitable as a litter-specific covariate.

Table F-9. Analysis of LSCs with respect to dose from Narotsky et al. (1995)

Relation of litter-specific covariates to dose			
Implants:	**none**		
damBW6:	**significant**		
		Mean	**Mean**
	TCE	Implants	damBW6
	0	9.5	176.0
	10.1	10.1	180.9
	32	9.1	174.9
	101	7.8	170.1
	320	10.4	174.5
	475	9.7	182.4
	633	9.6	185.3
	844	8.9	182.9
	1,125	9.6	184.2
Using expt as covariate, e.g., damBW6 ~ TCE.mg.kgd + expt			
Linear regression		$p = 0.7486$	$p = 0.0069$
AoV (ordered factor)		$p = 0.1782$	$p = 0.0927$

Two LSCs were considered, with analyses summarized in Table F-9. The number of implants is unrelated to dose, as inferred from regression and ANOVA, and was considered as a LSC for eye defects. As number of implants is part of the definition for the endpoint of prenatal loss, it is not considered as a LSC for prenatal loss. A second LSC, the dam body weight on GD 6 (damBW6) was significantly related to dose and is unsuitable as a litter-specific covariate.

F.4.2.2.1. Fetal eye defects

The nested log-logistic and Rai-VanRyzin models were fitted to the number of pups with eye defects reported by Narotsky et al. (1995), with the results summarized in Table F-10.

Table F-10. Results of nested log-logistic and Rai-VanRyzin model for fetal eye defects from Narotsky et al. (1995), on the basis of applied dose (mg/kg/day in drinking water)

Model	LSC?[a]	IC?	AIC	Pval	BMR	BMD	BMDL
NLOG	Y	Y	255.771	0.3489	0.05	875.347	737.328[b]
NLOG	Y	N	259.024	0.0445	0.05	830.511	661.629
NLOG	N	Y	270.407	0.2281	0.05	622.342	206.460
NLOG	N	N	262.784	0.0529	0.10	691.93	542.101
NLOG	N	N	262.784	0.0529	0.05	427.389	264.386
NLOG	N	N	262.784	0.0529	0.01	147.41	38.7117[c]
RAI	Y	Y	274.339	0.1047	0.05	619.849	309.925
RAI	Y	N	264.899	0.0577	0.05	404.788	354.961
RAI	N	Y	270.339	0.2309	0.05	619.882	309.941
RAI	N	N	262.481	0.0619	0.10	693.04	346.52
RAI	N	N	262.481	0.0619	0.05	429.686	214.843
RAI	N	N	262.481	0.0619	0.01	145.563	130.938[c]

[a]LSC analyzed was implants.
[b]Graphical fit at the origin exceeds observed control and low-dose responses and slope is quite flat (see Figure F-5), fitted curve does not represent the data well.
[c]Indicates model selected.

RAI = Rai-VanRyzin model

Results for the nested log-logistic model suggested a better model fit with the inclusion of the LSC and IC, based on AIC. However, the graphical fit (see Figure F-5) is strongly sublinear and high at the origin where the fitted response exceeds the observed low-dose responses for the control group and two low-dose groups. An alternative nested log-logistic model without either LSC or IC (see Figure F-6), which fits the low-dose responses better, was selected. Given that this model had no LSC and no IC, the nested log-logistic model reduces to a quantal log-logistic model. Parameter estimates and the *p*-values were essentially the same for the two models (see Table F-11). A similar model selection can be justified for the Rai-Van Ryzin model (see Figure F-7). Because no LSC and no IC were needed, this endpoint was modeled with quantal models, using totals of implants and losses for each dose group, which allowed choice from a wider range of models (those results appear with quantal model results in this appendix).

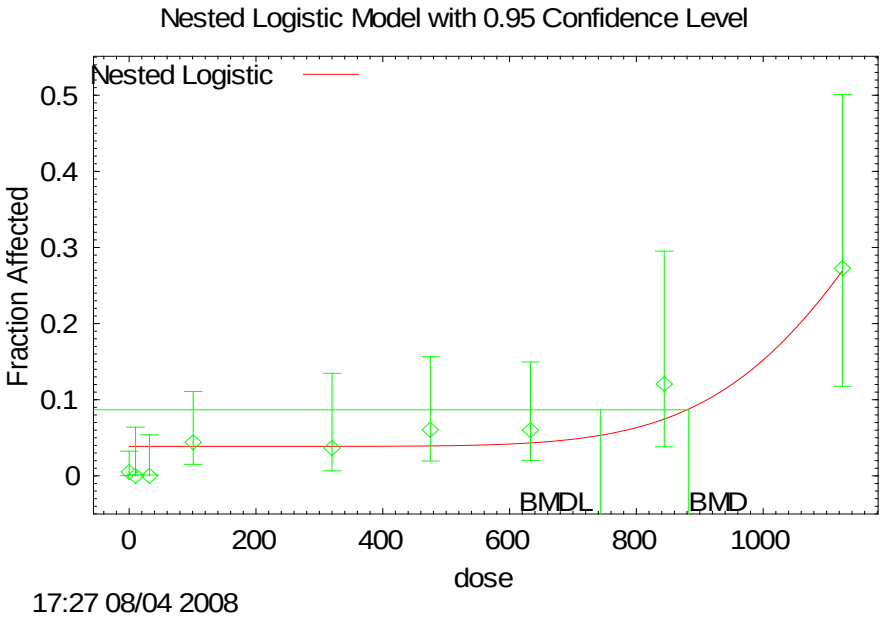


Figure F-5. BMD modeling of fetal eye defects from Narotsky et al. (1995) using nested log-logistic model, with applied dose, with both LSC and IC, using a BMR of 0.05 extra risk.

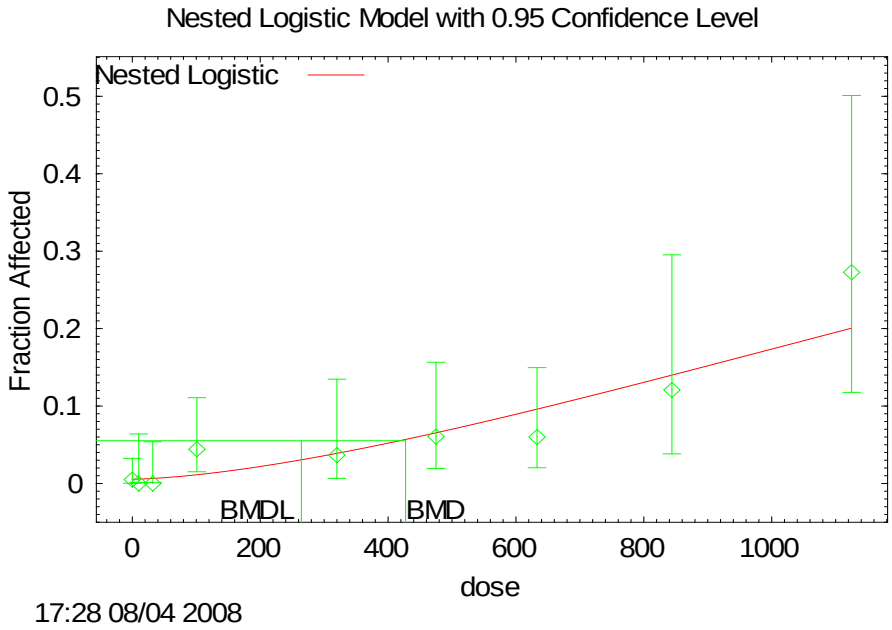


Figure F-6. BMD modeling of fetal eye defects from Narotsky et al. (1995) using nested log-logistic model, with applied dose, without either LSC or IC, using a BMR of 0.05 extra risk.

Table F-11. Comparison of results of nested log-logistic (without LSC or IC) and quantal log-logistic model for fetal eye defects from Narotsky et al. (1995)

Model	Parameter			BMD_{05}	$BMDL_{05}$
	Alpha	Beta	Rho		
Nested	0.00550062	-12.3392	1.55088	427.4	264.4
Quantal	0.00549976	-12.3386	1.55079	427.4	260.2

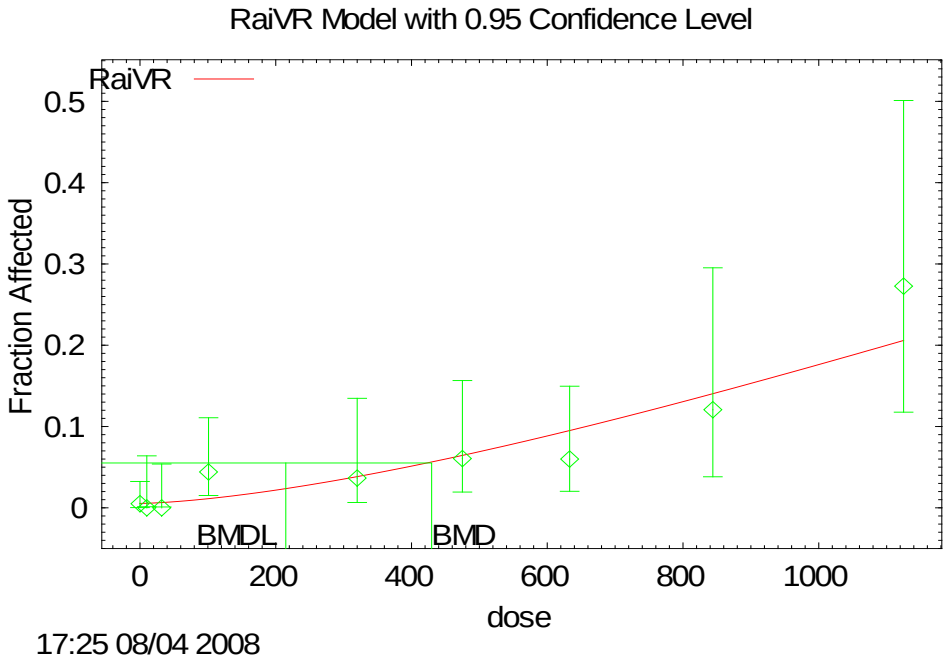


Figure F-7. BMD modeling of fetal eye defects from Narotsky et al. (1995) using nested Rai-VanRyzin model, with applied dose, without either LSC or IC, using a BMR of 0.05 extra risk.

F.4.2.2.2. Narotsky et al. (1995) prenatal loss

The nested log-logistic and Rai-VanRyzin models were fitted to prenatal loss reported by Narotsky et al. (1995), with the results summarized in Table F-12.

Table F-12. Results of nested log-logistic and Rai-VanRyzin model for prenatal loss from Narotsky et al. (1995), on the basis of applied dose (mg/kg/day in drinking water)

Model	LSC?[a]	IC?	AIC	Pval	BMR	BMD	BMDL
NLOG	Y	Y	494.489	0.2314	0.10	799.723	539.094
NLOG	Y	N	627.341	0.0000	0.10	790.96	694.673
NLOG	N	N	628.158	0.0000	0.10	812.92	725.928
NLOG	N	Y	490.766	0.2509	0.10	814.781	572.057
NLOG	N	Y	490.766	0.2509	0.05	738.749	447.077
NLOG	**N**	**Y**	**490.766**	**0.2509**	**0.01**	**594.995**	**252.437[b]**
RAI	Y	Y	491.859	0.3044	0.10	802.871	669.059
RAI	Y	N	626.776	0.0000	0.10	819.972	683.31
RAI	N	N	626.456	0.0000	0.10	814.98	424.469
RAI	N	Y	488.856	0.2983	0.10	814.048	678.373
RAI	N	Y	488.856	0.2983	0.05	726.882	605.735
RAI	**N**	**Y**	**488.856**	**0.2983**	**0.01**	**562.455**	**468.713[b]**

[a]LSC analyzed was dam body weight on GD 6.
[b]Indicates model selected.

The BMDS nested models require a LSC, so dam body weight on GD6 ("damBW6") was used as the LSC. However, damBW6 is significantly related to dose and, so, is not a reliable LSC. Number of implants could not be used as a LSC because it was identified as number at risk in the BMDS models. These issues were obviated because the model selected did not employ the LSC.

For the nested log-logistic models, the AIC is much larger when the IC is dropped, so the IC is needed in the model. The LSC can be dropped (and is also suspect because it is correlated with dose). The model with IC and without LSC was selected on the basis of AIC (shown in Figure F-8). For the Rai-VanRyzin models, the model selection was similar to that for the nested log-logistic, leading to a model with IC and without LSC, which had the lowest AIC (shown in Figure F-9).

F.4.3. Model Selections and Results

The final model selections and results for noncancer dose-response modeling are presented in Table F-13.

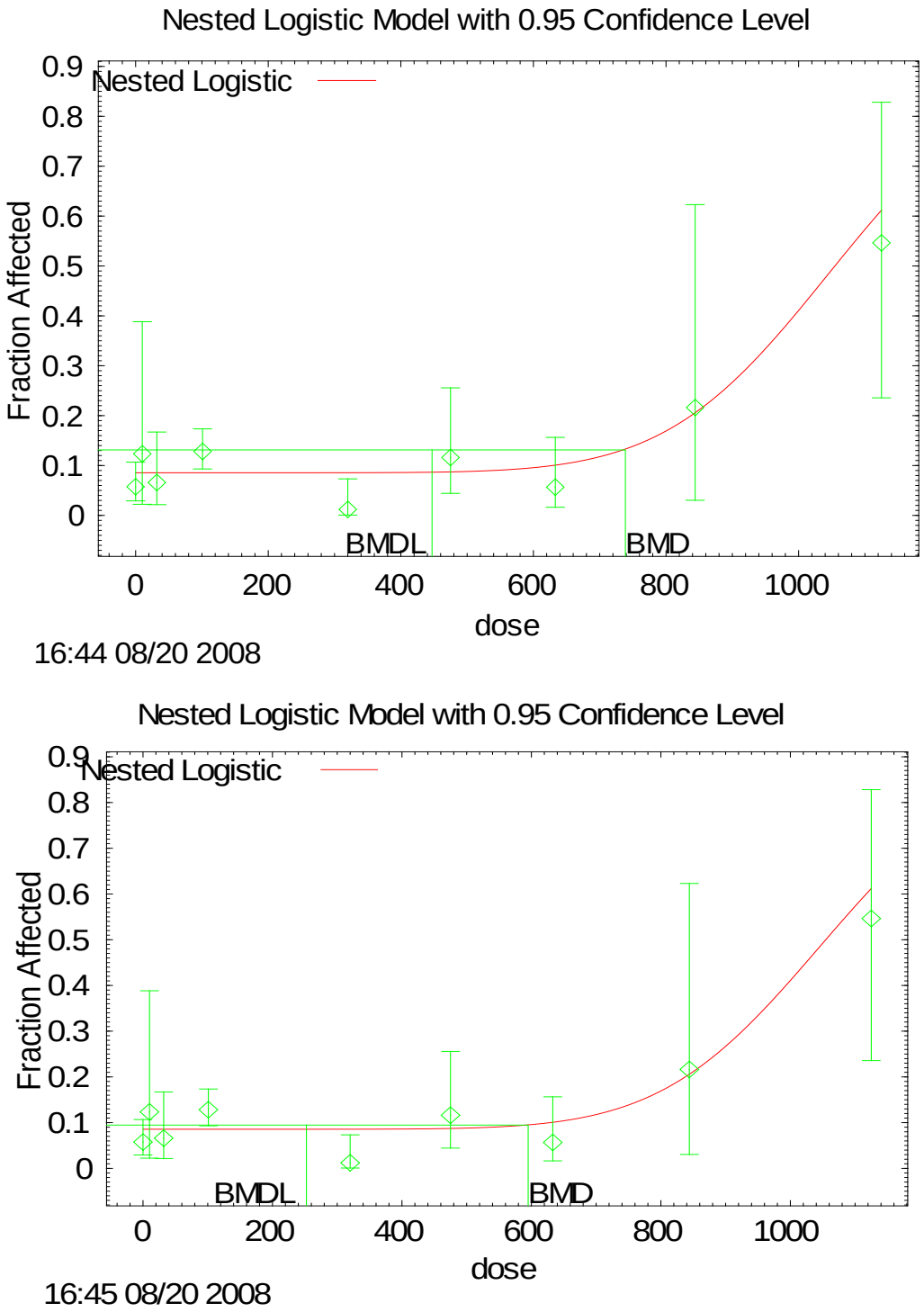
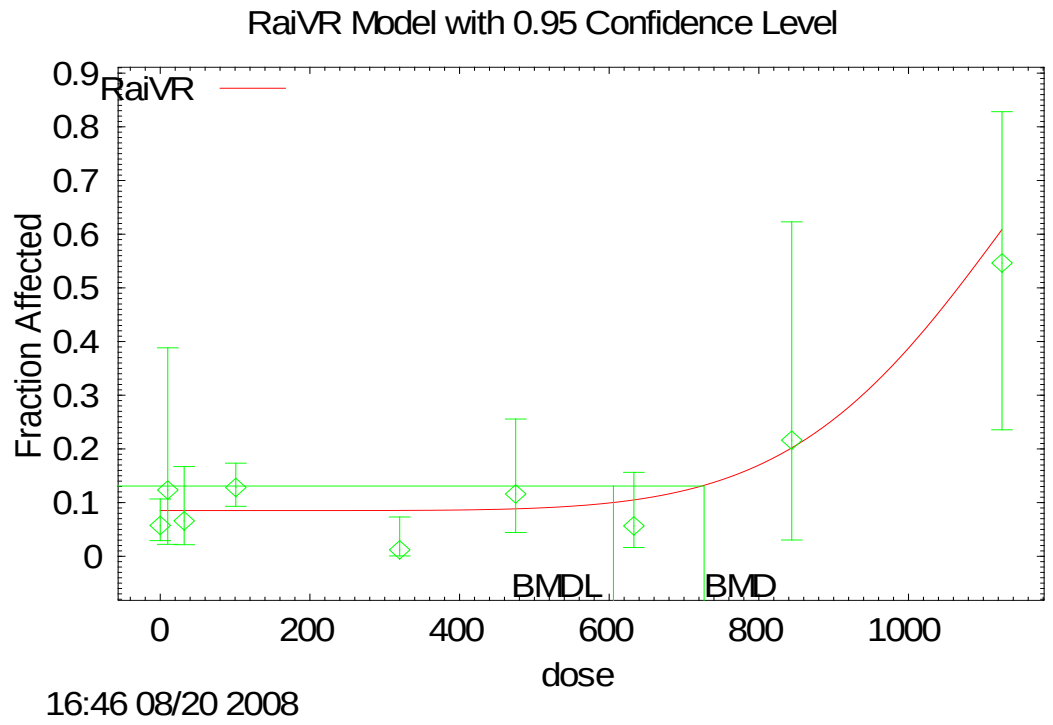


Figure F-8. BMD modeling of prenatal loss reported in Narotsky et al. (1995) using nested log-logistic model, with applied dose, without LSC, with IC, using a BMR of 0.05 extra risk (top panel) or 0.01 extra risk (bottom panel).



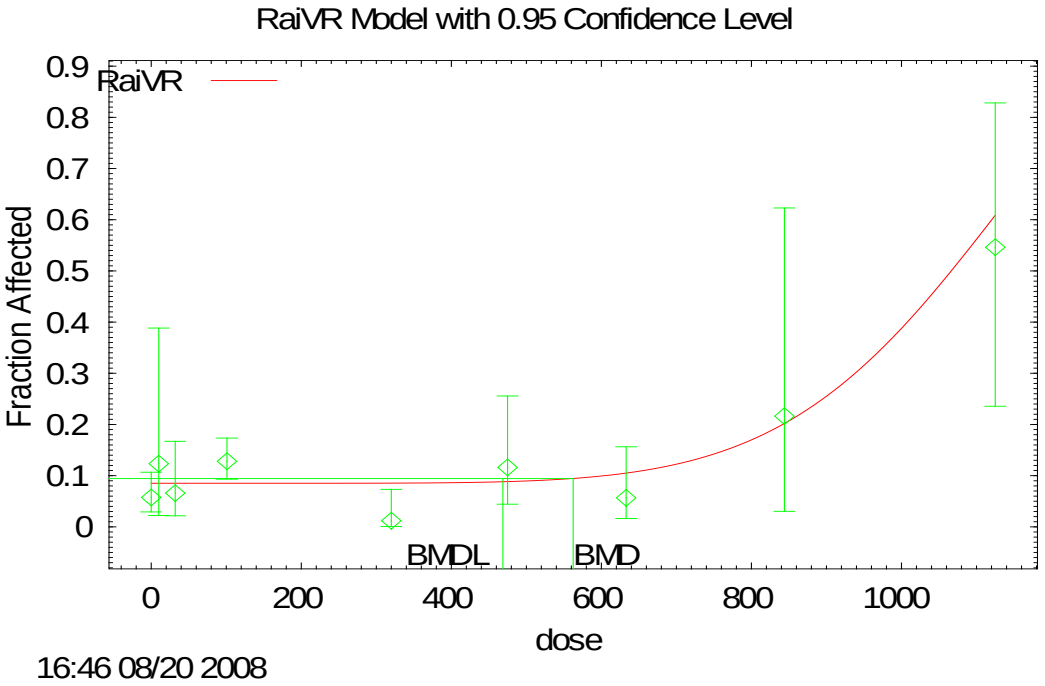


Figure F-9. BMD modeling of prenatal loss reported in Narotsky et al. (1995) using nested Rai-VanRyzin model, with applied dose, without LSC, with IC, using a BMR of 0.05 extra risk (top panel) or 0.01 extra risk (bottom panel).

Table F-13. Model selections and results for noncancer dose-response analyses

GRP	Study/run abbrev.	Species	Sex	Strain	Exposure route	Endpoint	Dose-metric	BMR type	BMR	BMD/ BMDL	BMDL	Model	Rep. BMD	Notes
Dichotomous models														
3	Chia et al. (1996)	Human	M	workers.elec.factory	inhal	N.hyperzoospermia	appl.dose	extra	0.1	2.14	1.43	loglogistic.1	3.06	
7	Narotsky et al. (1995)	Rat	F	F344	oral.gav	N.pups.eye.defects	appl.dose	extra	0.01	1.46	60.1	multistage	806	a
13	Narotsky et al. (1995)	Rat	F	F344	oral.gav	N.dams.w.resorbed.litters	appl.dose	extra	0.01	5.47	32.2	multistage.2	570	
13	Narotsky et al. (1995)	Rat	F	F344	oral.gav	N.dams.w.resorbed.litters	AUCCBld	extra	0.01	5.77	17.5	multistage.2	327	
13	Narotsky et al. (1995)	Rat	F	F344	oral.gav	N.dams.w.resorbed.litters	TotMetabBW34	extra	0.01	1.77	77.5	weibull	156	
14	Johnson et al. (2003).drophi	Rat	F	Sprague.Dawley	oral.dw	N.litters.abnormal.hearts	appl.dose	extra	0.1	2.78	0.0146	loglogistic.1	0.0406	b
36	Griffin et al. (2000b)	mice	F	MRL++	oral.dw	portal.infiltration	appl.dose	extra	0.1	2.67	13.4	loglogistic.1	35.8	
38	Maltoni et al. (1986)	Rat	M	Sprague.Dawley	inhal	megalonucleocytosis	appl.dose	extra	0.1	1.22	40.2	multistage	49.2	c
38	Maltoni et al. (1986)	Rat	M	Sprague.Dawley	inhal	megalonucleocytosis	ABioactDCVCBW34	extra	0.1	1.18	0.0888	loglogistic	0.105	
38	Maltoni et al. (1986)	Rat	M	Sprague.Dawley	inhal	megalonucleocytosis	AMetGSHBW34	extra	0.1	1.19	0.086	loglogistic	0.102	
38	Maltoni et al. (1986)	Rat	M	Sprague.Dawley	inhal	megalonucleocytosis	TotMetabBW34	extra	0.1	1.13	53.8	weibull	61	d
39	Maltoni et al. (1986)	Rat	M	Sprague.Dawley	oral.gav	megalonucleocytosis	appl.dose	extra	0.1	1.53	33.8	multistage.2	51.8	e
39	Maltoni et al. (1986)	Rat	M	Sprague.Dawley	oral.gav	megalonucleocytosis	ABioactDCVCBW34	extra	0.1	1.60	0.0594	multistage.2	0.0948	
39	Maltoni et al. (1986)	Rat	M	Sprague.Dawley	oral.gav	megalonucleocytosis	AMetGSHBW34	extra	0.1	1.65	0.0605	multistage.2	0.0977	
39	Maltoni et al. (1986)	Rat	M	Sprague.Dawley	oral.gav	megalonucleocytosis	TotMetabBW34	extra	0.1	1.41	20.5	multistage.2	29	e
49	NTP (1988)	Rat	F	Marshall	oral.gav	toxic nephropathy	appl.dose	extra	0.05	1.45	9.45	loglogistic.1	28.9	
49	NTP (1988)	Rat	F	Marshall	oral.gav	toxic nephropathy	ABioactDCVCBW34	extra	0.05	1.45	0.0132	loglogistic.1	0.0404	
49	NTP (1988)	Rat	F	Marshall	oral.gav	toxic nephropathy	AMetGSHBW34	extra	0.05	1.46	0.0129	loglogistic.1	0.0397	
49	NTP (1988)	Rat	F	Marshall	oral.gav	toxic nephropathy	TotMetabBW34	extra	0.05	1.45	2.13	loglogistic.1	6.5	

Table F-13. Model selections and results for noncancer dose-response analyses (continued)

GRP	Study/run abbrev.	Species	Sex	Strain	Exp. route	Endpoint	Dose-metric	BMR type	BMR	BMD/BMDL	BMDL	Model	Rep. BMD	Notes
Nested dichotomous models														
NA	Johnson et al. (2003).drophi	rat	F	Sprague.Dawley	oral.dw	N.pups.abnormal.hearts	appl.dose	extra	0.01	3.12	0.0207	loglogistic.IC	0.711	b
NA	Johnson et al. (2003).drophi	rat	F	Sprague.Dawley	oral.dw	N.pups.abnormal.hearts	TotOxMetabBW34	extra	0.01	3.12	0.0142	loglogistic.IC		b
NA	Johnson et al. (2003).drophi	rat	F	Sprague.Dawley	oral.dw	N.pups.abnormal.hearts	AUCCBld	extra	0.01	3.12	0.000648	loglogistic.IC		b
NA	Narotsky et al. (1995)	rat	F	F344	oral.gav	N.prenatal.loss	appl.dose	extra	0.01	1.2	469	RAI.IC	814	
Continuous models														
2	Land et al. (1981)	mouse	M	(C57B1xC3H)F1	inhal	pct.abnormal.sperm	appl.dose	standard	0.5	1.33	46.9	polynomial.constvar	125	
6	Carney et al. (2006)	rat	F	Sprague-Dawley (Crl:CD)	inhal	gm.wgt.gain.GD6.9	appl.dose	relative	0.1	2.5	10.5	hill	62.3	
8	Narotsky et al. (1995)	rat	F	F344	oral.gav	gm.wgt.gain.GD6.20	appl.dose	relative	0.1	1.11	108	polynomial.constvar	312	
19	Crofton and Zhao (1997)	rat	M	Long-Evans	inhal	dB.auditory.threshold(16kHz)	appl.dose	absolute	10	1.11	274	polynomial.constvar	330	
21	George et al. (1986)	rat	F	F344	oral.food	litters	appl.dose	standard	0.5	1.69	179	polynomial.constvar	604	
23	George et al. (1986)	rat	F	F344	oral.food	live.pups	appl.dose	standard	0.5	1.55	152	polynomial.constvar	470	
26	George et al. (1986)	rat	F	F344	oral.food	Foffspring.BWgm.day21	appl.dose	relative	0.05	1.41	79.7	polynomial.constvar	225	

Table F-13. Model selections and results for noncancer dose-response analyses (continued)

GRP	Study/run abbrev.	Species	Sex	Strain	Exp. route	Endpoint	Dose-metric	BMR type	BMR	BMD/BMDL	BMDL	Model	Rep. BMD	Notes
34sq	Moser et al. (1995)+perscom	rat	F	F344	oral.gav	no.rears	appl.dose	standard	1	1.64	248	polynomial.constvar	406	b,f
49	George et al. (1986)	rat	F	F344	oral.food	traverse.time.21do	appl.dose	relative	1	1.98	72.6	power	84.9	
51	Buben and O'Flaherty (1985)	mouse	M	SwissCox	oral.gav	Liverwt.pctBW	appl.dose	relative	0.1	1.26	81.5	hill.constvar	92.8	
51	Buben and O'Flaherty (1985)	mouse	M	SwissCox	oral.gav	Liverwt.pctBW	AMetLiv1BW34	relative	0.1	1.08	28.6	polynomial.constvar	28.4	
51	Buben and O'Flaherty (1985)	mouse	M	SwissCox	oral.gav	Liverwt.pctBW	TotOxMetabBW34	relative	0.1	1.08	37	polynomial.constvar	36.7	
58	Kjellstrand et al. (1983a)	mouse	M	NMRI	inhal	Liverwt.pctBW	appl.dose	relative	0.1	1.36	21.6	hill	30.4	
58	Kjellstrand et al. (1983a)	mouse	M	NMRI	inhal	Liverwt.pctBW	AMetLiv1BW34	relative	0.1	1.4	22.7	hill	32.9	
58	Kjellstrand et al. (1983a)	mouse	M	NMRI	inhal	Liverwt.pctBW	TotOxMetabBW34	relative	0.1	1.3	73.4	hill	97.7	
60.Rp	Kjellstrand et al. (1983a)	mouse	M	NMRI	inhal	Kidneywt.pctBW	appl.dose	relative	0.1	1.17	34.7	polynomial	47.1	
60.Rp	Kjellstrand et al. (1983a)	mouse	M	NMRI	inhal	Kidneywt.pctBW	AMetGSHBW34	relative	0.1	1.18	0.17	polynomial	0.236	
60.Rp	Kjellstrand et al. (1983a)	mouse	M	NMRI	inhal	Kidneywt.pctBW	TotMetabBW34	relative	0.1	1.17	71	polynomial	95.2	
63	Woolhiser et al. (2006)	rat	F	CD (Sprague-Dawley)	inhal	Antibody.Forming Cells	appl.dose	standard	1	1.94	31.2	power.constvar	60.6	b
62	Woolhiser et al. (2006)	rat	F	CD (Sprague-Dawley)	inhal	Antibody.Forming Cells	AUCCBld	standard	1	1.44	149	polynomial	214	

Table F-13. Model selections and results for noncancer dose-response analyses (continued)

GRP	Study/run abbrev.	Species	Sex	Strain	Exp. route	Endpoint	Dose-metric	BMR type	BMR	BMD/BMDL	BMDL	Model	Rep. BMD	Notes
62	Woolhiser et al. (2006)	rat	F	CD (Sprague-Dawley)	inhal	Antibody.Forming Cells	TotMetabBW34	standard	1	1.5	40.8	polynomial	61.3	
65	Woolhiser et al. (2006)	rat	F	CD (Sprague-Dawley)	inhal	kidney.wt.per100gm	appl.dose	relative	0.1	4.29	15.7	hill.constvar	54.3	
65	Woolhiser et al. (2006)	rat	F	CD (Sprague-Dawley)	inhal	kidney.wt.per100gm	ABioactDCVCBW34	relative	0.1	4.27	0.0309	hill.constvar	0.103	
65	Woolhiser et al. (2006)	rat	F	CD (Sprague-Dawley)	inhal	kidney.wt.per100gm	AMetGSHBW34	relative	0.1	4.28	0.032	hill.constvar	0.107	
65	Woolhiser et al. (2006)	rat	F	CD (Sprague-Dawley)	inhal	kidney.wt.per100gm	TotMetabBW34	relative	0.1	1.47	40.8	polynomial.constvar	52.3	
67	Woolhiser et al. (2006)	rat	F	CD (Sprague-Dawley)	inhal	liver.wt.per100gm	appl.dose	relative	0.1	4.13	25.2	hill.constvar	70.3	
67	Woolhiser et al. (2006)	rat	F	CD (Sprague-Dawley)	inhal	liver.wt.per100gm	AMetLiv1BW34	relative	0.1	1.53	46	polynomial.constvar	56.1	
67	Woolhiser et al. (2006)	rat	F	CD (Sprague-Dawley)	inhal	liver.wt.per100gm	TotOxMetabBW34	relative	0.1	1.53	48.9	polynomial.constvar	59.8	

[a]Eight-stage multistage model.
[b]Dropped highest dose.
[c]Three-stage multistage model.
[d]Weibull selected over log-logistic with the same AIC on basis of visual fit (less extreme curvature).
[e]Second-order MS selected on basis of visual fit (less extreme curvature).
[f]Square-root transformation of original individual count data.

Applied dose BMDLs are in units of ppm in air for inhalation exposures and mg/kg/day for oral exposures. Internal dose BMDLs are in dose-metric units. Reporting BMD is BMD using a BMR of 0.1 extra risk for dichotomous models, and 1 control SD for continuous models.
Log-logistic = unconstrained log-logistic; log-logistic.1 = constrained log-logistic; multistage = multistage with #stages=dose groups-1; multistage.n = n-stage multistage; log-logistic.IC = nested log-logistic with IC, without LSC; RAI.IC = Rai-VanRyzin model with IC, without LSC; zzz.constvar = continuous model zzz with constant variance (otherwise variance is modeled).
Rep. = reporting

F.5. DERIVATION OF POINTS OF DEPARTURE

F.5.1. Applied Dose Points of Departure

For oral studies in rodents, the POD on the basis of applied dose in mg/kg/day was taken to be the BMDL, NOAEL, or LOAEL. NOAELs and LOAELs were adjusted for intermittent exposure to their equivalent continuous average daily exposure (for BMDLs, the adjustments were already performed prior to BMD modeling).

For inhalation studies in rodents, the POD on the basis of applied dose in ppm was taken to be the BMDL, NOAEL, or LOAEL. NOAELs and LOAELs were adjusted for intermittent exposure to their equivalent continuous average daily exposure (for BMDLs, the adjustments were already performed prior to BMD modeling). These adjusted concentrations are considered HECs, in accordance with U.S. EPA (1994a), as TCE is considered a Category 3 gas (systemically acting) and has a blood-air partition coefficient in rodents greater than that in humans.[14]

F.5.2. PBPK Model-Based Human Points of Departure

As discussed in Section 5.1.3, the PBPK model was used for simultaneous interspecies (for endpoints in rodent studies), intraspecies, and route-to-route extrapolation based on the estimates from the PBPK model of the internal dose points of departure (idPOD) for each candidate critical study/endpoints. The following supplementary data files contain figures showing the derivation of the HEDs and HECs for the median (50^{th} percentile) and sensitive (99^{th} percentile) individual from the (rodent or human) study idPOD. In each case, for a specific study/endpoint(s)/sex/species (in the figure main title), and for a particular dose-metric (Y-axis label), the horizontal line shows the original study idPOD (a BMDL, NOAEL, or LOAEL as noted) and where it intersects with the human 99^{th} percentile (open square) or median (closed square) exposure-internal-dose relationship:

1. HECs from human inhalation studies ("Supplementary data for TCE assessment: Non-cancer HECs plots from human inhalation studies," 2011)
2. HECs from rodent inhalation studies ("Supplementary data for TCE assessment: Non-cancer HECs plots from rodent inhalation studies," 2011)
3. HECs from rodent oral studies ("Supplementary data for TCE assessment: Non-cancer HECs plots from rodent oral studies," 2011)
4. HEDs from human inhalation studies ("Supplementary data for TCE assessment: Non-cancer HEDs plots from human inhalation studies," 2011)
5. HEDs from rodent inhalation studies ("Supplementary data for TCE assessment: Non-cancer HEDs plots from rodent inhalation studies," 2011)

[14] The posterior population median estimate for the TCE blood:air partition coefficient was 14 in the mouse [Table 3-37], 19 in the rat [Table 3-38], and 9.2 in the human [Table 3-39].

6. HEDs from rodent oral studies ("Supplementary data for TCE assessment: Non-cancer HEDs plots from rodent oral studies," 2011)

The original study internal doses are based on the median estimates from about 2,000 "study groups" (for rodent studies) or "individuals" (for human studies), and corresponding exposures for the human median and 99th percentiles were derived from a distribution of 2,000 "individuals." In both cases, the distributions reflect combined uncertainty (in the population means and variances) and population variability.

In addition, as part of the uncertainty/variability analysis described in Section 5.1.4.2, the POD for studies/endpoints for which BMD modeling was done was replaced by the LOAEL or NOAEL. This was done to because there was no available tested software for performing BMD modeling in such a context and because of limitations in time and resources to develop such software. However, the relative degree of uncertainty/variability should be adequately captured in the use of the LOAEL or NOAEL. The graphical depiction of the HEC_{99} or HED_{99} using these alternative PODs is shown in the following supplementary data files:

1. HECs from rodent inhalation studies ("Supplementary data for TCE assessment: Non-cancer HECs altPOD plots from rodent inhalation studies," 2011)
2. HECs from rodent oral studies ("Supplementary data for TCE assessment: Non-cancer HECs altPOD plots from rodent oral studies," 2011)
3. HEDs from rodent inhalation studies ("Supplementary data for TCE assessment: Non-cancer HEDs altPOD plots from rodent inhalation studies," 2011)
4. HEDs from rodent oral studies ("Supplementary data for TCE assessment: Non-cancer HEDs altPOD plots from rodent oral studies," 2011)

F.6. SUMMARY OF POINTS OF DEPARTURE (PODs) FOR STUDIES AND EFFECTS SUPPORTING THE INHALATION RfC AND ORAL RfD

This section summarizes the selection and/or derivation of PODs from the critical and supporting) studies and effects that support the inhalation RfC and oral RfD. In particular, for each endpoint, the following are described: dosimetry (adjustments of continuous exposure, PBPK dose-metrics), selection of BMR and BMD model (if BMD modeling was performed), and derivation of the HEC or dose for a sensitive individual (if PBPK modeling was used). Section 5.1.3.1 discusses the dose-metric selection for different endpoints.

F.6.1. NTP (NTP, 1988)—BMD Modeling of Toxic Nephropathy in Rats

The supporting endpoint here is toxic nephropathy in female Marshall rats (NTP, 1988), which was the most sensitive sex/strain in this study, although the differences among different sex/strain combinations was not large (BMDLs differed by threefold or less).

F.6.1.1. Dosimetry and BMD Modeling

Rats were exposed to 500 or 1,000 mg/kg/day, 5 days/week, for 104 weeks. The primary dose-metric was selected to be average amount of DCVC bioactivated/$kg^{¾}$/day, with median estimates from the PBPK model for the female Marshall rats in this study of 0.47 and 1.1.

Figure F-10 shows BMD modeling for the dichotomous models used (see Section F.5.1, above). The log-logistic model with slope constrained to ≥1 was selected because: (1) the log-logistic model with unconstrained slope yielded a slope estimate <1 and (2) it had the lowest AIC.

The idPOD of 0.0132 mg DCVC bioactivated/$kg^{¾}$/day was a BMDL for a BMR of 5% extra risk. This BMR was selected because toxic nephropathy is a clear toxic effect. This BMR required substantial extrapolation below the observed responses (about 60%); however, the response level seemed warranted for this type of effect and the ratio of the BMD to the BMDL was not large (1.56 for the selected model).

F.6.1.2. Derivation of HEC_{99} and HED_{99}

The HEC_{99} and HED_{99} are the lower 99^{th} percentiles for the continuous HEC and continuous human ingestion dose that lead to a human internal dose equal to the rodent idPOD. The derivation of the HEC_{99} of 0.0056 ppm and HED_{99} of 0.00338 mg/kg/day for the 99^{th} percentile for uncertainty and variability are shown in Figure F-11. These values are used as this supporting effect's POD to which additional UFs are applied.

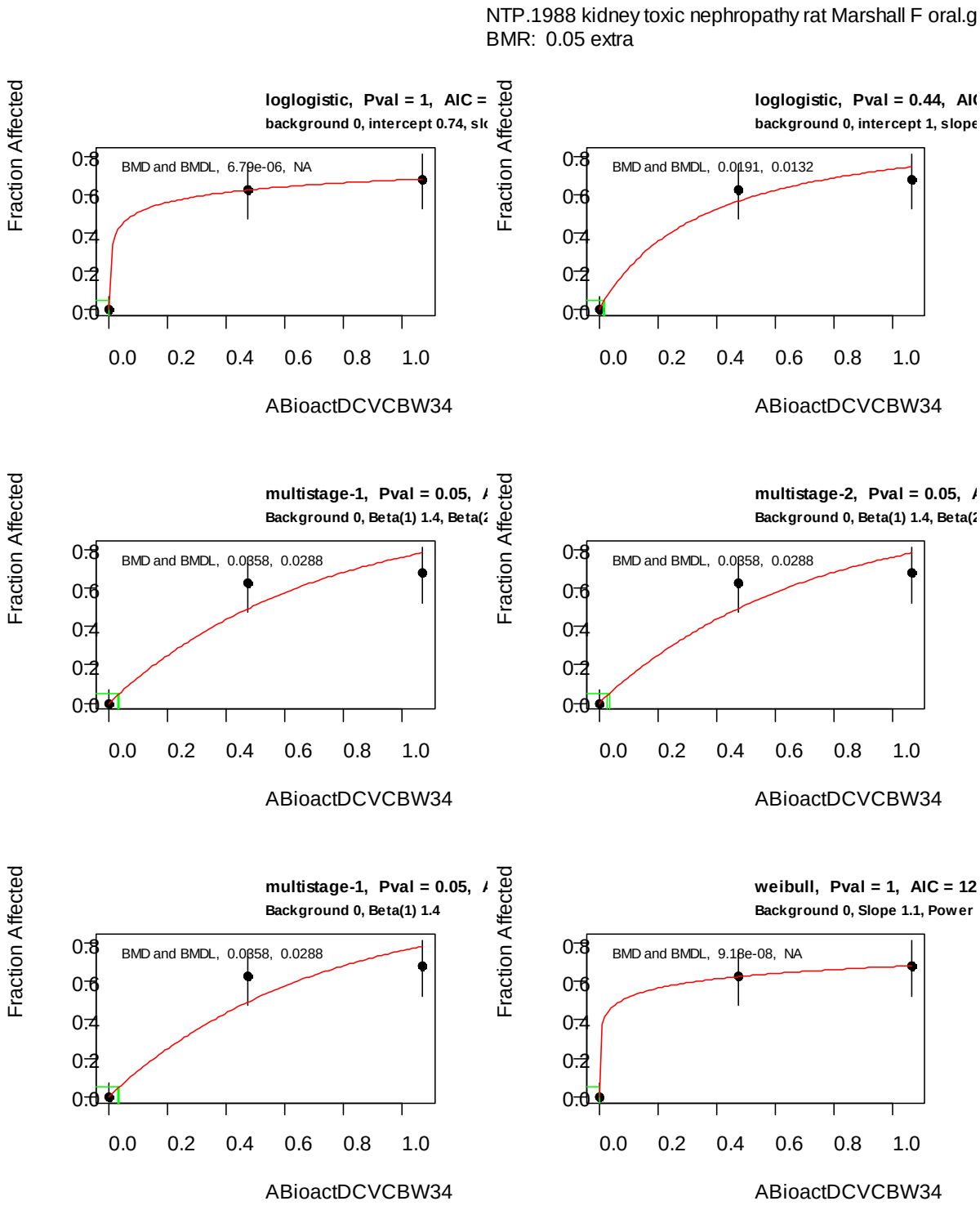


Figure F-10. BMD modeling of NTP (1988) toxic nephropathy in female Marshall rats.

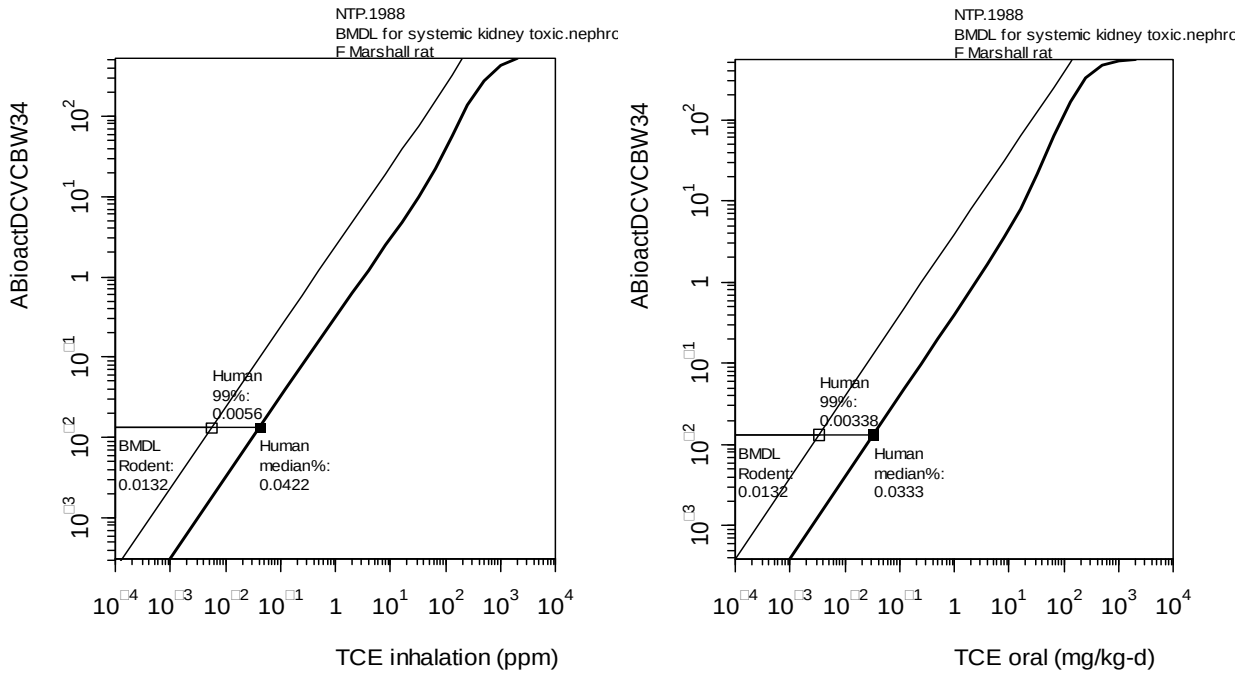


Figure F-11. Derivation of HEC_{99} and HED_{99} corresponding to the rodent idPOD from NTP (1988) toxic nephropathy in rats.

F.6.2. Woolhiser et al. (2006)—BMD Modeling of Increased Kidney Weight in Rats

The endpoint here is increased kidney weights in female Sprague-Dawley (Sprague-Dawley) rats (Woolhiser et al., 2006), which was considered a supporting effect for the RfD.

F.6.2.1. Dosimetry and BMD Modeling

Rats were exposed to 100, 300, and 1,000 ppm, 6 hours/day, 5 days/week, for 4 weeks. The primary dose-metric was selected to be average amount of DCVC bioactivated/$kg^{¾}$/day, with median estimates from the PBPK model for this study of 0.038, 0.10, and 0.51.

Figure F-12 shows BMD modeling for the continuous models used (see Section F.5.2, above). The Hill model with constant variance was selected because it had the lowest AIC and because other models with the same AIC either were a power model with power parameter <1 or had poor fits to the control data set.

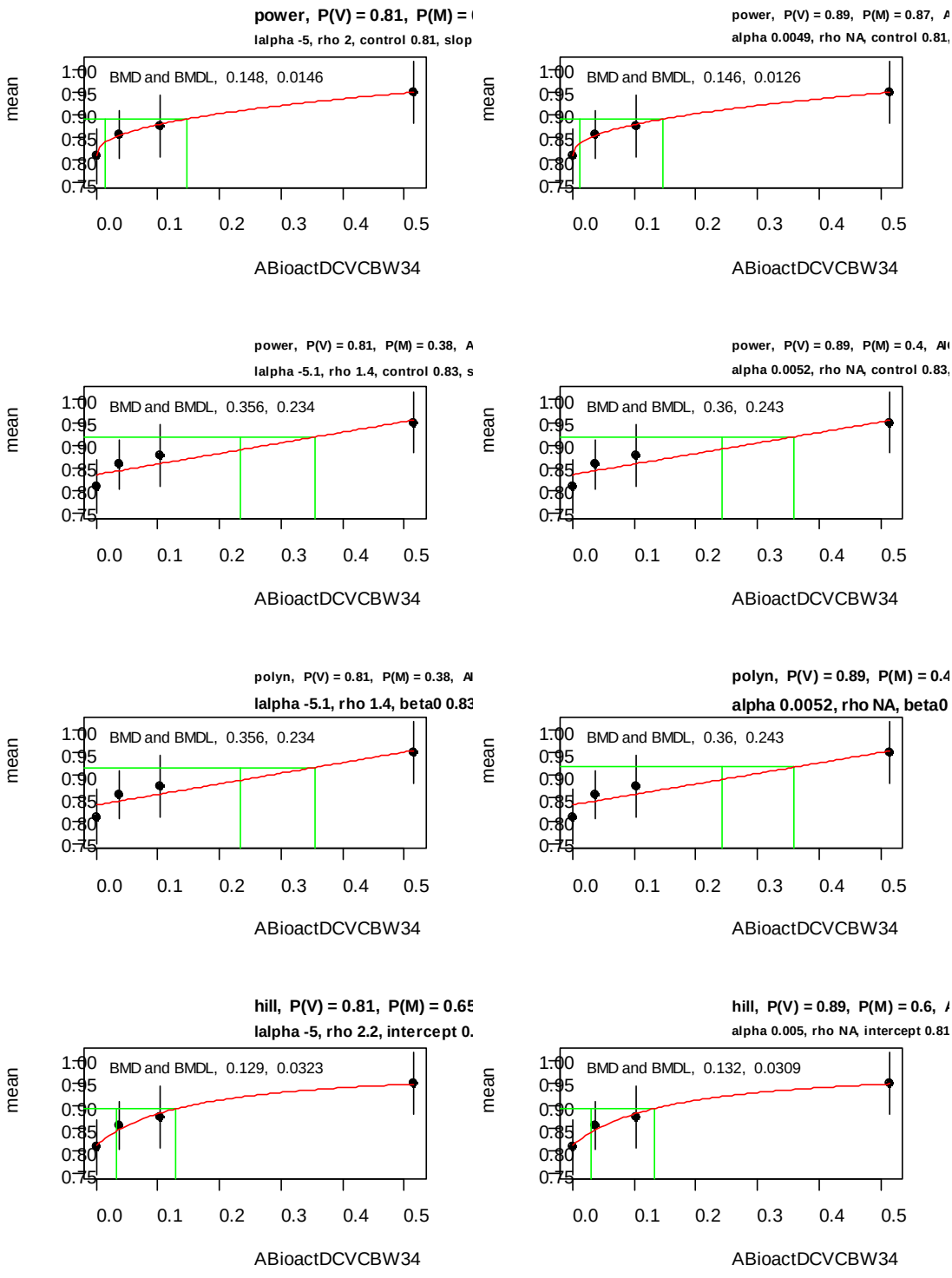


Figure F-12. BMD modeling of Woolhiser et al. (2006) for increased kidney weight in female Sprague-Dawley rats.

The idPOD of 0.0309 mg DCVC bioactivated/kg^{¾}/day was a BMDL for a BMR of 10% weight change, which is the BMR typically used by U.S. EPA for body weight and organ weight changes. The response used in each case was the organ weight as a percentage of body weight, to account for any commensurate decreases in body weight, although the results did not differ much when absolute weights were used instead.

F.6.2.2. Derivation of HEC_{99} and HED_{99}

The HEC_{99} and HED_{99} are the lower 99th percentiles for the continuous HEC and continuous human ingestion dose that lead to a human internal dose equal to the rodent idPOD. The derivation of the HEC_{99} of 0.0131 ppm and HED_{99} of 0.00791 mg/kg/day for the 99th percentile for uncertainty and variability are shown in Figure F-13. These values are used as this effect's POD to which additional UFs are applied, and the resulting candidate RfD value is supportive of the RfD.

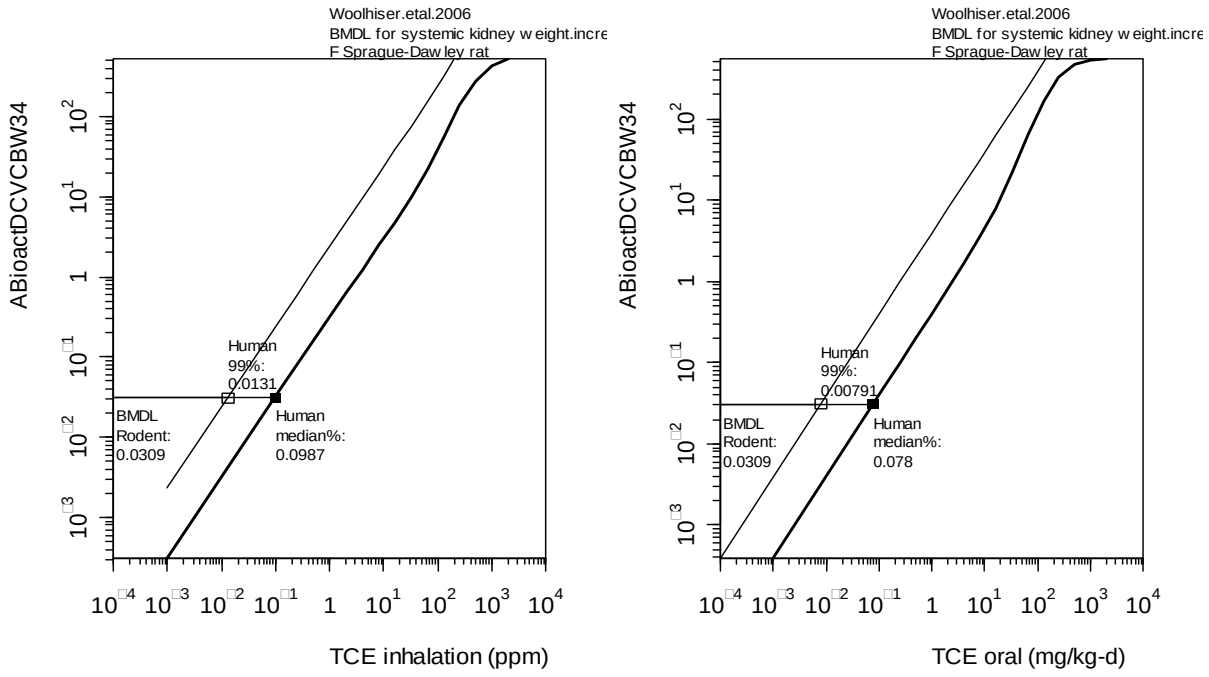


Figure F-13. Derivation of HEC_{99} and HED_{99} corresponding to the rodent idPOD from Woolhiser et al. (2006) for increased kidney weight in rats.

F.6.3. Keil et al. (2009)—LOAEL for Decreased Thymus Weight in Mice

The critical endpoint here is decreased thymus weight in female $B6C3F_1$ mice (Keil et al., 2009).

F.6.3.1. Dosimetry

Mice were exposed to 1,400 and 14,000 ppb of TCE in drinking water, with an average dose estimated by EPA to be 0.35 and 3.5 mg/kg/day, for 30 weeks, based on the average of subchronic and chronic values for generic body weight and water consumption rates for female B6C3F1 mice (U.S. EPA, 1988). The dose-response relationships were sufficiently supralinear that BMD modeling failed to produce an adequate fit. The primary dose-metric was selected to be the average amount of TCE metabolized/$kg^{¾}$/day. The lower dose group was the LOAEL, and the median estimate from the PBPK model at that exposure level was 0.139 mg TCE metabolized/$kg^{¾}$/day, which is used as the rodent idPOD.

F.6.3.2. Derivation of HEC_{99} and HED_{99}

The HEC_{99} and HED_{99} are the lower 99th percentiles for the continuous HEC and continuous human ingestion dose that lead to a human internal dose equal to the rodent idPOD. The derivation of the HEC_{99} of 0.0332 ppm and HED_{99} of 0.0482 mg/kg/day for the 99th percentile for uncertainty and variability are shown in Figure F-14. These values are used as this critical effect's POD to which additional UFs are applied.

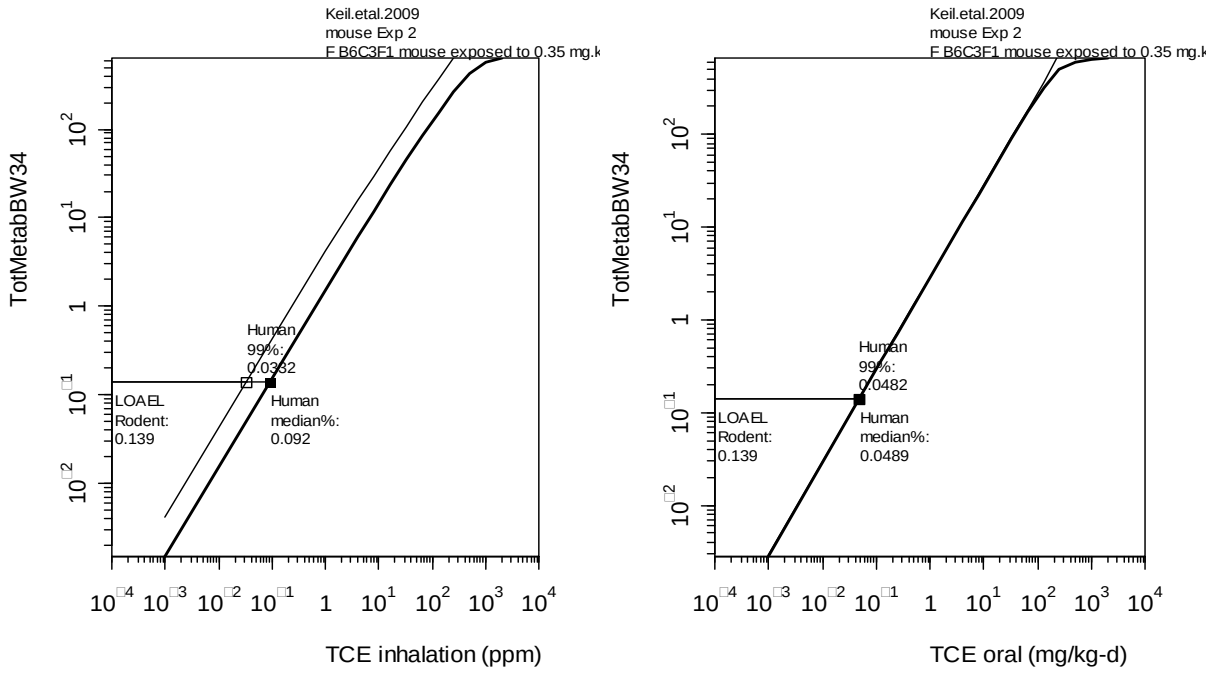


Figure F-14. Derivation of HEC_{99} and HED_{99} corresponding to the rodent idPOD from Keil et al. (2009) for decreased thymus weight in mice.

F.6.4. Johnson et al. (2003)—BMD Modeling of Fetal Heart Malformations in Rats

The critical endpoint here is increased fetal heart malformations in female Sprague-Dawley rats (Johnson et al., 2003).

F.6.4.1. Dosimetry and BMD Modeling

Rats were exposed to 2.5, 250, 1.5, or 1,100 ppm TCE in drinking water for 22 days (GDs 1–22). The primary dose-metric was selected to be average amount of TCE metabolized by oxidation/$kg^{3/4}$/day, with median estimates from the PBPK model for this study of 0.00031, 0.033, 0.15, and 88.

As discussed previously in Section F.4.2.1, from results of nested log-logistic modeling of these data, with the highest dose group dropped, the idPOD of 0.0142 mg TCE metabolized by oxidation/$kg^{3/4}$/day was a BMDL for a BMR of 1% increased in incidence in pups. A 1% extra risk of a pup having a heart malformation was used as the BMR because of the severity of the effect; some of the types of malformations observed could have been fatal.

F.6.4.2. Derivation of HEC_{99} and HED_{99}

The HEC_{99} and HED_{99} are the lower 99th percentiles for the continuous HEC and continuous human ingestion dose that lead to a human internal dose equal to the rodent idPOD. The derivation of the HEC_{99} of 0.00365 ppm and HED_{99} of 0.00515 mg/kg/day for the 99th percentile for uncertainty and variability are shown in Figure F-15. These values are used as this critical effect's POD to which additional UFs are applied.

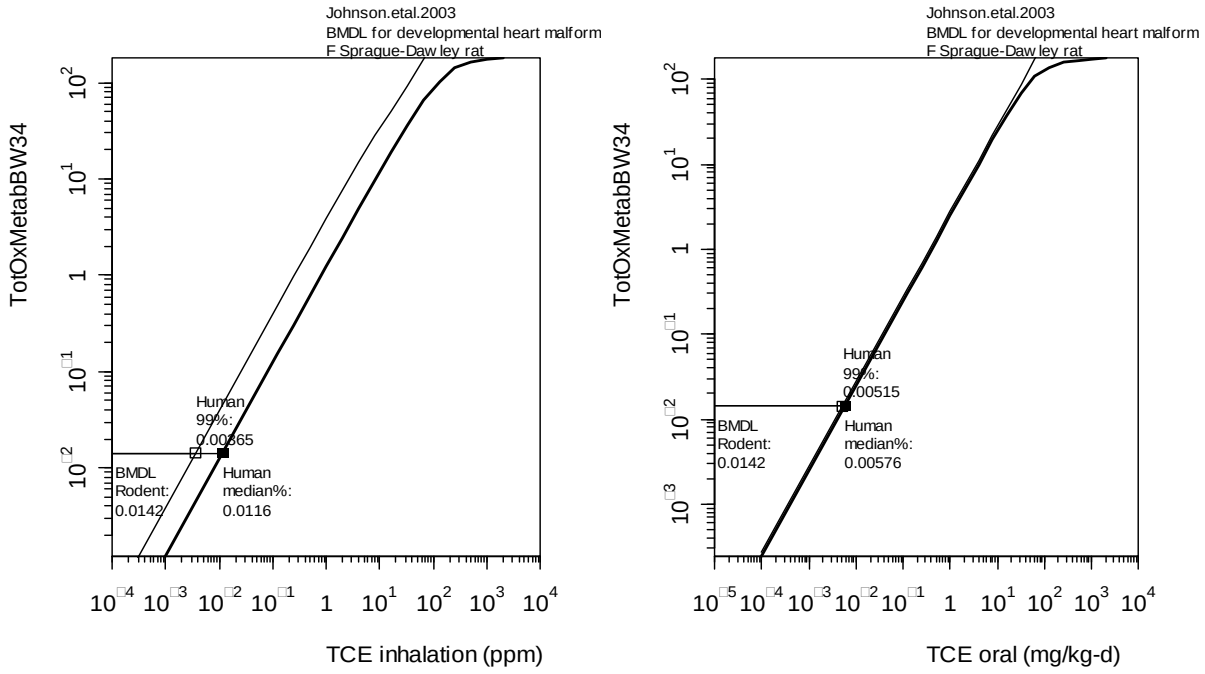


Figure F-15. Derivation of HEC_{99} and HED_{99} corresponding to the rodent idPOD from Johnson et al. (2003) for increased fetal cardiac malformations in female Sprague-Dawley rats using the total oxidative metabolism dose-metric.

F.6.5. Peden-Adams et al. (2006)—LOAEL for Decreased PFC Response and Increased Delayed-Type Hypersensitivity in Mice

The critical endpoints here are decreased PFC response and increased delayed-type hypersensitivity in mice exposed pre- and postnatally (Peden-Adams et al., 2006).

Mice were exposed to 1,400 and 14,000 ppb in drinking water, with an average dose in the dams estimated by the authors to be 0.37 and 3.7 mg/kg/day, from GD 0 to postnatal ages of 3 or 8 weeks. The dose-response relationships were sufficiently supralinear that BMD modeling failed to produce an adequate fit. In addition, because of the lack of an appropriate PBPK model and parameters to estimate internal doses given the complex exposure pattern (placental and lactational transfer, and pup ingestion postweaning), no internal dose estimates were made. Therefore, the LOAEL of 0.37 mg/kg/day on the basis of applied dose was used as the critical effect's POD to which additional UFs are applied.

G. TCE CANCER DOSE-RESPONSE ANALYSES WITH RODENT CANCER BIOASSAY DATA

G.1. DATA SOURCES

TCE cancer endpoints were identified in Maltoni et al. (1986), NCI (1976), NTP (1990, 1988), Fukuda et al. (1983), and Henschler et al. (1980). These data were reviewed and tabulated in spreadsheets, and the numbers were verified. All endpoint data identified by authors as having a statistically significant response to dose were tabulated, and data that had marginally significant trends with dose were also reviewed. For all endpoints for which dose-response model estimates were presented, trends were verified using the Cochran-Armitage or the Poly-3 test.

G.1.1. Numbers at Risk

The numbers of animals at risk are not necessarily those used by the authors; instead, the number alive at 52 weeks was used (if the first cancer of the type of interest was observed at later than 52 weeks) or the number alive at the week when the first cancer of the type of interest was observed. In general, the data of Maltoni et al. (1986) were presented in this way, in their tables titled "Incidence of the different types of tumors referred to specific corrected numbers." In a few cases in Maltoni et al. (1986), the time of first occurrence was later than 52 weeks, so an alternative number at risk was used from another column (for another cancer) in the same table having a first occurrence close to 52 weeks. For NTP (1990, 1988) and NCI (1976), the week of the first observation and the numbers alive at that week were determined from the appendix tables. For Fukuda et al. (1983), the reported "effective number of mice" in their Table 2 was used, which is consistent with numbers alive at 40–42 weeks (when the first tumor, a thymic lymphoma, was observed) in their mortality curve. For Henschler et al. (1980), the number of mice alive at week 36 (from their Figure 1), which is when the first tumor was observed (according to their Figure 2), was used.

In cases in which there is high early mortality or differential mortality across dose groups and the individual animal data are available, a more involved analysis that takes into account animals at risk at different times (ages) is preferred (e.g., the poly-3 approach or time-to-tumor modeling; see Section G.7). The more rudimentary approach of adjusting the denominator to account for animals alive at the time of the first tumor entails some inaccuracy (bias) in estimating the animals at risk compared to a more involved analysis accounting more completely for time. However, it is generally agreed that it is better to use such an adjustment than to use no adjustment at all (Haseman et al., 1984; Gart et al., 1979; Hoel and Walburg, 1972).

G.1.2. Cumulative Incidence

Maltoni et al. (1986) conducted a lifetime study, in which rodents were exposed for 104 weeks (rats) or 78 weeks (mice), and allowed to live until they died "naturally." Maltoni et al. (1986) reported cumulative incidence on this basis, and it was not possible to determine incidence at any fixed time, such as 104 weeks on study. For Henschler et al. (1980), the number of mice with tumors observed by week 104 (their Figure 2) was used. The cumulative incidence reported by Fukuda et al. (1983) at 107 weeks (after 104 weeks of exposure) was used. For the NCI (1976) and NTP (1990, 1988) studies, the reported cumulative incidence at 103–107 weeks (study time varied by study and species) was used.

G.2. INTERNAL DOSE-METRICS AND DOSE ADJUSTMENTS

PBPK modeling was used to estimate levels of dose-metrics corresponding to different exposure scenarios in rodents and humans (see Section 3.5). The selection of dose-metrics for specific organs and endpoints is discussed under Section 5.2. Internal dose-metrics were selected based on applicability to each major affected organ. The dose-metrics used with our cancer dose-response analyses are shown in Table G-1.

Table G-1. Internal dose-metrics used in dose-response analyses, identified by "X"

Dose-metric units	Liver	Lung	Kidney	Other
ABioactDCVCBW34 (mg/wk-kg$^{3/4}$)	0	0	X	0
AMetGSHBW34 (mg/wk-kg$^{3/4}$)	0	0	X	0
AMetLiv1BW34 (mg/wk-kg$^{3/4}$)	X	0	0	0
AMetLngBW34 (mg/wk-kg$^{3/4}$)	0	X	0	0
AUCCBld (mg-hr/L-wk)	0	X	0	X
TotMetabBW34 (mg/wk-kg$^{3/4}$)	0	0	X	X
TotOxMetabBW34 (mg/wk-kg$^{3/4}$)	X	X	0	0

The PBPK model requires the rodent body weight as an input. For most of the studies, central estimates specific to each species, strain, and sex (and substudy) were used. These were estimated by medians of body weights digitized from graphics in Maltoni et al. (1986), by medians of weekly averages in NTP (1990, 1988), and by averages over the study duration of weekly mean body weights tabulated in NCI (1976).

For the studies by Fukuda et al. (1983) and Henschler et al. (1980), mouse body weights were not available. After reviewing body weights reported for similar strains by two laboratories[15] and in the other studies reported for TCE, it was concluded that a plausible range

[15] http://phenome.jax.org/pub-cgi/phenome/mpdcgi?rtn=meas%2Fdatalister&req=Cbody+weight&pan=2&noomit=&datamode=measavg, http://www.hilltoplabs.com/public/growth.html.

for lifetime average body weight is 20–35 g, with a median near 28 g. For these two studies, internal dose-metrics for these three average body weights (20, 28, and 35 g) were computed. The percentage differences between the internal dose-metrics for the intermediate body weight of 28 g and the low and high average body weight of 20 and 35 g were then evaluated. Internal dose-metrics were little affected by choice of body weight. For all dose-metrics, the differences were less than ±13%. A body weight of 28 g was used for these two studies.

The medians (from the Markov chain Monte Carlo posterior distribution) for each of the dose-metrics for the rodent were used in quantal dose-response analyses. The median is probably the most appropriate posterior parameter to use as a dose-metric, as it identifies a "central" measure and it is also a quantile, making it more useful in nonlinear modeling. The "multistage" dose-response functions are nonlinear. One is interested in estimating the expected response. The expected value of a nonlinear function of dose is under- or overestimated when the mean (expected value) of the dose is used, depending on whether the function is concave or convex. (This is Jensen's Inequality: for a real convex function f(X), $f[E(X)] \leq E[f(X)]$.) For the dose-response function, one is interested in E[f(X)], so using E(X) (estimated by the posterior mean) as the dose-metric will not necessarily predict the mean response. Using the posterior median rather than the mean as the dose-metric should lead to a response function that is closer to the median response. However, if the estimated dose-response function is close to linear, this source of distortion may be small, and the mean response might be predicted reasonably well by using the posterior mean as the dose-metric. The mean and median are expected to be rather different because the posterior distributions are skewed and approximately lognormal. Therefore, results based on the posterior median and the posterior mean dose-metrics were compared before deciding to use the median.

G.3. DOSE ADJUSTMENTS FOR INTERMITTENT EXPOSURE

The nominal applied dose was adjusted for exposure discontinuity (e.g., exposure for 5 days/week and 6 hours/day reduced the dose by the factor [(5/7) × (6/24)]), and for exposure durations less than full study time (up to 2 years) (e.g., the dose might be reduced by a factor [78 week/104 week]). The PBPK dose-metrics took into account the daily and weekly discontinuity to produce an equivalent dose for continuous exposure. The NCI (1976) gavage study applied one dose for weeks 1–12 and another, slightly different dose for weeks 13–78; PBPK dose-metrics were produced for both dose regimes and then time-averaged (e.g., average dose = (12/78) × D1 + (66/78) × D2). For Henschler et al. (1980), Maltoni et al. (1986), and NCI (1976), a further adjustment of (exposure duration/study duration) was made to account for the fact that exposures ended prior to terminal sacrifice, so that the dose-metrics reflect average weekly values over the exposure period. Finally, for NCI (1976), the dose-metrics were then

adjusted for early sacrifice[16] (at 91 weeks rather than 104 weeks) by a factor of (91 wk/ 104 wk)^3.[17]

G.4. RODENT TO HUMAN DOSE EXTRAPOLATION

Adjustments for rodent-to-human extrapolation were applied to the final results—the BMD, BMDL, and cancer slope factor (potency), which is calculated as BMR/BMDL, e.g., $0.10/BMDL_{10}$.

For the PBPK dose-metrics, a ratio between human and laboratory animal internal dose was determined by methods described in Section 3.5. The cancer slope factor is relevant only for very low extra risk (typically on the order of 10^{-4}–10^{-6}), thus very low dose, and it was determined that the relation between human and animal internal dose was linear in the low-dose range for each of the dose-metrics used, hence this ratio was multiplied by the animal dose (or divided into the cancer slope factor) to extrapolate animal to human dose or concentration.

For the experimentally applied dose, default interspecies extrapolation approaches were used. These are provided for comparison to results based on PBPK metrics. To extrapolate animal inhalation exposure to human inhalation exposure, the "equivalent" HEC (i.e., the exposure concentration in humans that is expected to give the same level of response that was observed in the test species) was assumed to be identical to the animal inhalation exposure concentration (i.e., "ppm equivalence"). This assumption is consistent with U.S. EPA recommendations (U.S. EPA, 1994a) for deriving a HEC for a Category 3 gas for which the blood:air partition coefficient in laboratory animals is greater than that in humans.[18] To extrapolate animal oral exposure to equivalent human oral exposure, animal dose was scaled up by body weight to the ¾-power using the factor $(BW_{Human}/BW_{Animal})^{0.75}$. To extrapolate animal inhalation exposure to human oral exposure, the following equation (Eq. G-1) was used;[19]

Animal, equivalent oral intake, mg/kg/day =

$$ppm \times [MW_{TCE}/24.45]^{20} \times MV \times (60 \text{ minutes/hour}) \times (10^3 \text{ mg/g}) \times [24 \text{ hour}/BW_{kg}] \quad \text{(Eq. G-1)}$$

with units

[16]For studies of <2 years (i.e., with terminal kills before 2 years), the doses are generally adjusted by the study length ratio to a power of 3 (i.e., a factor [length of study in week/104 week]^3) to reflect the fact that the animals were not observed for the full standard lifetime (1980).

[17]For studies of <2 years (i.e., with terminal kills before 2 years), the doses are generally adjusted by the study length ratio to a power of 3 (i.e., a factor [length of study in week/104 week]^3) to reflect the fact that the animals were not observed for the full standard lifetime (1980).

[18] The posterior population median estimate for the TCE blood:air partition coefficient was 14 in the mouse [Table 3-37], 19 in the rat [Table 3-38], and 9.2 in the human [Table 3-39].

[19]ToxRisk version 5.3, © 2000–2001 by the KS Crump Group, Inc.

[20]Molecular weight of TCE is 131.39; there are 24.45 L of perfect gas per g-mol at standard temperature and pressure.

$$\text{ppm} \times [\text{g/mol} \div \text{L/mol}] \times \text{L/minute} \times (\text{minutes/hour}) \times (\text{mg/g}) \times [\text{hour/day} \div \text{kg}] \quad \text{(Eq. G-2)}$$

which reduces to

$$\text{ppm} \times [7.738307 \times \text{MV}/\text{BW}_{kg}] \quad \text{(Eq. G-3)}$$

where

ppm = animal inhalation concentration, $1/10^6$, unitless
MV = minute volume (breathing rate) at rest, L/minute.

Minute volume (MV) was estimated using equations from U.S. EPA (1994b, p. 4–27),

$$\text{Mouse} \quad \ln(\text{MV}) = 0.326 + 1.05 \times \ln(\text{BW}_{kg}) \quad \text{(Eq. G-4)}$$

$$\text{Rat} \quad \ln(\text{MV}) = -0.578 + 0.821 \times \ln(\text{BW}_{kg}). \quad \text{(Eq. G-5)}$$

Animal equivalent oral intake was converted to human equivalent oral intake by multiplying by the rodent to human ratio of body weights to the power +0.25.[21]

To extrapolate animal oral exposure to equivalent human inhalation exposure, the calculation above was reversed to extrapolate the animal inhalation exposure.

G.5. COMBINING DATA FROM RELATED EXPERIMENTS IN MALTONI ET AL. (1986)

Data from Maltoni et al. (1986) required decisions regarding whether to combine related experiments for certain species and cancers.

In experiment BT306, which used $B6C3F_1$ mice, males experienced unusually low survival, reportedly because of the age of the mice at the outset and resulting aggression. The protocol was repeated (for males only), with an earlier starting age, as experiment BT306bis, and male survival was higher (and typical for such studies). The rapid male mortality in experiment BT306 apparently censored later-developing cancers, as suggested by the low frequency of liver cancers for males in BT306 as compared to BT306bis. Data for the two experiments clearly cannot legitimately be combined. Therefore, only experiment BT306bis males were used in the analyses.

Experiments BT304 and BT304bis, on rats, provide evidence in male rats of leukemia, carcinomas of the kidney, and testicular (Leydig cell) tumors, and provide evidence in female rats for leukemia. Maltoni et al. (1986) stated "Since experiments BT 304 and BT 304bis on

[21]Find whole-animal intake from mg/kg/d × $\text{BW}_{\text{Animal}}$. Scale this allometrically by $(\text{BW}_{\text{Human}}/\text{BW}_{\text{Animal}})^{0.75}$ to extrapolate whole-human intake. Divide by human body weight to find mg/kg/d for the human. The net effect is Animal mg/kg/d × $(\text{BW}_{\text{Animal}}/\text{BW}_{\text{Human}})^{0.25}$ = Human mg/kg/d.

Sprague-Dawley rats were performed at the same time, exactly in the same way, on animals of the same breed, divided by litter distribution within the two experiments, they have been evaluated separately and comprehensively." The data were also analyzed separately and in combination.

The data and modeling results for these tumors in the BT304 and BT304bis experiments are tabulated in Tables G-2 through G-5. It was decided that it was best to combine the data for the two experiments. There were no consistent differences between experiments, and no firm basis for selecting one of them. Our final analyses are, therefore, based on the combined numbers and tumor responses for these two experiments.

Table G-2. Experiments BT304 and BT304bis, female Sprague-Dawley rats, Maltoni et al. (1986). Number alive is reported for week of first tumor observation in either males or females.[a] These data were not used for dose-response modeling because there is no consistent trend (for the combined data, there is no significant trend by the Cochran-Armitage test, and no significant differences between control and dose groups by Fisher's exact test).

Exposure concentration (ppm)	Number alive	Number of rats with this cancer	Proportion with cancer	Multistage model fit statistics[b]				
				Model order	*p*-Value	AIC	BMD_{10}	$BMDL_{10}$
	Experiment BT304, female rats, leukemias, N alive at 7 wks							
0	105	7	0.067	No adequately fitting model				
100	90	6	0.067					
300	90	0	0.000					
600	90	7	0.078					
	Experiment BT304bis, female rats, leukemias, N alive at 7 wks							
0	40	0	0.000	1	0.202	70.4	127	58.7
100	40	3	0.075					
300	40	2	0.050					
600	40	4	0.100					
	Experiments BT304 and BT304bis, female rats, leukemias, combined data							
0	145	7	0.048	3	0.081	227	180	134
100	130	9	0.069					
300	130	2	0.015					
600	130	11	0.085					

[a]First tumor occurrences were not reported separately by sex.
[b]Models of orders 3 were fitted; the highest-order nonzero coefficient is reported in column "Model order." BMDL was estimated for extra risk of 0.10 and confidence level 0.95. Exposure concentrations were multiplied by (7/24) × (5/7) = 0.20833 before fitting the models, to adjust for exposure periodicity (i.e., the time-averaged concentrations were about 20% of the nominal concentrations).

Table G-3. Experiments BT304 and BT304bis, male Sprague-Dawley rats, Maltoni et al. (1986): leukemias. Number alive is reported for week of first tumor observation in either males or females.[a]

Exposure concentration (ppm)	Number alive	Number of rats with this cancer	Proportion with cancer	Multistage model fit statistics[b]				
				Model order	*p*-Value	AIC	BMD_{10}	$BMDL_{10}$
	Experiment BT304, male rats, leukemias, N alive at 7 wks							
0	95	6	0.063	1	0.429	238	NA	NA
100	90	10	0.111					
300	90	11	0.122					
600	89	9	0.101					
	Experiment BT304bis, male rats, leukemias, N alive at 7 wks							
0	39	3	0.077	3	0.979	102	143	71.9
100	40	3	0.075					
300	40	3	0.075					
600	40	6	0.150					
	Combined data for BT304 and BT304bis, male rats, leukemias							
0	134	9	0.067	1	0.715	337	269	111
100	130	13	0.100					
300	130	14	0.108					
600	129	15	0.116					

[a]First tumor occurrences were not reported separately by sex.
[b]Models of orders 3 were fitted; the highest-order nonzero coefficient is reported in column "Model order." BMDL was estimated for extra risk of 0.10 and confidence level 0.95. Exposure concentrations were multiplied by (7/24) × (5/7) = 0.20833 before fitting the models, to adjust for exposure periodicity (i.e., the time-averaged concentrations were about 20% of the nominal concentrations). "NA" indicates the BMD or BMDL could not be solved because it exceeded the highest dose.

Table G-4. Experiments BT304 and BT304bis, male Sprague-Dawley rats, Maltoni et al. (1986): kidney adenomas + carcinomas. Number alive is reported for week of first tumor observation in either males or females.[a]

Exposure concentration (ppm)	Number alive	Number of rats with this cancer	Proportion with cancer	Multistage model fit statistics[b]				
				Model order	*p*-Value	AIC	BMD_{10}	$BMDL_{10}$
	Experiment BT304 male rats, kidney adenomas + carcinomas, N alive at 47 wks							
0	87	0	0.000	3	0.318	50.1	173	134
100	86	1	0.012					
300	80	0	0.000					
600	85	4	0.047					
	Experiment BT304bis, male rats, kidney adenomas + carcinomas, N alive at 53 wks							
0	34	0	0.000	3	0.988	13.0	266	173
100	32	0	0.000					
300	36	0	0.000					
600	38	1	0.027					
	Combined data for BT304 and BT304bis, male rats, kidney adenomas + carcinomas							
0	121	0	0.000	3	0.292	60.5	181	144
100	118	1	0.008					
300	116	0	0.000					
600	123	5	0.041					

[a]First tumor occurrences were not reported separately by sex.
[b]Models of orders three were fitted; the highest-order nonzero coefficient is reported in column "Model order." BMDL was estimated for extra risk of 0.10 and confidence level 0.95. Exposure concentrations were multiplied by (7/24) × (5/7) = 0.20833 before fitting the models, to adjust for exposure periodicity (i.e., the time-averaged concentrations were about 20% of the nominal concentrations). "NA" indicates the BMD or BMDL could not be solved because it exceeded the highest dose.

Table G-5. Experiments BT304 and BT304bis, male Sprague-Dawley rats, Maltoni et al. (1986): testis, Leydig cell tumors. Number alive is reported for week of first tumor observation.[a]

Exposure concentration (ppm)	Number alive	Number of rats with this cancer	Proportion with cancer	Multistage model fit statistics[b]				
				Model order	*p*-Value	AIC	BMD_{10}	$BMDL_{10}$
	Experiment BT304, male rats, Leydig cell tumors, N alive at 47 wks							
0	87	5	0.057	1	0.0494	309	41.5	29.2
100	86	11	0.128					
300	80	24	0.300					
600	85	22	0.259					
	Experiment BT304bis, male rats, Leydig cell tumors, N alive at 53 wks							
0	34	1	0.029	1	0.369	117	54.5	30.9
100	32	5	0.156					
300	36	6	0.167					
600	38	9	0.237					
	Combined data for BT304 and BT304bis, male rats, Leydig cell tumors							
0	121	6	0.050	1	0.0566	421	44.7	32.7
100	116	16	0.138					
300	116	30	0.259					
600	122	31	0.254					

[a]Numbers alive reported for weeks as close as possible to week 52 (first tumors observed at weeks 81 and 62, respectively, for the two experiments).
[b]Models of orders three were fitted; the highest-order nonzero coefficient is reported in column "Model order." BMDL was estimated for extra risk of 0.10 and confidence level 0.95. Exposure concentrations were multiplied by (7/24) × (5/7) = 0.20833 before fitting the models, to adjust for exposure periodicity (i.e., the time-averaged concentrations were about 20% of the nominal concentrations). "NA" indicates the BMD or BMDL could not be solved because it exceeded the highest dose.

G.6. DOSE-RESPONSE MODELING RESULTS

Using BMDS, the multistage quantal model was fitted using the applicable dose metrics for each combination of study, species, strain, sex, organ, and BMR (extra risk) value under consideration. A multistage model of order one less than the number of dose groups (g) was fitted. This means that, in some cases, the fitted model could be strictly nonlinear at low dose (estimated coefficient "b1" was zero), and in other cases, higher-order coefficients might be estimated as zero so the resulting model would not necessarily have order (#groups-1). Because more parsimonious, 1st-order models often fit such data well, based on our extensive experience and that of others (Nitcheva et al., 2007), if the resulting model was not a 1st-order multistage, then lower-order models were also fitted, down to a 1st-order multistage model. This permitted us to screen results efficiently.

A supplementary data file ("Supplementary data for TCE assessment: Cancer rodents plots," 2011) shows the fitted model curves. The graphics include observations (as proportions [i.e., cumulative incidence divided by number at risk]), the estimated multistage curve (solid red line), and estimated BMD, with a BMDL. Vertical bars show 95% CIs for the observed proportions. Printed above each plot are some key statistics (necessarily rounded) for model goodness of fit and estimated parameters. Printed in the plots at upper left are the BMD and BMDL for the rodent data, in the same units as the rodent dose. Within the plot at lower right are human exposure values (BMDL and cancer slope factor for continuous inhalation and oral exposures) corresponding to the rodent BMDL. For applied doses, the human equivalent values were calculated by "default" methods,[22] as discussed above, and then only for the same route of exposure as the rodent, and they are in units of rodent dose. For internal dose-metrics, the human values are based upon the PBPK rodent-to-human extrapolation, as discussed in Section 5.2.1.2.

Another supplementary data file ("Supplementary data for TCE assessment: Cancer rodents results," 2011) presents the data and model summary statistics, including goodness-of-fit measures (χ^2 goodness-of-fit *p*-value, AIC), parameter estimates, BMD, BMDL, and "cancer slope factor" ("CSF"), which is the extra risk divided by the BMDL. Much more descriptive information appears also, including the adjustment terms for intermittent exposure, and the doses before applying those adjustments. The group "GRP" numbers are arbitrary, and are the same as GRP numbers in the plots. There is one line in this table for each dose-response graph in the preceding document. Input data for the analyses are in a separate supplementary data file ("Supplementary data for TCE assessment: Cancer rodents input data," 2011). Finally, the values and model selections for the results used in Section 5.2 are summarized in another supplementary data file (primary dose-metrics in bold) ("Supplementary data for TCE assessment: Cancer rodents model selections," 2011).

[22]For oral intake, dose (BMDL) is multiplied by the ratio of animal to human body weight (60 kg female, 70 kg male) taken to the ¼ power. For inhalation exposures, ppm equivalence is assumed.

G.7. MODELING TO ACCOUNT FOR DOSE GROUPS DIFFERING IN SURVIVAL TIMES

Differential mortality among dose groups can potentially interfere with (i.e., censor) the occurrence of late-appearing cancers. Usually the situation is one of greater mortality rates at higher doses, caused by toxic effects, or, sometimes, by cancers other than the cancer of interest. Statistical methods of estimation (for the cancer of interest) in the presence of competing risks assume uninformative censoring.

For bioassays with differential early mortality occurring primarily before the time of the 1st tumor or 52 weeks (whichever came first), the effects of early mortality were largely accounted for by adjusting the tumor incidence for animals at risk, as described above, and the dose-response data were modeled using the multistage model.

If, however, there was substantial overlap between the appearances of cancers and progressively differential mortality among dose groups, it was necessary to apply methods that take into account individual animal survival times. Two such methods were used here: time-to-tumor modeling and the poly-3 method of adjusting numbers at risk. Three such studies were identified, all with male rats (see Table 5-34). Using both survival-adjustment approaches, BMDs and BMDLs were obtained and unit risks derived. Section 5.2.1.3 presents a comparison of the results for the three data sets and for various dose-metrics.

G.7.1. Time-to-Tumor Modeling

The first approach used to take into account individual survival times was application of the multistage Weibull (MSW) time-to-tumor model. This model has the general form

$$P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t - t_0)^z], \qquad \text{(Eq. G-6)}$$

where $P(d,t)$ represents the probability of a tumor by age t for dose d, and parameters $z \geq 1$, $t_0 \geq 0$, and $q_i \geq 0$ for $i = 0,1,...,k$, where k = the number of dose groups; the parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death. The MSW model likelihood accounts for the left-censoring inherent in "incidental" observations of nonfatal tumors discovered upon necropsy and the right-censoring inherent in deaths not caused by fatal tumors. All of our analyses used the model for incidental tumors, which has no t_0 term, and which assumes that the tumors are nonfatal (or effectively so, to a reasonable approximation). This seems reasonable because the tumors of concern appeared relatively late in life and there were multiple competing probable causes of death (especially toxic effects) operating in these studies (also note that cause of death was not reported by the studies used). It is difficult to formally evaluate model fit with this model because there is no

applicable goodness-of-fit statistic with a well-defined asymptotic distribution. However, plots of fitted vs. observed responses were examined.

A computer program ("MSW") to implement the multistage Weibull time-to-tumor model was designed, developed and tested for U.S. EPA by Battelle Columbus (Ohio). The MSW program obtains maximum likelihood estimates for model parameters and solves for the BMDL (lower confidence limit for BMD) using the profile-likelihood method. The model, with documentation for methodology (statistical theory and estimation, and numerical algorithms) and testing, was externally reviewed by experts in June 2007. Reviews were generally positive and confirmed that the functioning of the computer code has been rigorously tested. (U.S. EPA and Battelle confirmed that MSW gave results essentially identical to those of "ToxRisk," a program no longer commercially issued or supported.) U.S. EPA's BMDS Web site provided reviewers' comments and U.S. EPA's responses.[23] The MSW program and reports on statistical and computational methodology and model testing are available on U.S. EPA's BMDS Web site (www.epa.gov/ncea/bmds).

Results of this modeling are shown in a supplementary data file ("Supplementary data for TCE assessment: Rodents time to tumor results, 2011").

G.7.2. Poly-3 Calculation of Adjusted Number at Risk

To obtain an independent estimate of a POD using different assumptions, it was thought desirable to compare time-to-tumor modeling to an alternative survival-adjustment technique, "poly-3 adjustment" (Portier and Bailer, 1989), applied to the same data. This technique was used to adjust the tumor incidence denominators based on the individual animal survival times. The adjusted incidence data then served as inputs for U.S. EPA's BMDS multistage model, and multistage model selection was conducted as described in Section 5.2.

A detailed exposition is given in Section 6.3.2 of Piegorsch and Bailer (Bailer and Piegorsch, 1997). Each tumor-less animal is weighted by its fractional survival time (survival time divided by the duration of the bioassay) raised to the power of 3 to reflect the fact that animals are at greater risk of cancer at older ages. Animals with tumors are given a weight of 1. The sum of the weights of all of the animals in an exposure group yields the effective survival-adjusted denominator. The "default" power of 3 (thus, "poly-3") was assumed, which was found to be representative for a large number of cancer types (Portier et al., 1986). Algebraically,

$$N_{adj} = \sum_i w_i \qquad \text{(Eq. G-7)}$$

[23]At http://www.epa.gov/ncea/bmds/response.html under title "2007 External Review of New Quantal Models;" use links to comments and responses.

where

w_i = 1 if tumor is present
w_i = $(t_i/T)^3$ if tumor is absent at time of death (t_i)
T = duration of study. N was rounded to the nearest integer.[24]

Calculations are reproduced in the time-to-tumor supplementary data file ("Supplementary data for TCE assessment: Rodents time to tumor results," 2011).

G.8. COMBINED RISK FROM MULTIPLE TUMOR SITES

For bioassays that exhibited more than one type of tumor response in the same sex and species (these studies have a row for "combined risk" in the "Endpoint" column of Table 5-34, Section 5.2), the cancer potency for the different tumor types combined was estimated. The combined tumor risk estimate describes the risk of developing tumors for *any* (not all together) of the tumor types that exhibited a TCE-associated tumor response; this estimate then represents the total excess cancer risk. The model for the combined tumor risk is also multistage, with the sum of the stage-specific multistage coefficients from the individual tumor models serving as the stage-specific coefficients for the combined risk model (i.e., for each q_i, $q_{i[combined]} = q_{i1} + q_{i2} + ... + q_{ik}$, where the q_is are the coefficients for the powers of dose and k is the number of tumor types being combined) (NRC, 1994; Bogen, 1990). This model assumes that the occurrences of two or more tumor types are independent. The resulting model equation can be readily solved for a given BMR to obtain a maximum likelihood estimate (BMD) for the combined risk. However, the confidence bounds for the combined risk estimate are not calculated by available modeling software. Therefore, a Bayesian approach was used to estimate confidence bounds on the combined BMD. This approach was implemented using the freely available WinBUGS software (Spiegelhalter et al., 2003), which applies Markov chain Monte Carlo computations. Use of WinBUGS has been demonstrated for derivation of a distribution of BMDs for a single multistage model (Kopylev et al., 2007) and can be straightforwardly generalized to derive the distribution of BMDs for the combined tumor load.

G.8.1. Methods

G.8.1.1. Single Tumor Sites

Cancer dose-response models were fitted to data using BMDS. These were multistage models with coefficients constrained to be non-negative. The order of model fitted was (g – 1), where g is the number of dose groups. For internal dose-metrics, the values shown in tables above were used.

[24]Notice that the assumptions required for significance testing and estimating variances of parameters are changed by this procedure. The Williams-Bieler variance estimator is described by Piegorsch and Bailer (1997). Our multistage modeling did not take this into account, so the resulting BMDL may be somewhat lower than could be obtained by more laborious calculations.

The multistage model was modified for U.S. EPA NCEA by Battelle (under contract EPC04027) to provide model-based estimates of extra risk at a user-specified dose and profile-likelihood CIs for that risk. Thus, CIs for extra risk in addition to BMDs could be reported.

G.8.1.2. Combined Risk From Multiple Tumor Sites

The multistage model identified by BMDS[25] was used in a WinBUGS script to generate posterior distributions for model parameters, the BMD and extra risk at the same dose specified for the BMDS estimates. The prior used for multistage parameters was the positive half of a normal distribution having a mean of zero and a variance of 10,000, effectively a very flat prior. The burn-in was of length 10,000, then 100,000 updates were made and thinned to every 10th update for sample monitoring. From a WinBUGS run, the sample histories, posterior distribution plots, summary statistics, and codas were archived.

Codas were then imported to R and processed using R programs to compute BMD and the extra risk at a specific dose for each tumor type. BMD and extra risk for the combined risk function (assuming independence) were also computed following Bogen (NRC, 1994, Chapter 11, Appendix I-1, Appendix I-2; 1990, Chapter IV). Results were summarized as percentiles, means, and modes (modes were based upon the smoothed posterior distributions). The extra risks across tumor types at a specific dose (10 or 100 was used) were also summed.

BMDLs for rodent internal doses, reported below, were converted to human external doses using the conversion factors in Tables G-6 and G-7 (based on PBPK model described in Section 3.5).

Table G-6. Rodent to human conversions for internal dose-metric TotOxMetabBW34

Route	Sex	Human (mean)
Inhalation, ppm	F	9.843477
	M	9.702822
Oral, mg/kg/d	F	15.72291
	M	16.4192

Table G-7. Rodent to human conversions for internal dose-metric TotMetabBW34

Route	Sex	Human (mean)
Inhalation, ppm	F	11.84204
	M	11.69996
Oral, mg/kg/d	F	18.76327
	M	19.6

[25]The highest-order model was used, e.g., if BMDS estimates were gamma = 0, beta.1 > 0, beta.2 = 0, beta.3 > 0, the model in WinBUGS allowed beta.2 to be estimated (rather than being fixed at zero).

The application of rodent to human conversion factors is as follows:

Given rodent internal dose *D* in some units of TotOxMetabBW34, divide by tabled value *Y* above to find human exposure in ppm or mg/kg/day.

Example: ppm (human) = *D*(rodent)/*Y*

ppm (human female mean) = 500 (internal units)/9.843477

= 50.80 ppm (Eq. G-8)

G.8.2. Results

The results follow in this order:

Applied doses

NCI (1976), Female B6C3F$_1$ mice, gavage, liver and lung tumors and lymphomas (see Tables G-8 through G-10 and Figures G-1 and G-2)

Maltoni (1986), Female B6C3F$_1$ mice, inhalation (expt. BT306), liver and lung tumors (see Tables G-11 through G-13 and Figures G-3 and G-4)

Maltoni (1986), Male Sprague-Dawley rats, inhalation (expt. BT304), kidney tumors, testis Leydig Cell tumors, and lymphomas (see Tables G-14 through G-16 and Figures G-5 and G-6)

Internal Doses

NCI (1976) Female B6C3F$_1$ mice, gavage, liver and lung tumors and lymphomas (see Tables G-17 through G-19 and Figures G-7 and G-8)

Maltoni (1986), Female B6C3F$_1$ mice, inhalation (expt. BT306), liver and lung tumors (see Tables G-20 through G-22 and Figures G-9 and G-10)

Maltoni (1986), Male Sprague-Dawley rats, inhalation (expt. BT304), kidney tumors, Testis Leydig Cell tumors, and lymphomas (see Tables G-23 through G-25 and Figures G-11 and G-12)

Table G-8. Female B6C3F$_1$ mice—applied doses: data

Dose[a]	N[b]	Liver HCCs	Lung adenomas + carcinomas	Hematopoietic lymphomas + sarcomas
0	18	0	1	1
356.4	45	4	4	5
713.3	41	11	7	6

[a]Doses were adjusted by a factor 0.41015625, accounting for exposure 5/7 days/week, exposure duration 78/91 weeks, and duration of study (91/104) ^{3}. Averaged applied gavage exposures were low-dose 869 mg/kg/day, high dose 1,739 mg/kg/day.
[b]Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study.

Source: NCI (1976).

Table G-9. Female B6C3F$_1$ mice—applied doses: model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	Model order, selected	Coefficient estimates equal zero	AIC	Largest[a] scaled residual	Goodness of fit *p*-value
Liver	2	γ	78.68	0	1
	1[a]	γ	77.52	-0.711	0.6698
Lung	2	NA	78.20	0	1
	1[a]	NA	76.74	-0.551	0.4649
Lymphomas + sarcomas	2	β2	77.28	0.113	0.8812
	1[a]	NA	77.28	0.113	0.8812

[a]Largest in absolute value.

Source: NCI (1976).

Table G-10. Female B6C3F$_1$ mice—applied doses: BMD and risk estimates (inferences for BMR of 0.05 extra risk at 95% confidence level)

	Liver HCCs	Lung adenomas + carcinomas	Hematopoietic lymphomas + sarcomas
Parameters used in model	q0, q1	q0, q1	q0, q1
p-Value for BMDS model	0.6698	0.6611	0.8812
BMD_{05} (from BMDS)	138.4	295.2	358.8
BMD_{05} (median, mode—WinBUGS)	155.5, 135.4	314.5, 212.7	352.3, 231.7
BMDL (BMDS)[a]	92.95	144.3	151.4
BMDL (5^{th} percentile, WinBUGS)	97.48	150.7	157.7
BMD_{05} for combined risk (median, mode, from WinBUGS)	84.99, 78.95		
BMDL for combined risk (5^{th} percentile, WinBUGS)	53.61		
BMDS maximum likelihood risk estimates			
Risk at dose 100	0.03640	0.01722	0.01419
Upper 95% confidence limit	0.05749	0.03849	0.03699
Sum of risks at dose 100	0.06781		
WinBUGS Bayes risk estimates			
Risk at dose 100: mean, median	0.0327, 0.0324	0.0168, 0.0161	0.0152, 0.0143
Upper 95% confidence limit	0.0513	0.0334	0.0319
Combined risk at dose 100 mean, median	0.06337, 0.0629		
Combined risk at dose 100, upper 95% confidence limit	0.09124		

[a]All CIs are at 5% (lower) or 95% (upper) level, one-sided.

Source: NCI (1976).

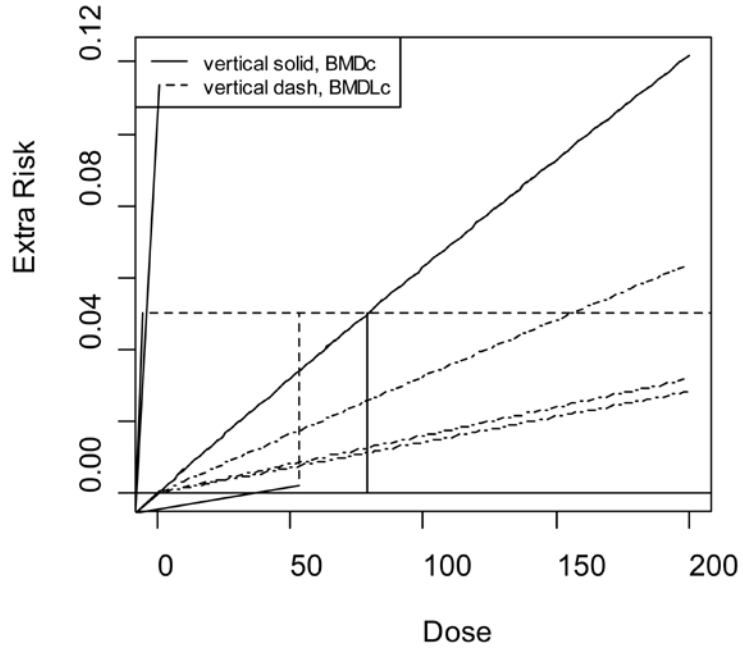


Figure G-1. Female B6C3F$_1$ mice—applied doses: combined and individual tumor extra-risk functions.

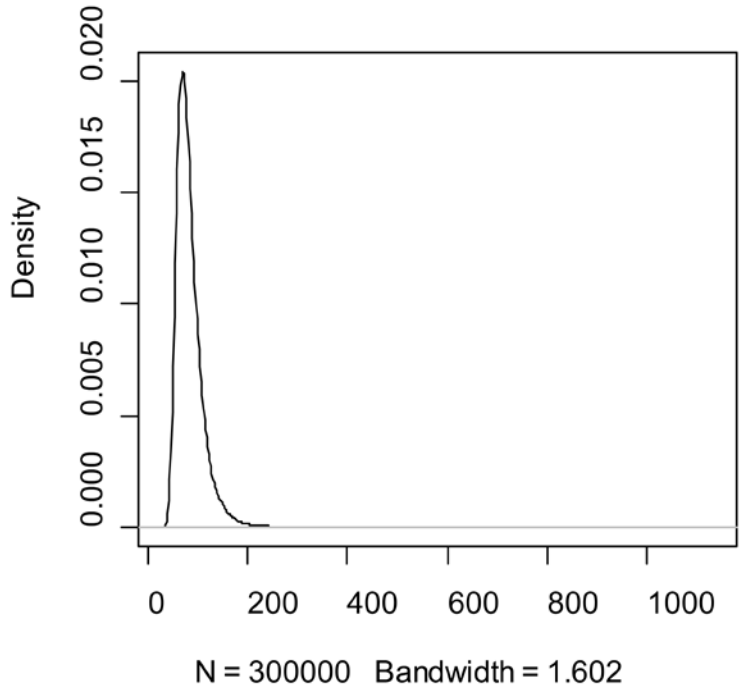


Figure G-2. Female B6C3F$_1$ mice—applied doses: posterior distribution of BMDc for combined risk.

Table G-11. $B6C3F_1$ female mice inhalation exposure—applied doses

Dose[a]	Liver hepatomas/N[b]	Lung adenomas + carcinomas/N[b]
0	3/88	2/90
15.6	4/89	6/90
46.9	4/88	7/89
93.8	9/85	14/87

[a]Doses adjusted by a factor 0.133928571, accounting for exposure 7/24 hours/day × 5/7 days/week, and exposure duration 78/104 weeks. Applied doses were 100, 300, and 600 ppm.
[b]Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study.

Source: Maltoni (1986).

Table G-12. $B6C3F_1$ female mice—applied doses: model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	Model order, selected	Coefficient estimates equal zero	AIC	Largest[a] scaled residual	Goodness of fit *p*-value
Liver	3	β2	154.91	0.289	0.7129
	2	β1	153.02	0.330	0.8868
	1[a]	NA	153.47	–0.678	0.7223
Lung	3	β2	195.91	0.741	0.3509
	2	β2	193.91	0.714	0.6471
	1[a]	NA	193.91	0.714	0.6471

[a]Largest in absolute value.

Source: Maltoni (1986).

Table G-13. $B6C3F_1$ female mice inhalation exposure—applied doses (inferences for 0.05 extra risk at 95% confidence level)

	Liver hepatomas	Lung adenomas + carcinomas
Parameters used in model	q0, q1	q0, q1
p-Value for BMDS model	0.7223	0.06471
BMD_{05} (from BMDS)	72.73	33.81
BMD_{05} (median, mode—WinBUGS)	71.55, 56.79	34.49, 31.65
BMDL (BMDS)[a]	37.13	21.73
ms combo.exe BMD_{05}c, BMDLc	32.12, 16.22	
BMD_{05} (5^{th} percentile, WinBUGS)	37.03	22.07
BMD_{05} for combined risk (median, mode, from WinBUGS)	23.07, 20.39	
BMDL for combined risk (5^{th} percentile, WinBUGS)	15.67	
BMDS maximum likelihood risk estimates		
Risk at dose 10	0.0070281	0.0150572
Upper 95% confidence limit	0.0151186	0.0250168
Sum of risks at dose 10	0.0220853	
WinBUGS Bayes risk estimates: means (medians)		
Risk at dose 10: mean, median	0.007377, 0.007138	0.01489, 0.01476
Upper 95% confidence limit	0.01374	0.02
Combined risk at dose 10: mean, median	0.02216, 0.02198	
Combined risk at dose 10: upper 95% confidence limit	0.03220	

[a]All CIs are at 5% (lower) or 95% (upper) level, one-sided.

Source: Maltoni (1986).

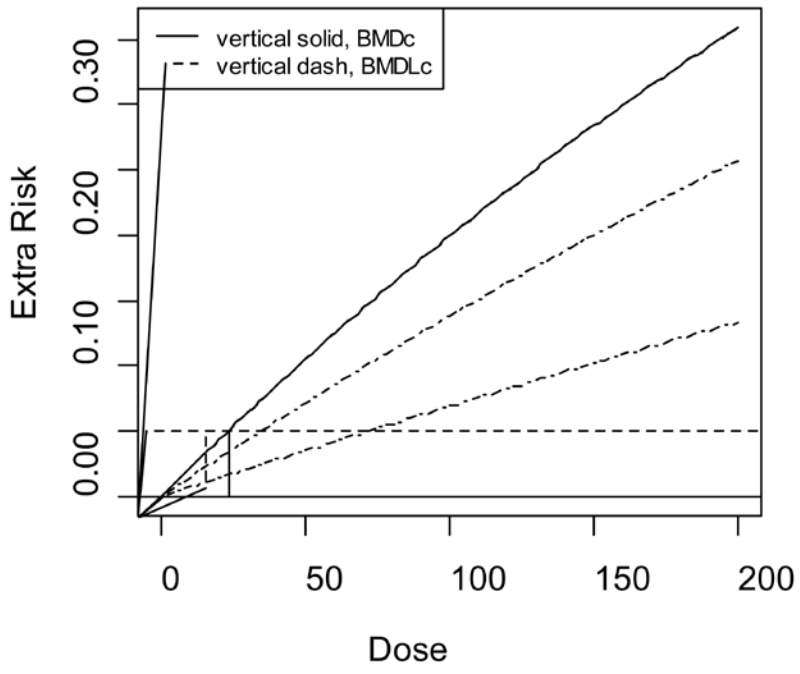


Figure G-3. B6C3F$_1$ female mice inhalation exposure—applied doses: combined and individual tumor extra-risk functions.

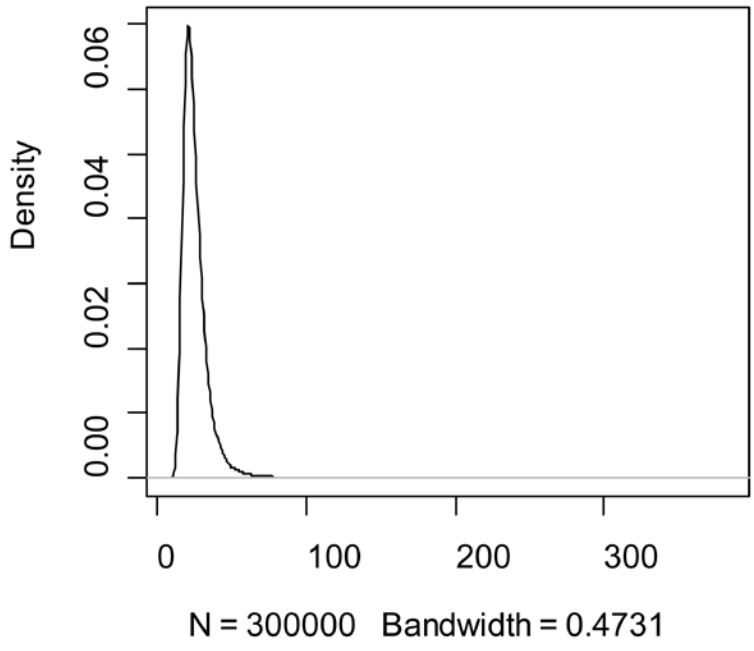


Figure G-4. B6C3F$_1$ female mice inhalation exposure—applied doses: posterior distribution of BMDc for combined risk.

Table G-14. Maltoni Sprague-Dawley male rats—applied doses

Dose[a]		Kidney adenomas + carcinomas/N[b]	Leukemias/N[b]	Testis, Leydig cell tumors/N[b]
0		0/121	9/134	6/121
20.8		1/118	13/130	16/116
62.5		0/116	14/130	30/116
125		5/123	15/129	31/122

[a]Doses adjusted by a factor 0.208333333, accounting for exposure 7 hours/day × 5/7 days/week. Applied doses were 100, 300, and 600 ppm.
[b]Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study.

Table G-15. Maltoni Sprague-Dawley male rats—applied doses: model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	Model order[a]	Coefficient estimates equal zero	AIC	Largest+ scaled residual	Goodness of fit *p*-value
Kidney	3	β1, β2	60.55	1.115	0.292
	2	γ	61.16	-1.207	0.253
	1[a]	γ	59.55	-1.331	0.4669
Leukemia	3	β2, β3	336.8	0.537	0.715
	2	β2	336.8	0.537	0.715
	1	NA	336.8	0.537	0.715
Dropping high dose	2	β2	243.7	0.512	0.529
	1[a]	NA	243.7	0.512	0.529
Testis	3	β2, β3	421.4	-1.293	0.057
	2	β2	421.4	-1.293	0.057
	1	NA	421.4	-1.293	0.057
Dropping high dose	2	β2	277.6	0.291	0.728
	1[a]	NA	277.6	0.291	0.728

[a]Model order selected + largest in absolute value.

Table G-16. Maltoni Sprague-Dawley male rats—applied doses

	Kidney adenomas + carcinomas	Leukemia (high dose dropped)	Testis, Leydig cell tumors (high dose dropped)
Parameters used in models	q0, q1	q0, q1	q0, q1
p-Value for BMDS model	0.4669	0.5290	0.7277
BMD_{01} (from BMDS)	41.47	14.5854	2.46989
BMD_{01} (median, mode—WinBUGS)	46.00, 35.71	12.32, 8.021	2.497, 2.309
BMDL (BMDS)[a]	22.66	5.52597	1.77697
BMDL (5^{th} percentile, WinBUGS)	23.23	5.362	1.789
BMD_{01} for combined risk (median, mode, from WinBUGS)	1.960, 1.826		
BMDL for combined risk (5^{th} percentile, WinBUGS)	1.437		
BMDS maximum likelihood risk estimates			
Risk at dose 10	0.0024208	0.0068670	0.0398747
Upper 95% confidence limit	0.0048995	0.0202747	0.0641010
Sum of risks at dose 10			
Risk at dose 1	0.0002423	0.0006888	0.0040609
Upper 95% confidence limit	0.0004911	0.0020462	0.0066029
Sum of risks at dose 1			
WinBUGS Bayes risk estimates: means (medians)			
Risk at dose 10: mean, median	0.002302, 0.002182	0.008752, 0.008120	0.03961, 0.03945
Upper 95% confidence limit	0.004316	0.01860	0.05462
Combined risk at dose 10, mean, median	0.05020, 0.04998		
Combined risk at dose 10, upper 95% confidence limit	0.06757		
Risk at dose 1: mean, median	2.305×10^{-4}, 2.184×10^{-4}	8.800×10^{-4}, 8.150×10^{4}	0.004037, 0.004017
Upper 95% confidence limit	4.325×10^{-4}	1.876×10^{-3}	0.005601
Combined risk at dose 1, mean, median	0.005143, 0.005114		
Combined risk at dose 1, upper 95% confidence limit	0.006971		

[a]All CIs are at 5% (lower) or 95% (upper) level, one-sided.

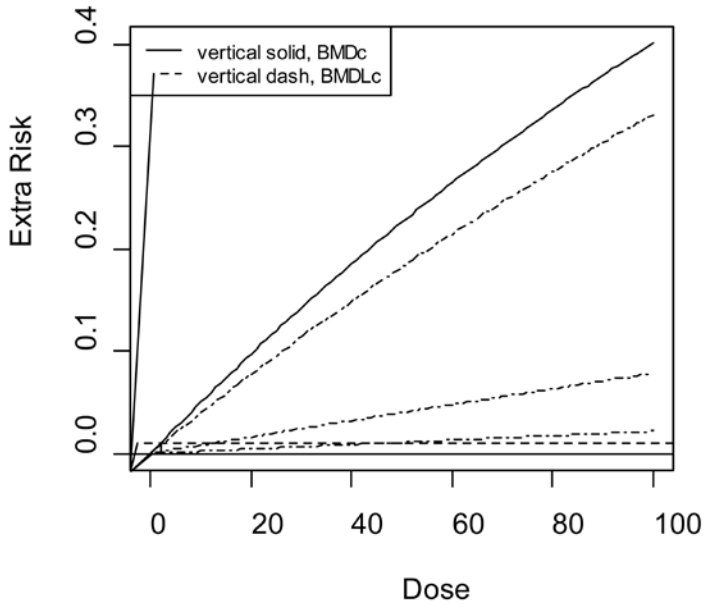


Figure G-5. Maltoni Sprague-Dawley male rats—applied doses: combined and individual tumor extra-risk functions.

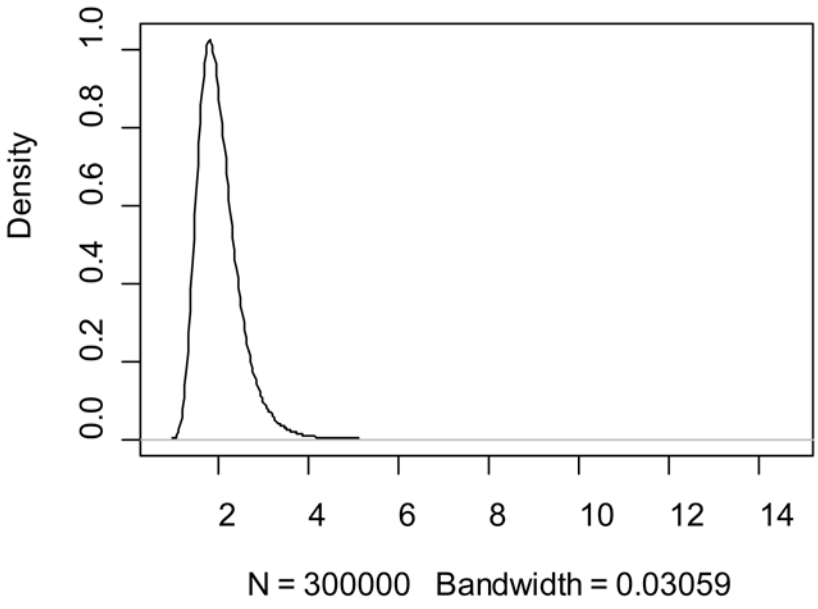


Figure G-6. Maltoni Sprague-Dawley male rats—applied doses: posterior distribution of BMDc for combined risk.

Table G-17. Female B6C3F$_1$ mice—internal dose-metric (total oxidative metabolism): data

Internal dose[a]	*N*[b]	Liver HCCs	Lung adenomas + carcinomas	Hematopoietic lymphomas + sarcomas
0	18	0	1	1
549.8	45	4	4	5
813.4	41	11	7	6

[a]Internal dose, Total Oxidative Metabolism, adjusted for body weight, units [mg/(wk-kg$^{3/4}$)]. Internal doses were adjusted by a factor 0.574219, accounting for exposure duration 78/91 weeks, and duration of study (91/104)^3. Before adjustment, the median internal doses were 957.48 and 1416.55 (mg/wk-kg$^{3/4}$).
[b]Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study.

Source: NCI (1976).

Table G-18. Female B6C3F$_1$ mice—internal dose: model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	BMD, BMDL	Model order[a]	Coefficient estimates equal zero	AIC	Largest+ scaled residual	Goodness of fit *p*-value
Liver	505, 284	2[a]	γ, β1	77.25	-0.594	0.7618
	367, 245	1	γ	78.86	-1.083	0.3542
Lung	742, 396	2[a]	β1	76.33	-0.274	0.7197
	780, 380	1	NA	76.74	-0.551	0.4649
Lymphomas + sarcomas	870, 389	2	NA	79.26	0	1
	839, 390	1[a]	NA	77.27	-0.081	0.9140

[a]Model order selected + largest in absolute value.

Source: NCI (1976).

Table G-19. Female B6C3F$_1$ mice—internal dose-metric (total oxidative metabolism): BMD and risk estimates (values rounded to 4 significant figures) (inferences for BMR of 0.05 extra risk at 95% confidence level)

	Liver HCCs	Lung adenomas + carcinomas	Hematopoietic lymphomas + sarcomas
Parameters used in models	q0, q1, q2	q0, q1, q2	q0, q1
p-Value for BMDS model	0.7618	0.7197	0.9140
BMD_{05} (from BMDS)	352.4	517.8	423.8
BMD_{05} (median, mode from WinBUGS)	284.8, 292.5	414.3, 299.9	409.8, 382.6
BMDL (BMDS)[a]	138.1	193.0	189.5
BMDL (5^{th} percentile, WinBUGS)	162.6	195.4	226.2
BMD_{05} for Combined Risk (median, mode, from WinBUGS)	136.1, 121.1		
BMDL for Combined Risk (5^{th} percentile, WinBUGS)	85.65		
BMDS maximum likelihood risk estimates			
Risk at dose 100	0.004123	0.001912	0.0120315
Upper 95% confidence limit	0.04039	0.02919	0.0295375
Sum of risks at dose 100			
WinBUGS Bayes risk estimates			
Risk at dose 100: mean, median	0.01468, 0.01311	0.01284, 0.01226	0.009552, 0.008286
Upper 95% confidence limit	0.03032	0.02590	0.021410
Combined risk at dose 100 mean, median	0.03663, 0.03572		
Combined risk at dose 100, upper 95% confidence limit	0.05847		

[a]All CIs are at 5% (lower) or 95% (upper) level, one-sided.

Source: NCI (1976).

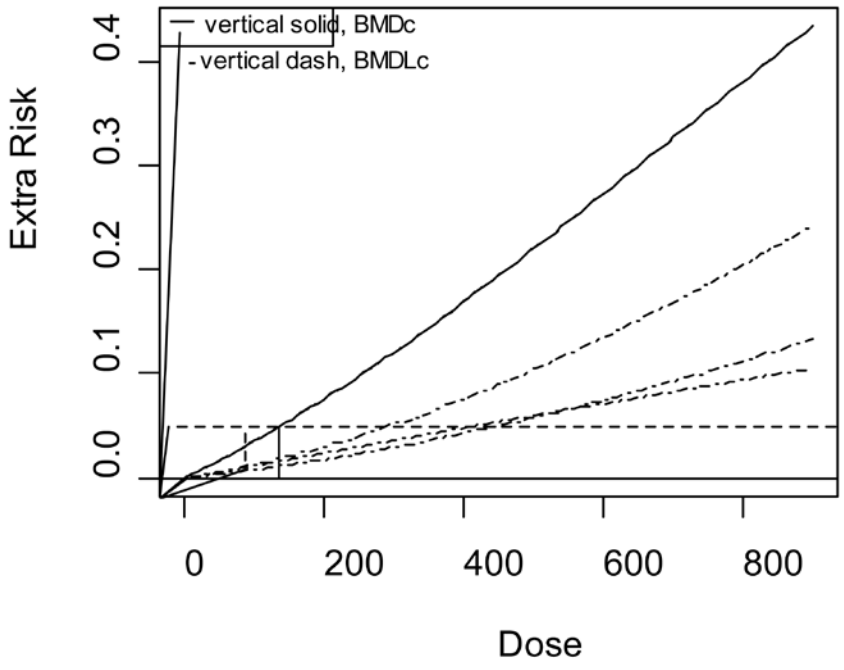


Figure G-7. Female B6C3F$_1$ mice—internal dose-metric (total oxidative metabolism): combined and individual tumor extra-risk functions.

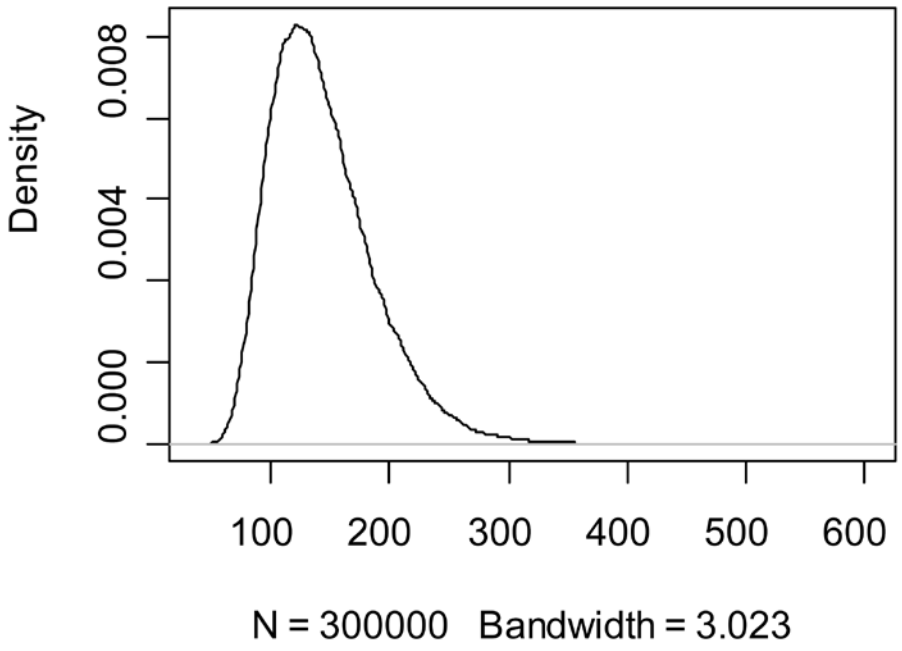


Figure G-8. Female B6C3F$_1$ mice—internal dose-metric (total oxidative metabolism): posterior distribution of BMDc for combined risk.

Table G-20. $B6C3F_1$ female mice inhalation exposure—internal dose-metric (total oxidative metabolism)

Internal dose[a]	Liver hepatomas/*N*[b]	Lung adenomas + carcinomas/*N*[b]
0	3/88	2/90
280.946	4/89	6/90
622.530	4/88	7/89
939.105	9/85	14/87

[a]Internal dose, Total Oxidative Metabolism, adjusted for body weight, units (mg/[wk-kg$^{3/4}$]). Internal doses were adjusted by a factor 0.75, accounting for exposure duration 78/104 weeks. Before adjustment, median internal doses were 374.5945, 830.0405, 1,252.14 (mg/[wk-kg$^{3/4}$]).
[b]Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study

Source: Maltoni (1986).

Table G-21. $B6C3F_1$ female mice—internal dose: model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	Model order, selected[a]	Coefficient estimates equal zero	AIC	Largest+ scaled residual	Goodness of fit *p*-value
Liver	3[a]	β1, β2	153.1	-0.410	0.8511
	2	β1	153.4	-0.625	0.7541
	1	NA	154	-0.816	0.5571
Lung	3	β2	195.8	-0.571	0.3995
	2	NA	195.9	-0.671	0.3666
	1[a]	NA	194	-0.776	0.6325

[a]Model order selected + largest in absolute value.

Source: Maltoni (1986).

Table G-22. $B6C3F_1$ female mice inhalation exposure—internal dose-metric (total oxidative metabolism) (inferences for 0.05 extra risk at 95% confidence level)

	Liver hepatomas	Lung adenomas + carcinomas
Parameters used in models	q0, q1, q2, q3	q0, q1
p-Value for BMDS model	0.5571	0.6325
BMD_{05} (from BMDS)	813.7	366.7
BMD_{05} (median, mode—WinBUGS)	672.9, 648.0	382.8, 372.1
BMDL (BMDS)[a]	419.7	244.6
ms_combo BMD_{05}c, BMDLc	412.76, 189.23	
BMDL (5^{th} percentile, WinBUGS)	482.7	251.1
BMD_{05} for combined risk (median, mode, from WinBUGS)	286.7, 263.1	
BMDL for combined risk (5^{th} percentile, WinBUGS)	199.5	
BMDS maximum likelihood risk estimates		
Risk at dose 100	0.006284	0.01389
Upper 95% confidence limit	0.01335	0.02215
Sum of risks at dose 100	0.02017	
WinBUGS Bayes risk estimates: means (medians)		
Risk at dose 100: mean, median	0.003482, 0.002906	0.01337, 0.01331
Upper 95% confidence limit,	0.008279	0.02022
Combined risk at dose 100 mean, median	0.01637, 0.01621	
Combined risk at dose 100, upper 95% confidence limit	0.02455	

[a]All CIs are at 5% (lower) or 95% (upper) level, one-sided.

Source: Maltoni (1986).

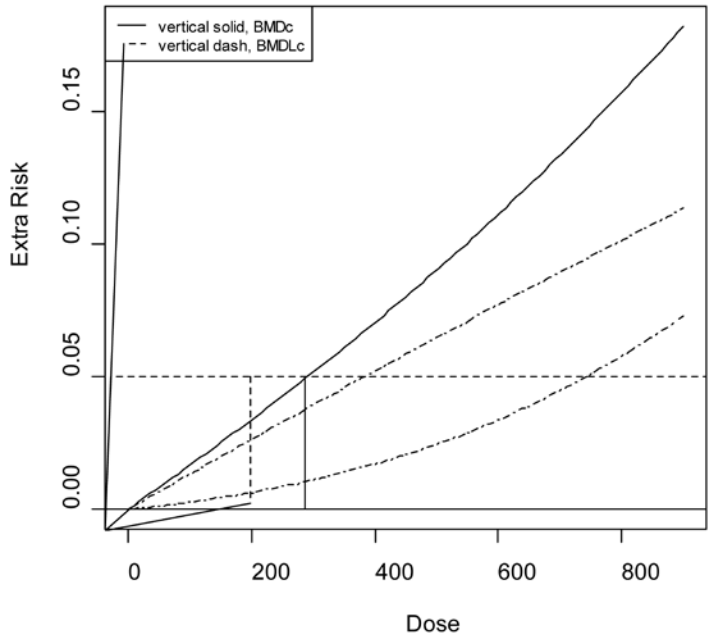


Figure G-9. B6C3F$_1$ female mice inhalation exposure—internal dose-metric: combined and individual tumor extra-risk functions.

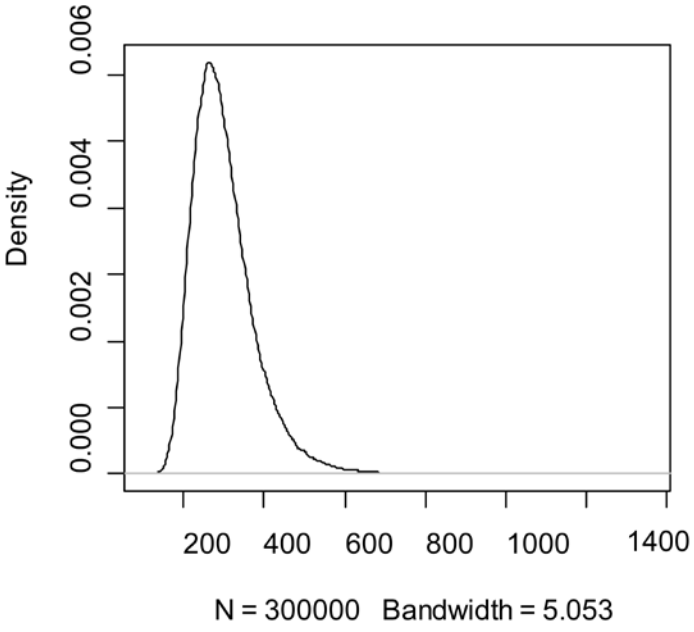


Figure G-10. B6C3F$_1$ female mice inhalation exposure—internal dose-metric: posterior distribution of BMDc for combined risk.

Table G-23. Maltoni Sprague-Dawley male rats—internal dose-metric (total metabolism)

Internal dose[a]	Kidney adenomas + carcinomas/*N*[b]	Leukemias/*N*[b]	Testis, Leydig cell tumors/*N*[b]
0	0/121	9/134	6/121
214.6540	1/118	13/130	16/116
507.0845	0/116	14/130	30/116
764.4790	5/123	15/129	31/122

[a]Internal dose, Total Oxidative Metabolism, adjusted for body weight, units [mg/(wk-kg$^{3/4}$)].
[b]Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study.

Table G-24. Maltoni Sprague-Dawley male rats—internal dose model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	Model order, selected	Coefficient estimates equal zero	AIC	Largest[a] scaled residual	Goodness of fit *p*-value
Kidney	3	γ, β2	61.35	–1.264	0.262
	2	γ	61.75	–1.343	0.246
	1[a]	γ	60.32	–1.422	0.370
Leukemias	3	β2, β3	336.5	0.479	0.828
	2	β2	336.5	0.479	0.828
	1[a]	NA	336.5	0.479	0.828
Testis, Leydig cell tumors	3	β2, β3	417.7	1.008	0.363
	2	β2	417.7	1.008	0.363
	1[a]	NA	417.7	1.008	0.363

[a]Largest in absolute value.

Table G-25. Maltoni Sprague-Dawley male rats—internal dose-metric (total metabolism) (inferences for 0.01 extra risk at 95% confidence level)

	Kidney adenomas + carcinomas	Leukemias	Testis, Leydig cell tumors
Parameters used in models	q0, q1	q0, q1	q0, q1
p-Value for BMDS model	0.3703	0.8285	0.3626
BMD_{01} (from BMDS)	295.1	145.8	26.65
BMD_{01} (median, mode—WinBUGS)			
BMDL (BMDS)[a]	161.3	65.29	20.32
BMDL (5^{th} percentile, WinBUGS)			
BMD_{01} for combined risk (median, mode, from WinBUGS)	20.97, 19.73		
BMDL for combined risk (5^{th} percentile, WinBUGS)	16.14		
BMDS maximum likelihood risk estimates			
Risk at dose 100	0.003400	0.0068694	0.0370162
Upper 95% confidence limit	0.0068784	0.0169134	0.0504547
Sum of risks at dose 100	0.04729		
Risk at dose 10	0.0003406	0.0006891	0.0037648
Upper 95% confidence limit	0.0006900	0.0017044	0.0051638
Sum of risks at dose 10	0.004795		
WinBUGS Bayes risk estimates: means (medians)			
Risk at dose 100: mean, median	0.003191, 0.003028	7.691×10^{-3}, 7.351×10^{-3}	0.03641, 0.03641
Upper 95% confidence limit	0.006044	1.539×10^{-2}	0.04769
Combined risk at dose 100—mean, median	0.04688, 0.04680		
Combined risk at dose 100, upper 95% confidence limit	0.060380		
Risk at dose 100—mean, median	3.196×10^{-4}, 3.032×10^{4}	7.726×10^{-4}, 7.376×10^{4}	0.003705, 0.003703
Upper 95% confidence limit	6.060000×10^{-4}	1.550000×10^{-3}	0.004874000
Combined risk at dose 10—mean, median	0.004793, 0.0047820		
Combined risk at dose 10, upper 95% confidence limit	0.006208		

[a]All CIs are at 5% (lower) or 95% (upper) level, one-sided.

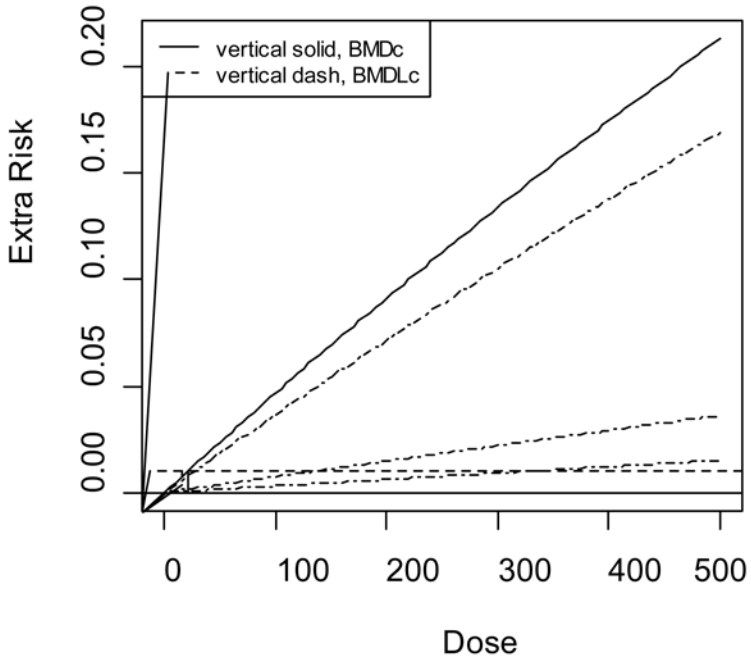


Figure G-11. Maltoni Sprague-Dawley male rats—internal dose-metric: combined and individual tumor extra-risk functions.

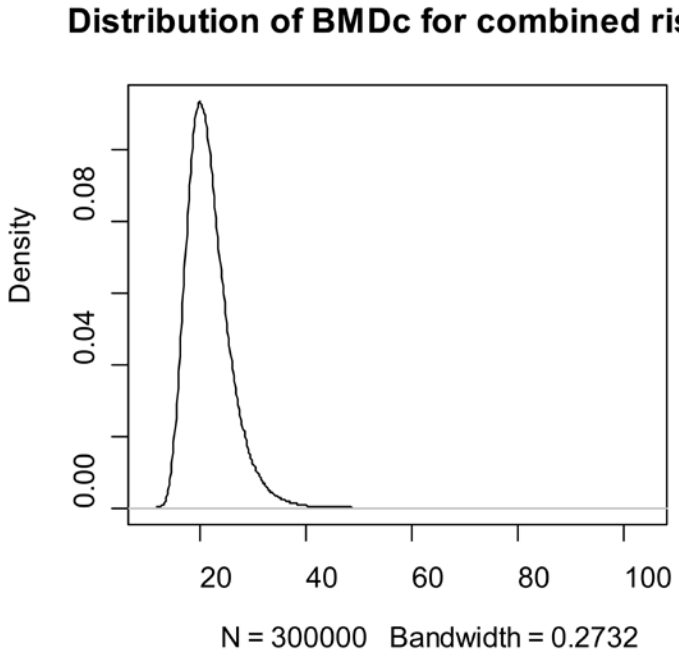


Figure G-12. Maltoni Sprague-Dawley male rats—internal dose-metric: posterior distribution of BMDc for combined risk.

G.9. PBPK-MODEL UNCERTAINTY ANALYSIS OF UNIT RISK ESTIMATES

As discussed in Section 5.2, an uncertainty analysis was performed on the unit risk estimates derived from rodent bioassays to characterize the impact of pharmacokinetic uncertainty. In particular, two sources of uncertainty are incorporated: (a) uncertainty in the rodent internal doses for each dose group in each chronic bioassay and (b) uncertainty in the relationship between exposure and the human population mean internal dose at low exposure levels.

A Bayesian approach provided the statistical framework for this uncertainty analysis. Rodent bioassay internal dose-response relationships were modeled with the multistage model, with general form:

$$P(id) = 1 - \exp[-(q_0 + q_1 id + q_2 id^2 + ... + q_k id^k)], \qquad \text{(Eq. G-9)}$$

where $P(id)$ represents the lifetime risk (probability) of cancer at *internal* dose id, and multistage parameters $q_i \geq 0$, for $i = 0, 1, ..., k$. Since the BMD (in internal dose units) for a given BMR can be derived from the multistage model parameters q_i, it is sufficient to estimate the posterior distribution of q_i given the combined bioassay data (for each dose group j, the number responding y_j, the number at risk n_j, and the administered dose d_j) and the rodent pharmacokinetic data, for which the posterior distribution can be derived using the Bayesian analysis of the PBPK model described in Section 3.5. In particular, the posterior distribution of q_i can be expressed as:

$$P(q_{[i]}|D_{bioassay}\, D_{pk}) \propto P(q_{[i]})\, P(y_{[j]}|\, q_{[i]}\, n_{[j]})\, P(id_{[j]}|d_{[j]}, D_{pk}) \qquad \text{(Eq. G-10)}$$

Here, the first term after the proportionality $P(q_{[i]})$ is the prior distribution of the multistage model parameters (assumed to be noninformative), the second term $P(y_{[j]}|q_{[i]}\, n_{[j]})$ is the likelihood of observing the bioassay response given a particular set of multistage parameters and the number at risk (the product of binomial distributions for each dose group), and $P(id_{[j]}|d_{[j]}, D_{pk})$ is the posterior distribution of the rodent internal doses $id_{[j]}$, given the bioassay doses and the pharmacokinetic data used to estimate the PBPK model parameters.

The distribution of unit risk ($UR_{id} = BMR/BMD$) estimates in units of "per internal dose" is then derived deterministically from the distribution of multistage model parameters:

$$P(UR_{id}|D_{bioassay}\, D_{pk\text{-}rodent}) = \int P(q_{[i]}|D_{bioassay}\, D_{pk\text{-}rodent})\, \delta[UR - BMR/BMD(q_{[i]})]\, dq_{[i]} \qquad \text{(Eq. G-11)}$$

Here δ is the Dirac delta-function. Then, the distribution of unit risk estimates in units of "per human exposure" (per mg/kg/day ingested or per continuous ppm exposure) is derived by converting the unit risk estimate in internal dose units:

$$P(UR_{human}|D_{bioassay}\ D_{pk\text{-}rodent}) = \int P(UR_{id}|D_{bioassay}\ D_{pk\text{-}rodent})\ P(id_{conversion}|D_{pk\text{-}human})$$
$$\delta(UR_{human} - UR_{id} \times id_{conversion})\ d\mathrm{id}_{conversion} \qquad \text{(Eq. G-12)}$$

Here, $id_{conversion}$ is the population mean of the ratio between internal dose and administered exposure at low dose (0.001 ppm or 0.001 mg/kg/day), and $P(id_{conversion}|D_{pk\text{-}human})$ is its posterior distribution from the Bayesian analysis of the human PBPK model.

This statistical model was implemented via Monte Carlo as follows. For each bioassay, for a particular iteration r ($r = 1 \ldots n_r$),

(1) A sample of rodent PBPK model *population* parameters $(\mu,\Sigma)_{rodent,r}$ was drawn from the posterior distribution. Using these population parameters, a single set of *group* rodent PBPK model parameters $\theta_{rodent,r}$ was drawn from the population distribution. As discussed in Section 3.5, for rodents, the population model describes the variability among groups of rodents, and the group-level parameters represent the "average" toxicokinetics for that group.

(2) Using $\theta_{rodent,r}$, the rodent PBPK model was run to generate a set of internal doses $id_{[j],r}$ for the bioassay.

(3) Using this set of internal doses $id_{[j],r}$, a sample $q_{[i],r}$ was selected from the distribution (conditional on $id_{[j],r}$) of multistage model parameters, generated using the WinBUGS, following the methodology of Kopylev et al. (2007).

(4) The unit risk in internal dose units $UR_{id,r} = BMR/BMD(q_{[i],r})$ was calculated based on the multistage model parameters.

(5) A sample of human PBPK model *population* parameters $(\mu,\Sigma)_{human,r}$ was drawn from the posterior distribution. Using these population parameters, multiple sets of *individual* human PBPK model parameters $\theta_{human,r,[s]}$ ($s = 1 \ldots n_s$) were generated. A continuous exposure scenario at low exposure was run for each individual, and the population mean internal dose conversion was derived by taking the arithmetic mean of the internal dose conversion for each individual: $id_{conversion,r} = \mathrm{Sum}(id_{conversion,r,s})/n_s$.

(6) The sample for the unit risk in units per human exposure was calculated by multiplying the sample for the unit risk in internal dose units by the sample for the population internal dose conversion: $UR_{human,r} - UR_{id,r} \times id_{conversion,r}$.

In practice, samples for each of the above distributions were "precalculated," and inferences were performed by re-sampling (with replacement) according to the scheme above. For the results described in Section 5.2, a total of $n_r = 15{,}000$ samples was used for deriving summary statistics. For calculating the unit risks in units of internal dose, the BMDs were derived by re-sampling from a total of 4.5×10^6 multistage model parameter values (1,500 rodent PBPK model parameters from the Bayesian analysis described in Section 3.5, for each of which there were conditional distributions of multistage model parameters of length 3,000 derived

using WinBUGS). The conversion to unit risks in units of human exposure was re-sampled from 500 population mean values, each of which was estimated from 500 sampled individuals.

A supplementary data file ("Supplementary data for TCE assessment: Cancer rodents uncertainty analysis," 2011) contains summary statistics (mean, and selected quantiles from 0.01 to 0.99) from these analyses, and is the source for the results presented in Chapter 5 (see Tables 5-41 and 5-42). Histograms of the distribution of unit risks in per unit human exposure are in separate supplementary data files for the rodent inhalation bioassays ("Supplementary data for TCE assessment: Cancer rodents uncertainty CSF-inhalation histograms, inhalation bioassays,") and for the rodent oral bioassays ("Supplementary data for TCE assessment: Cancer rodents uncertainty CSF-oral histograms, oral bioassays," 2011). Route-to-route extrapolated unit risks are in other supplementary data files for inhalation unit risks extrapolated from oral bioassays ("Supplementary data for TCE assessment: Cancer rodents uncertainty CSF-inhalation historams, oral bioassays," 2011) and for oral unit risks extrapolated from inhalation bioassays ("Supplementary data for TCE assessment: Cancer rodents uncertainty CSF-oral histograms, inhalation bioassay," 2011)). Each figure shows the uncertainty distribution for the male and female combined population risk per unit exposure (transformed to base-10 logarithm), with the exception of testicular tumors, for which only the population risk per unit exposure for males is shown.

H. LIFETABLE ANALYSIS AND WEIGHTED LINEAR REGRESSION BASED ON RESULTS FROM CHARBOTEL ET AL. (2006)

H.1. LIFETABLE ANALYSIS

A spreadsheet illustrating the extra-risk calculation for the derivation of the lower 95% bound on the effective concentration associated with a 1% extra risk (LEC_{01}) for RCC incidence is presented in Table H-1.

H.2. EQUATIONS USED FOR WEIGHTED LINEAR REGRESSION OF RESULTS FROM CHARBOTEL ET AL. (2006) [SOURCE: ROTHMAN (1986), P. 343–344]

Linear model: $RR = 1 + bX$

where RR = risk ratio, X = exposure, and b = slope.

b can be estimated from the following equation:

$$\hat{b} = \frac{\sum_{j=2}^{n} w_j x_j \hat{RR}_j - \sum_{j=2}^{n} w_j x_j}{\sum_{j=2}^{n} w_j x_j^{\,2}} \qquad \text{(Eq. H-1)}$$

where j specifies the exposure category level and the reference category ($j = 1$) is ignored.

The standard error of the slope can be estimated as follows:

$$SE(\hat{b}) \approx \sqrt{\frac{1}{\sum_{j=2}^{n} w_j x_j^{\,2}}} \quad . \qquad \text{(Eq. H-2)}$$

The weights, w_j, are estimated from the CIs (as the inverse of the variance):

$$Var(\hat{RR}_j) \approx \hat{RR}_j^{\,2} Var[\ln(\hat{RR}_j)] \approx \hat{RR}_j^{\,2} \times \left[\frac{\ln(\overline{RR}_j) - \ln(\underline{RR}_j)}{2 \times 1.96}\right]^2 \qquad \text{(Eq. H-3)}$$

where $\overline{RR}_j$ is the 95% upper bound on the RR_j estimate (for the jth exposure category) and $\underline{RR}_j$ is the 95% lower bound on the RR_j estimate.

Table H-1. Extra-risk calculation[a] for environmental exposure to 1.82 ppm TCE (the LEC_{01} for RCC incidence)[b] using a linear exposure-response model based on the categorical cumulative exposure results of Charbotel et al. (2006), as described in Section 5.2.2.1.2.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Interval number (*i*)	**Age interval**	**All cause mortality (× 10^5/yr)**	**RCC incidence (× 10^5/yr)**	**All cause hazard rate (*h**)**	**Prob. of surviving interval (*q*)**	**Prob. of surviving up to interval (*S*)**	**RCC cancer hazard rate (*h*)**	**Cond. prob. of RCC incidence in interval (*Ro*)**	**Exp. duration mid interval (*x*time)**	**Cum. exp. mid interval (*x*dose)**	**Exposed RCC hazard rate (*hx*)**	**Exposed all cause hazard rate (*h***x*)**	**Exposed prob. of surviving interval (*qx*)**	**Exposed prob. of surviving up to interval (*Sx*)**	**Exposed cond. prob. of RCC in interval (*Rx*)**
1	<1	685.2	0	0.0069	0.9932	1.0000	0.000000	0.000000	0.5	2.77	0.000000	0.0069	0.9932	1.0000	0.000000
2	1–4	29.9	0	0.0012	0.9988	0.9932	0.000000	0.000000	3	16.61	0.000000	0.0012	0.9988	0.9932	0.000000
3	5–9	14.7	0	0.0007	0.9993	0.9920	0.000000	0.000000	7.5	41.52	0.000000	0.0007	0.9993	0.9920	0.000000
4	10–14	18.7	0.1	0.0009	0.9991	0.9913	0.000005	0.000005	12.5	69.20	0.000006	0.0009	0.9991	0.9913	0.000006
5	15–19	66.1	0.1	0.0033	0.9967	0.9903	0.000005	0.000005	17.5	96.88	0.000006	0.0033	0.9967	0.9903	0.000006
6	20–24	94	0.2	0.0047	0.9953	0.9871	0.000010	0.000010	22.5	124.56	0.000013	0.0047	0.9953	0.9871	0.000013
7	25–29	96	0.7	0.0048	0.9952	0.9824	0.000035	0.000034	27.5	152.24	0.000049	0.0048	0.9952	0.9824	0.000048
8	30–34	107.9	1.6	0.0054	0.9946	0.9777	0.000080	0.000078	32.5	179.91	0.000117	0.0054	0.9946	0.9777	0.000114
9	35–39	151.7	3.2	0.0076	0.9924	0.9725	0.000160	0.000155	37.5	207.59	0.000245	0.0077	0.9924	0.9724	0.000237
10	40–44	231.7	6.3	0.0116	0.9885	0.9651	0.000315	0.000302	42.5	235.27	0.000504	0.0118	0.9883	0.9650	0.000484
11	45–49	352.3	11	0.0176	0.9825	0.9540	0.000550	0.000520	47.5	262.95	0.000919	0.0180	0.9822	0.9537	0.000869
12	50–54	511.7	17.3	0.0256	0.9747	0.9373	0.000865	0.000801	52.5	290.63	0.001507	0.0262	0.9741	0.9367	0.001393
13	55–59	734.8	26.2	0.0367	0.9639	0.9137	0.001310	0.001175	57.5	318.31	0.002375	0.0378	0.9629	0.9124	0.002127
14	60–64	1,140.1	36.2	0.0570	0.9446	0.8807	0.001810	0.001549	62.5	345.99	0.003409	0.0586	0.9431	0.8786	0.002909
15	65–69	1,727.4	44.6	0.0864	0.9173	0.8319	0.002230	0.001777	67.5	373.67	0.004358	0.0885	0.9153	0.8286	0.003456
16	70–74	2,676.4	49	0.1338	0.8747	0.7631	0.002450	0.001750	72.5	401.35	0.004961	0.1363	0.8726	0.7584	0.003518
17	75–59	4,193.2	51.6	0.2097	0.8109	0.6675	0.002580	0.001554	77.5	429.03	0.005407	0.2125	0.8086	0.6617	0.003223
18	80–84	6,717.2	44.4	0.3359	0.7147	0.5412	0.002220	0.001021	82.5	456.71	0.004809	0.3384	0.7129	0.5351	0.002183
							***Ro* =**	0.010736						***Rx* =**	0.020586
Extra risk = (*Rx* – *Ro*)/(1 – *Ro*) = 0.00996															

Column A: interval index number (i).
Column B: 5-year age interval (except <1 and 1–4) up to age 85.
Column C: all-cause mortality rate for interval i ($\times 10^5$/year) [2004 data from CDC (2007)].
Column D: RCC incidence rate for interval i ($\times 10^5$/year) (2001–2005 SEER data [http://seer.cancer.gov]).
Column E: all-cause hazard rate for interval i (h^*_i) [= all-cause mortality rate × number of years in age interval].[c]
Column F: probability of surviving interval i without being diagnosed with RCC (q_i) [= $\exp(-h^*_i)$].
Column G: probability of surviving up to interval i without having been diagnosed with RCC (S_i) [$S_1 = 1$; $S_i = S_{i-1} \times q_{i-1}$, for i > 1].
Column H: RCC incidence hazard rate for interval i (h_i) [= RCC incidence rate × number of years in interval].
Column I: conditional probability of being diagnosed with RCC in interval i [= $(h_i/h^*_i) \times S_i \times (1-q_i)$] (i.e., conditional upon surviving up to interval i without having been diagnosed with RCC) [Ro, the background lifetime probability of being diagnosed with RCC = the sum of the conditional probabilities across the intervals].
Column J: exposure duration (in years) at mid-interval ($x$time).
Column K: cumulative exposure mid-interval ($x$dose) [= exposure level (i.e., 1.82 ppm) × 365/240 × 20/10 × $x$time] (365/240 × 20/10 converts continuous environmental exposures to corresponding occupational exposures).
Column L: RCC incidence hazard rate in exposed people for interval i (hx_i) [= $h_i \times (1 + \beta \times x\text{dose})$, where $\beta = 0.001205 + (1.645 \times 0.0008195) = 0.002554$] [0.001205 per ppm × year is the regression coefficient obtained from the weighted linear regression of the categorical results (see Section 5.2.2.1.2). To estimate the LEC_{01} (i.e., the 95% lower bound on the continuous exposure giving an extra risk of 1%), the 95% upper bound on the regression coefficient is used (i.e., MLE + 1.645 × SE).
Column M: all-cause hazard rate in exposed people for interval i (h^*x_i) [= $h^*_i + (hx_i - h_i)$].
Column N: probability of surviving interval i without being diagnosed with RCC for exposed people (qx_i) [= $\exp(-h^*x_i)$].
Column O: probability of surviving up to interval i without having been diagnosed with RCC for exposed people (Sx_i) [$Sx_1 = 1$; $Sx_i = Sx_{i-1} \times qx_{i-1}$, for i > 1].
Column P: conditional probability of being diagnosed with RCC in interval i for exposed people [= $(hx_i/h^*x_i) \times Sx_i \times (1-qx_i)$] (Rx, the lifetime probability of being diagnosed with RCC for exposed people = the sum of the conditional probabilities across the intervals).

[a]Using the methodology of BEIR IV (1988).
[b]The estimated 95% lower bound on the continuous exposure level of TCE that gives a 1% extra lifetime risk of RCC.
[c]For the cancer incidence calculation, the all-cause hazard rate for interval i should technically be the rate of either dying of any cause or being diagnosed with the specific cancer during the interval, i.e., the all-cause mortality rate for the interval + the cancer-specific incidence rate for the interval—the cancer-specific mortality rate for the interval [so that a cancer case isn't counted twice, i.e., upon diagnosis and upon death]) × number of years in interval. This adjustment was ignored here because the RCC incidence rates are small compared with the all-cause mortality rates.

I. EPA RESPONSE TO MAJOR PEER REVIEW AND PUBLIC COMMENTS

I.1. PBPK MODELING (SAB REPORT SECTION 1): COMMENTS AND EPA RESPONSE

I.1.1. SAB Overall Comments:

The Panel commended the updated PBPK model (Chiu et al., 2009; Evans et al., 2009) for dose-response assessment. The Panel found that while the PBPK model was generally well presented, its description was incomplete in that mass-balance equations were not presented. The Panel provided suggestions to improve model documentation and clarity, including clearer descriptions of the strategy behind the model structure and the biological relevance of each model equation. Model assumptions need to be more clearly described and the consequences of potential violations of these assumptions should be discussed. In addition, a more detailed justification was needed for the handling of between-animal variability in the model. The Panel agreed that use of the Bayesian framework for estimation and characterization of the PBPK model parameter uncertainties was appropriate. However, a more thorough description was needed for the choice of prior distributions, the Bayesian fitting methodology, and the fit of the posterior distribution for each model parameter. The Panel also generally endorsed the hierarchical calibration approach that uses the posterior results in mice to establish the rat priors, and the rat posterior results to set the human priors. The Panel also recommended performance of a local sensitivity analysis to identify key model parameters that drive changes in modeling results.

I.1.2. Major SAB Recommendations and EPA Response:

I.1.2.1. PBPK Model Structure (SAB Report Section 1a)

- Provide a better description of the final model structure and, in particular, provide a revised model structure diagram that identifies model parameters with model states and pathways (flows).

EPA response: EPA accepts this recommendation and has provided revised model structure diagrams in Appendix A, Section A.4.1.

- Clarify the strategy behind the model structure and describe the biological relevance of each model equation.

EPA response: EPA accepts this recommendation and has clarified the model structure and equations, and their biological relevance, in Appendix A, Section A.4.1.

- Document model assumptions and discuss the consequences of potential violations of these assumptions (e.g., impacts on bias and accuracy).

EPA response: EPA accepts this recommendation and has expanded the discussion of limitations of the model to include added discussion of model assumptions and the consequences of potential violations in Section 3.5.7.4.

- Provide a more detailed justification for how between animal variability is accounted for in the model.

EPA response: EPA accepts this recommendation and has expanded the discussion of how between animal variability is addressed in the model in Section 3.5.5.2.

I.1.2.2. Bayesian Statistical Approach (SAB Report Section 1b)

- Present better descriptions and/or details on the choice of prior distributions, the Bayesian fitting methodology and fit of the posterior distribution for each model parameter.

EPA response: EPA accepts this recommendation and has added a description of the choice of prior distribution functions in Section 3.5.5.2; presented a description of the overall Bayesian posterior distribution function used in the parameter fitting in Section A.4.4; and added graphical presentation to Section A.5.1 of the posterior distributions, in comparison with the prior distribution, for each model parameter. In addition, the use of the terms "population" and "group" have been clarified throughout Chapter 3 and Appendix A.

- Provide some information on correlations around posterior medians for species-specific parameters.

EPA response: EPA accepts this recommendation and provided tables of correlation coefficients in Appendix A, Section A.5.1.

- Supply more information on the model ordinary differential equations and on the likelihood function used in the Bayesian estimation.

EPA response: EPA accepts this recommendation and has supplied more information on the model ordinary differential equations in Appendix A, Section A.4.1, and more information on the likelihood function in Appendix A, Section A.4.3.4.

I.1.2.3. Parameter Calibration (SAB Report Section 1c)

- Improve the quality and the description of the assumptions underlying the use of the hierarchical approach to parameter calibration. Help the reader to understand the extent to which these assumptions are used consistently throughout the parameter calibration process.

EPA response: EPA accepts this recommendation and revised Table A-4 to clarify the scaling assumptions consistently used throughout the parameter calibration process, and revised Section 3.5.5.3 to clarify the description of the assumptions underlying the hierarchical approach.

I.1.2.4. Model Fit Assessment and Dose-Metric Projections (SAB Report Section 1d)

- Move some graphical presentations from the linked graphics documents into the body of the report or into Appendix A.

EPA response: EPA accepts this recommendation and has moved (in a more condensed form) graphical presentations of the PBPK model predictions as compared to the in vivo data to the body of Appendix A.

- Incorporate more discussion on model fit and in particular indicate areas where the model fits well and areas where it did not fit well. Tie this discussion somehow to Table 3-41.

EPA response: EPA accepts this recommendation and has incorporated more discussion of model fit in Section 3.5.6.3 indicating areas where the model fits well and areas where it did not fit well. This discussion is tied to the Table previously labeled "3-41," as recommended. In addition, the interpretation of the residual error GSD is more closely tied to this revised discussion.

- Include graphs that show predicted vs. observed values for all data points used in the analysis (one graph per endpoint).

EPA response: EPA accepts this recommendation and has added graphics showing predicted vs. observed values for all data points used in the analysis (one graph per endpoint) to Section 3.5.6.3. The width of the residual error GSDs are also included on these graphs for comparison. In addition, this is tied to the revised discussion on model fit and the Table previously labeled "3-41."

- To help readers identify which parameters are better specified than others, provide a table of model parameters listed in reverse order by the width of their posterior variability (width of the IQR or width of 95% CI).

EPA response: EPA accepts this recommendation and has added a table to Section 3.5.6.2 of model parameters listed in reverse order by the width of their posterior variability, indicated by the width of 95% CI.

- Identify those parameters with very different prior and posterior distributions and discuss why this might be a reasonable result of the parameter calibration process. An alternative would be to provide a table where parameters are ranked based on the percent change of the posterior from the prior.

EPA response: EPA accepts this recommendation and has included a table in Section 3.5.6.2 that indicates the fold-change between the prior and posterior medians. This table is already sorted by reverse order of the width of the posterior variability (see previous recommendation). In order to identify those parameter with more different priors and posteriors, the fold-change was bolded if the change was greater than threefold. It is noted in the revised text for

Section 3.5.6.2 that those parameters with shifts >3-fold had prior CIs greater (sometimes substantially) than 100-fold, so that such shifts are reasonable in that context.

- Clarify which parameters are related to variability and which address parameter uncertainty. Separate the discussion of the two types of parameters.

EPA response: EPA accepts this recommendation and has replaced the tables in Section 3.5.6.2 that previously showed combined uncertainty and variability with tables that separately summarize parameter uncertainty and variability. This separation of uncertainty and variability has the added benefit of removing the appearance that posterior parameter distributions appear flatter than prior distributions, since posterior parameter uncertainty should always be less than or equal to prior parameter uncertainty. In addition, the text of Section 3.5.6.2 has been revised to discuss separately estimates of the central tendency of the population from estimates of population variability.

I.1.2.5. Lack of Adequate Sensitivity Analysis (SAB Report Section 1e)

- Perform a local sensitivity analysis, starting from the final fitted PBPK model, to assess how small changes in model parameter estimates impact predictions. Provide graphical presentations of the sensitivity of the model to changes in key model parameters in the final documentation.

EPA response: EPA accepts this recommendation and has conducted a local sensitivity analysis starting from the final fitted PBPK model, and assessing how small changes (5% increase or decrease) in model parameter estimates impact predictions. Two types of model predictions are analyzed. First, in Section 3.5.6.4, the sensitivity of predictions of calibration data is assessed, including a graphical presentation of the number of data points that are sensitive to each parameter. Second, in Section 3.5.7.2, the sensitivity of prediction of dose-metrics is assessed, including a graphical presentation of the sensitivity coefficient for each parameter and dose-metric. The results of these local sensitivity analyses confirms that the calibration data inform the value of most model parameters, with the remaining parameters either informed by substantial prior information or having little sensitivity with respect to dose metric predictions.

I.1.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with the extent and degree of variability of GSH conjugation in humans predicted by the PBPK model.

EPA response: In accordance with SAB recommendations (see response below in Section I.5.2.3), EPA has revised the discussions in Sections 3.3 and 3.5 to reflect the uncertainty in GSH conjugation predictions in humans.

- Some public commenters disagreed with the extent of population variability predicted by the PBPK model for some parameters.

EPA response: The External Review Draft reported posterior distributions as lumped uncertainty and variability. For the parameters raised as a concern in the comments, the high apparent variability is actually predominantly uncertainty, so the extent of population variability is not exceedingly high. In accordance with SAB recommendations (see response above in Section I.1.2.4), EPA has revised the description of posterior parameters to separate uncertainty and variability, providing additional clarity to the posterior predictions.

- Some public commenters recommended that EPA perform a sensitivity analysis on the PBPK model.

EPA response: In accordance with SAB recommendations (see response above in Section I.1.2.5), EPA has conducted a local sensitivity analysis of the PBPK model.

- Some public commenters recommended that EPA incorporate additional data in its PBPK model.

EPA response: In accordance with SAB recommendations (see response below in Section I.5.2.2), EPA incorporated additional data on TCA bioavailability in the TCA submodel of the PBPK model. Other data cited by the commenters were evaluated in Appendix A for the purposes of additional validation, but were not directly incorporated in the PBPK model.

I.2. META-ANALYSES OF CANCER EPIDEMIOLOGY (SAB REPORT SECTION 2): COMMENTS AND EPA RESPONSE

I.2.1. SAB Overall Comments:

The Panel agreed that EPA's updated meta-analyses for kidney cancer, lymphoma and liver cancer followed the NRC (2006) recommendations. The Panel agreed with EPA's conclusions that TCE increased the risk for the three cancers studied, based on appropriate inclusion criteria for studies, the methods of conducting the meta-analysis that included consideration of bias and confounding, and the robustness of the findings based on the tests for heterogeneity and sensitivity. The Panel also suggested performing a meta-analysis for lung cancer to further support the absence of smoking as a possible confounder.

I.2.2. Major SAB Recommendations and EPA Response:

- Provide a rationale for the three cancer sites selected for the meta-analysis. The rationale could be nicely summarized in a table.

EPA Response: EPA accepts this recommendation and has added text to Section 4.1 and Appendix C.

- Consider including meta-analysis for lung cancer for confounding purposes or other sites for comparison for which some association with TCE exposure has been reported in epidemiologic studies, such as childhood leukemia and cervical cancer. It might also be possible to provide this information without a formal meta-analysis.

EPA Response: EPA accepts this recommendation and has included a meta-analysis for lung cancer in Appendix C. Additionally, in the discussion in Chapter 4 of the possible role of smoking in confounding the association between TCE exposure and kidney cancer, EPA compares the RR estimates for lung and kidney cancers in five smoking cohorts and discusses the expected contribution by smoking to kidney cancer in Raaschou-Nielsen et al. (2003), which was estimated as 1–6%, far smaller than the 20–40% excess reported in this study. Meta-analyses were not conducted for other cancer types for which there may have been suggestive associations because there was inadequate reporting in the cohort studies, and for childhood leukemia, there were too few studies of sufficient quality.

- Provide measures of heterogeneity such as the I^2 statistic for each meta-analysis. Although this information was provided and accurately explained in Appendix C, it was mischaracterized at several points in the primary document. For example, the summary of the kidney cancer meta-analysis on p. 4-167 of the primary document states that "there was no observable heterogeneity across the studies for any of the meta-analyses," but Appendix C indicates "the I^2 value of 38% suggested the extent of the heterogeneity was low-to-moderate." Non-significant heterogeneity is indeed observed heterogeneity.

EPA Response: EPA accepts this recommendation and has provided measures of heterogeneity in the primary document. EPA has also corrected this sentence in Section 4.4.2.5; it now reads "there was no observable heterogeneity for any of the meta-analyses of the 15 studies and no indication of publication bias."

- Evaluate the likely impact of converting ORs to RR estimates [i.e., using the method of Greenland (2004) or Zhang and Yu (1998)], and decide if necessary to perform these conversions for the meta-analysis.

EPA Response: The papers cited by the SAB describe methods for correcting ORs in studies of common outcomes. Each of the cancer types for which EPA did meta-analyses has a background incidence <10% and is thus considered a rare disease, so no correction should be necessary. In the case of rare diseases, only high ORs might notably overestimate RRs. In the TCE studies, only Hardell et al. (1994) reported an OR high enough to be of potential concern, a Mantel-Haenszel-adjusted OR of 7.2 for NHL. According to Zhang and Yu (1998), the Mantel-Haenszel adjustment is a suitable way to estimate the RR; in fact, in the example they provide, the Mantel-Haenszel adjustment outperforms the adjustment they are proposing. Furthermore, according to McNutt et al. (2003), the Zhang and Yu method is incorrect when applied to an adjusted OR and will produce a biased estimate when confounding is present. Additionally, the model-based methods for estimating a RR from a case-control study described by Greenland

(2004) are only applicable when one has the raw data. Thus, neither of the papers cited by the SAB provides a satisfactory way to convert the Hardell et al. (1994) OR. However, any overestimation that might occur by treating the Hardell et al. (1994) OR as an RR estimate is negligible in the overall analysis. Removing the study all together only decreases the RRm from 1.23 to 1.21, and the latter result is still statistically significant (p = 0.004).

- Change the terminology regarding the meta-analysis results for 'lymphoma' to 'non-Hodgkin lymphoma' throughout the document.

EPA Response: EPA accepts this recommendation and has revised the terminology throughout the document.

I.2.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters requested a glossary of epidemiology terms be included in the document.

EPA response: EPA did not implement this recommendation, as definitions of epidemiologic terms can be easily found from authoritative sources on the internet.

- Some public commenters suggested that EPA examine the TCE subregistry for information about the association between TCE and cancer.

EPA response: EPA did not implement this recommendation with respect to cancer, as the ATSDR TCE subregistry provides only limited information on cancer outcomes, as analyses are for total cancers and less informative than for cancer types. EPA did consider, however, observations on neurotoxicity and other noncancer outcomes.

- Some public commenters disagreed with the meta-analysis conclusions from the External Review Draft, noting heterogeneity of findings, lack of consistent exposure-response, and other methodological problems. These comments noted that while EPA's meta-analysis methods and summaries are generally consistent with recent published summaries of this literature, the commenters did not agree with EPA's interpretation. These comments asserted that it is more accurate to report the epidemiologic evidence as "mixed" rather than "consistent" or "robust."

  Other public commenters agreed with the meta-analysis conclusions from the External Review Draft, noting that epidemiologic studies are usually biased towards the null, making it harder to detect a true causal relationship.

EPA response: In accordance with the SAB review, EPA maintains its meta-analysis conclusions. EPA agrees with the public commenters that the characterization of the general association between overall TCE exposure and cancer is "modest"; this was one of the points explicitly brought out in the discussion in Section 4.11.2.1.2 concerning the strength of the association. EPA also carefully considered the questions raised by the public commenters

regarding consistency of the results and regarding alternative explanations for these findings. This consideration is discussed in detail in Section 4.11.2.1. A strength of the meta-analytic approach is its ability to assess heterogeneity among studies, which is of particular importance in situations in which the overall RR estimate is modest and in situations in which results from individual studies may be quite imprecise because of sample size limitations. In reviewing the available data, including the results of the meta-analyses, EPA noted that chance was not supported as an explanation for the findings, nor was there support for confounding by other known or suspected risk factors as an explanation for the results.

I.3. NONCANCER HAZARD ASSESSMENT (SAB REPORT SECTION 3): COMMENTS AND EPA RESPONSE

I.3.1. SAB Overall Comments:

EPA has provided a comprehensive synthesis of the available evidence regarding the effects of TCE and its major metabolites on the CNS, kidney, liver, immune system, male reproductive system, and developing fetus. One issue of concern was the inconsistencies between reported levels of GSH conjugation pathway metabolites. The Panel recommended that the impact of these divergent levels be more transparently presented. The Panel recommended inclusion of the potential for TCE-induced immune dysfunctions (i.e., immunosuppression, autoimmunity, inappropriate and/or excessive inflammation) to mechanistically underlie other adverse health endpoints.

I.3.2. Major SAB Recommendations and EPA Response:

- If additional endpoints of renal dysfunction (e.g., diuresis, increased glucose excretion) were present in the reported studies, they should be included in the report. Often, only one or two parameters of renal function and histopathology were presented. A better overall description of renal dysfunction should be presented if available (especially for animal studies).

EPA Response: EPA accepts this recommendation, and has added the information to all studies where such data are available.

- There should be a better description of the location of the renal lesion, including nephron segment, if known. For example, TCE and DCVC appeared to affect the proximal tubule at the level of the outer stripe of the medulla (S3 segment of proximal tubule). Is this the site of lesions seen with other TCE metabolites? Explaining the role (or lack of a role) of any other TCE metabolites in TCE nephrotoxicity could be strengthened by comparing the sites of the renal lesion.

EPA Response: EPA accepts this recommendation, and has added the information to all studies where such data are available.

- On page 4-338, please clarify the use of the phrase, "subpopulation levels," on lines 31 and 33.

EPA Response: EPA accepts this recommendation, and has clarified the use of "subpopulations."

- A statement should be added that the spectrum of TCE-induced immune dysfunctions (immunosuppression, autoimmunity, inappropriate and/or excessive inflammation) included in this EPA draft report has the potential to produce adverse effects that are seen well beyond lymphoid organs and involving several other physiological tissues and systems. The types of immune-inflammatory dysfunctions described in this report have been observed to affect function and risk of disease in the nervous system (e.g., loss of hearing), the skin, the respiratory system, the liver, the kidney, the reproductive system (e.g., male sterility), and the cardiovascular system (e.g., heart disease, atherosclerosis).

EPA Response: EPA accepts this recommendation, and has added statements to Sections 4.6 and 4.6.3.1 that immume-related and inflammatory effects, particularly cell-mediated immunity, may influence a broader range of disease, including cancer.

- A statement should be added to emphasize the cell-mediated immune effects of TCE as some of this has been supported by the human epidemiology data and the issue is pertinent to risk of cancer.

EPA Response: See previous response.

I.3.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with EPA's draft conclusion that TCE poses a human health hazard for developmental cardiac effects due to limitations in the available data.

EPA response: In accordance with the SAB review, EPA acknowledges the limitations of the available data, but maintains its conclusion that TCE poses a human health hazard for developmental cardiac effects.

- Some public commenters disagreed with EPA's draft conclusion TCE poses a human health hazard for immunotoxicity because additional confirmatory studies are needed.

EPA response: In accordance with the SAB review, EPA concludes that adequate data are available to conclude that TCE poses a human health hazard for immunotoxicity.

I.4. CARCINOGENIC WEIGHT OF EVIDENCE (SAB REPORT SECTION 4): COMMENTS AND EPA RESPONSE

I.4.1. SAB Overall Comments:

The Panel agreed with EPA's conclusion that TCE is "carcinogenic to humans" by all routes of exposure. This is based on convincing evidence of a causal association between TCE exposure and kidney cancer, compelling evidence for lymphoma, and more limited evidence for

liver cancer as presented in the draft document. The epidemiologic data, in the aggregate, were quite strong. The summary risk estimates from the meta-analyses provided a clear indication of a cancer hazard from TCE. In addition, both animal data and toxicokinetic information provide biological plausibility and support the epidemiologic data.

I.4.2. Major SAB Recommendations and EPA Response:

- The immune effects as highlighted in the hazard assessment should be referred to in the conclusion, especially in the criteria of biological plausibility and coherence because of the relationship between immune system dysfunction and cancer risk.

EPA Response: EPA accepts this recommendation, and has added a statement to Section 4.11.2.1.6 that immune-related effects should also be considered in the biologic plausibility of TCE carcinogenicity.

- Although the summary evaluation focused on the scientific evidence and meta-analysis for kidney, lymphoma, and liver cancers, there is also some suggestive evidence for TCE as a risk factor for cancer at other sites including bladder, esophagus, prostate, cervix, breast, and childhood leukemia. This evidence that also supports the conclusion should be mentioned in the summary evaluation (Section 4.11.2.1).

EPA Response: EPA accepts this recommendation, and has added a statement mentioning the suggestive evidence of association between TCE and other types of cancer in Section 4.11.2.1.10.

- Add a paragraph describing the definition of lymphoma as used in IRIS. Change the terminology regarding the meta-analysis to 'non-Hodgkin lymphoma' instead of 'lymphoma', throughout the document. The term 'NHL' more accurately describes the intent of the analysis as well as the overwhelming majority of cases in the analysis, despite changing classification schemes. The focus of the meta-analysis on NHL and the exact classifications the meta-analysis includes where it may diverge from classical NHL (as in studies that included CLL) should be clearly explained in both Appendix C and in the Hazard Characterization document (Section 4.6.1.2.2).

EPA Response: EPA accepts this recommendation and has added text describing the definition of NHL as used in the assessment, in addition to revising the terminology and indicating divergent case definitions in both Appendix C and Section 4.6.1.2.2.

- To assist the reader, please include references in the summary section (Section 4.11.2). For example, "The other 13 high-quality studies [note: besides Hardell and Hansen] reported elevated RR estimates with overall TCE exposure that were not statistically significant." References for statements like this would be helpful. The Panel counted fewer than 13 studies in the meta-analysis after subtracting out Hardell and Hansen, and not all of these showed elevated risk estimates, so it would be helpful for the reader to know which 13 studies this statement refers to.

EPA Response: EPA accepts this recommendation and has added references to conclusions in Section 4.11.2.1.

I.4.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with EPA's draft conclusion in the External Review Draft that TCE is "carcinogenic to humans," judging the epidemiologic evidence to be inadequate due to limitations of the body of evidence. Limitations cited by these comments include exposure assessment limitations, potential unmeasured confounding, potential selection biases, and inconsistent findings across groups of studies. Comments also cited limitations in the experimental animal data, such as conflicting or negative experimental animal data for kidney and immune tumors. These comments suggested that a classification of "likely to be carcinogenic in humans" or "suggestive evidence of carcinogenicity" would be more appropriate. Some of these comments cited the NAS/NRC *Contaminated Water Supplies at Camp Lejeune: Assessing Potential Health Effects* (NRC, 2009) as support.

  Other public commenters supported EPA's draft conclusion in the External Review Draft that TCE is "carcinogenic to humans."

EPA response: In accordance with the SAB review, EPA concludes that TCE is "Carcinogenic to humans." EPA also notes that the NRC (2009) Camp Lejeune report was discussed during the SAB review meetings. The meeting minutes from the June 24, 2010 teleconference call state that "Panelists discussed the extent to which the EPA draft risk assessment document should discuss or compare its findings and conclusions to those of the 2009 NAS Report on Camp Lejeune. It was generally agreed that it was not necessary to compare EPA conclusions to all of the other reviews, particularly in view of the different criteria applied across reviews, different studies used across assessments and different scopes of each review and the fact that the current draft risk assessments carries out a meta-analysis that was not considered in the 2009 NAS review" (SAB, 2010a). In the meeting minutes from the December 15, 2010 SAB quality review teleconference call, the Panel chair stated that "the material reviewed by the Panel was different from what the NAS had reviewed" (SAB, 2010a, b).

I.5. ROLE OF METABOLISM (SAB REPORT SECTION 5): COMMENTS AND EPA RESPONSE

I.5.1. SAB Overall Comments:

The Panel agreed with EPA's conclusion that oxidative metabolites of TCE were likely responsible for mediating the liver effects. The Panel recommended that EPA examine studies that provided quantitative assessment of TCA and DCA formation after TCE exposure. Dose-response modeling, similar to that performed for tetrachloroethylene, may be considered by EPA

to provide scientifically-based information on relative contribution, or lack thereof, of TCA and/or DCA to the liver carcinogenesis effect of TCE.

EPA has provided a clear and comprehensive summary of the available evidence that metabolites derived from GSH conjugation of TCE mediate kidney effects. The Panel noted that uncertainties exist with regard to the extent of formation of the dichlorovinyl metabolites of TCE between humans and rodents. The issue of quantitative assessment of the metabolic flux of TCE through the GSH pathway vs. the oxidative metabolism pathway needs to be considered carefully. A more complete discussion of the strengths and limitations of the analytical methodologies used should be provided to address the large discrepancies in estimates of DCVG formation.

I.5.2. Major SAB Recommendations and EPA Response:

I.5.2.1. Mediation of TCE-Induced Liver Effects by Oxidative Metabolism (SAB Report Section 5a)

- No major recommendations in this section.

I.5.2.2. Contribution of TCA to Adverse effects on the Liver (SAB Report Section 5b)

- The EPA should examine studies that provide quantitative assessment of TCA and DCA formation after TCE exposure in vivo and draw conclusions with regards to the relative amount and kinetics of the oxidative metabolites of interest for liver toxicity.

EPA response: Most studies of TCA following TCE exposure have already been incorporated into the PBPK model-based analyses, and previous studies of DCA following TCE exposure are limited by the rapid clearance of DCA at low concentrations and analytical artifacts in DCA detection. Section 4.5.6.1 has been revised to include discussion of the studies by Delinksy et al. (2005) and Kim et al. (2009), which use more reliable analytic methods to quantify DCA formation after TCE exposure *in vivo*.

- A careful evaluation of the concentration-time kinetics is needed to achieve certainty in the comparisons of liver effects and the conclusions drawn by the EPA, which suggest that TCA-induced adverse liver effects do not explain those observed with TCE. Equally important is to fully consider the bioavailability of TCE itself with regards to the vehicle effects between studies.

EPA response: EPA assumes that the first part of this comment refers to the issue of TCA bioavailability, which is mentioned in the narrative text preceding this recommendations. EPA has incorporated into Section 4.5.6.2.1 an updated analysis of TCA bioavailability and its impact on conclusions regarding the role of TCA in TCE-induced hepatomegaly (Chiu, 2011). With respect to TCE vehicle effects, there are not enough kinetic data using different vehicles to quantitatively address vehicle effects. However, it is noted that if they are important, they may

be a significant contributor to the residual variability in the combined analysis of TCE-induced hepatomegaly.

- The body of the document could be further strengthened by reporting EPA's evaluation on the strength of the specific criteria used for phenotypic classification described in each study discussed, and noting where specific criteria were not reported. While most of this information was included in the appendix, the EPA may consider constructing a summary table for Section 4.5.6.

EPA response: Section 4.5.6.3.3.1 has been revised to note that no specific criteria are usually given as to what constitutes "basophilic" or "eosinophilic," with the one exception of Carter et al. (2003) noted. For immunochemical staining, it is noted that some studies use negative controls as a comparison.

- Dose-response modeling, similar to that performed for PERC, may be considered by the EPA to provide science-based information on relative contribution, or lack thereof, of TCA and/or DCA to the apical liver carcinogenesis effect of TCE. While data gaps exist and there are limitations in the comparisons between independent cancer bioassays, the document should clearly state what the limitations are should such analysis be deemed futile.

EPA response: EPA agrees that a quantitative analysis of the relative contributions of TCA and/or DCA to TCE-induced liver carcinogenesis would be useful if feasible. However, as noted in the revised Section 4.5.6.3.2.5, such an analysis is precluded by the high degree of heterogeneity both within and across the databases for TCE and its oxidative metabolites. The revised section gives notes substantial variability across bioassays in characteristics such as study duration, control group incidences, and apparent carcinogenic potency, thus precluding either quantitative analysis.

- The draft assessment may be strengthened by including information from human use of DCA in clinical practice.

EPA response: Human data on use of DCA in clinical practice is summarized in EPA's Toxicological Review of Dichloroacetic Acid (2003b), and reference has been made to this document in Section 4.5.6. In particular, it is noted that data on DCA in humans are scarce and complicated by the fact that available studies have predominantly focused on individuals who have a pre-existing (usually severe) disease.

I.5.2.3. Role of GSH-Conjugation Pathway on TCE-Induced Kidney Effects (SAB Report Section 5c)

- The issue of quantitative assessment of the metabolic flux of TCE through the GSH pathway vs. the oxidative metabolism pathway should be considered carefully since uncertainties exist with regard to the extent of formation of the dichlorovinyl metabolites

of TCE between humans and rodents. EPA may need to provide appropriate reservations to the conclusions based on the limited data for GSH metabolites.

- The discussion of how each of the in vitro and in vivo data sets were used to estimate DCVG formation parameters for the PBPK model should be more transparent indicating strengths and weaknesses in the database.

EPA responses: EPA accepts these two related recommendations. EPA has revised Section 3.3.3.2 to articulate additional reservations as to its conclusions regarding the except of formation of dichlorovinyl metabolites of TCE between rodents and humans, and to be more transparent regarding the strengths and weaknesses in vitro and in vivo datasets. In addition, cross-references to this discussion have been added in the context of the PBPK model parameters and predictions to Sections 3.5.4.3, 3.5.5.1, 3.5.6.3.3, 3.5.7.3.1, 3.5.7.3.2, 3.5.7.4, and 3.5.7.5.

I.5.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with EPA's conclusion that DCA may play a role in TCE-induced liver effects. These commenters also recommended that EPA take into account the bioavailability of TCA in its evaluation of liver effects.

EPA response: In accordance with SAB recommendations, EPA has re-examined the data on the contributions of TCA and/or DCA to TCE-induced liver effects, including incorporation of data on TCA bioavailability, in Section 4.4. However, EPA's conclusion remains that TCA cannot adequately account for account the liver effects of TCE.

- Some public commenters disagreed with EPA's conclusion that GSH-conjugation-derived metabolites play the primary role in TCE-induced nephrotoxicity and nephrocarcinogenicity.

EPA response: EPA maintains its conclusions, and notes that both the SAB review and the NRC (2006) report support the conclusion that the GSH pathway is primarily responsible for TCE-induced kidney effects.

I.6. MODE OF ACTION (SAB REPORT SECTION 6): COMMENTS AND EPA RESPONSE

I.6.1. SAB Overall Comments:

The Panel agreed that the weight of evidence supports a mutagenic mode of action for TCE-induced kidney tumors. However, the Panel concluded that the weight of evidence also supported an mode of action involving cytotoxicity and compensatory cell proliferation and including these may more accurately reflect kidney tumor formation than does a mutagenic mechanism alone. The combination of cytotoxicity, proliferation and DNA damage together may be a much stronger mode of action than any individual components.

The Panel agreed that the data are inadequate to conclude that any of the TCE-induced cancer and noncancer effects in rodents are not relevant to humans.

The Panel agreed that there is inadequate support for peroxisome proliferator activated receptor alpha (PPARα) agonism and its sequellae being key events in TCE-induced human liver carcinogenesis. Recent data from animal models (Yang et al., 2007) suggest that activation of PPARα is an important, but not limiting, factor for the development of mouse liver tumors, and additional molecular events may be involved. The Panel viewed the mode of action for liver carcinogenicity in rodents as complex rather than unknown. It is likely that key events from several pathways may operate leading to acute, subchronic, and chronic liver toxicity of TCE.

I.6.2. Major SAB Recommendations and EPA Response:

I.6.2.1. Hazard Assessment and Mode of Action (SAB Report Section 6a)

- The impact of the inconsistencies in data on the quantity of GSH pathway metabolites formed in humans and rodents should be presented more transparently.

EPA Response: EPA accepts this recommendation, and has added discussion and/or mention of the quantitative uncertainties with respect to GSH conjugation wherever relevant throughout the document.

- In the body of the document, mode-of-action information should be systematized and broken down into key events for each proposed mode of action. The EPA may consider using a tabular format to facilitate the ease of evaluation. Information on supporting/refuting (if any) evidence (with appropriate references indicated), human relevance (if available), and "strength" of each line of evidence/study should be included.

EPA Response: EPA accepts this recommendation, and has added tables summarizing the proposed modes of action and conclusions for kidney and liver carcinogenesis.

- EPA should consider tabular summaries by specific metabolites when studies used metabolite exposure rather than the parent compound.

EPA Response: EPA considered this recommendation, but decided against adding the tables for the metabolites because in most cases (TCA, DCA, and CH), those studies are described and tabulated in detail in other toxicological reviews.

- Data gaps should be clearly identified to help guide future research.

EPA Response: EPA considered this recommendation, and decided to focus on data gaps related to dose-response, as these will have the greatest impact on any future revision to the Toxicological Review. These research needs are now included as a separate section at the end of Chapter 5.

- Key conclusions supporting/refuting each key event should be presented in bullet form indicating where in the document a more detailed narrative/tables can be found.

EPA Response: EPA accepts this recommendation, and has included key conclusions in the summary mode-of-action tables described above for kidney and liver carcinogenesis.

I.6.2.2. Mode of Action for TCE-Induced Kidney Tumors (SAB Report Section 6b)

- Modify the relevant text to reflect that the available data do, in fact, provide support for TCE-induced kidney tumors involving cytotoxicity and compensatory cell proliferation, possibly in combination with a mutagenic mode of action, although not to the extent that support for a mutagenic mode of action was provided.

EPA Response: EPA accepts this recommendation and has included additional discussion along the lines suggested to the section on kidney tumor mode of action.

I.6.2.3. Inadequate Support for PPARα Agonism and its Sequellae Being Key Events in TCE-Induced Liver Carcinogenesis (SAB Report Section 6c)

- Graphical or tabular presentation of these data to strengthen the comparative analysis between metabolites and chemicals.
- Including some of the analyses that compare the receptor transactivation potency and the carcinogenic potential of TCA, DCA and other model peroxisome proliferators from Guyton et al. (2009) to strengthen the arguments.

EPA Response: EPA accepts these recommendations, and has added the tabular presentation of quantitative differences among PPARα agonists and the quantitative analyses comparing carcinogenic potential and the receptor transactivation potency or other short-term markers of PPARα activation from Guyton et al. (2009) to strengthen the comparative analysis and arguments.

I.6.2.4. Inadequate Data to specify Key Events and Modes of Action Involved in Other TCE-Induced Cancer and Noncancer Effects (SAB Report Section 6d)

- No major recommendations in this section.

I.6.2.5. Human Relevance of TCE-Induced Cancer and Noncancer Effects in Rodents (SAB Report Section 6e)

- The impact of potential overestimation of the extent of the GSH pathway in humans in Section 4.4.7 (Kidney) must be transparent

EPA Response: EPA accepts this recommendation, and has added discussion and/or mention of the quantitative uncertainties with respect to GSH conjugation wherever relevant throughout the document.

- The mode of action for carcinogenicity should be described as complex rather than unknown in Section 4.5.7.4. Mode of Action. With respect to conclusions regarding the liver, while the complete mode of action in animals may not be clear at this time, complex is a more appropriate descriptor since it is likely that key events from several pathways may operate leading to acute, subchronic, and chronic liver toxicity of TCE.

EPA Response: EPA accepts this recommendation, and has rephrased the liver mode of action conclusions in Section 4.5.7.4 along the lines suggested.

I.6.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with EPA's conclusion that a mutagenic mode of action is operative for TCE-induced kidney tumors, recommending instead that a mode of action involving cytotoxicity is involved.

EPA response: EPA maintains its conclusion, in accordance with the SAB review (see Section I.6.1, above), that a mutagenic mode of action is operative for TCE-induced kidney tumors. However, in accordance with the SAB recommendations (see Section I.6.2.2, above) and in partial response to this public comment, EPA has added additional discussion of the data supporting a mode of action involving cytotoxicity.

- Some public commenters disagreed with EPA's conclusion that there is inadequate support for PPARα agonism and its sequellae being key events in TCE-induced hepatocarcinogenesis. Other public commenters agreed with EPA's conclusions.

EPA response: In accordance with the SAB recommendations (see Section I.6.2.3, above), EPA has provided additional analysis to support its conclusions.

- Some public commenters disagreed with EPA's conclusion that a cytotoxic mode of action was inadequately supported for TCE-induced lung tumors, citing analogies to other chemicals and other indirect data.

EPA response: EPA has added discussion of data from other compounds hypothesized to have the same mode of action for inducing mouse lung tumors. However, in accordance with the SAB review, EPA still concludes that there are inadequate data to specify the key events and modes of action involved in TCE-induced lung cancer and noncancer effects.

I.7. SUSCEPTIBLE POPULATIONS (SAB REPORT SECTION 7): COMMENTS AND EPA RESPONSE

I.7.1. SAB Overall Comment:

The Panel found that EPA's hazard assessment provided a good review of potentially susceptible populations, and identified factors (genetics, lifestage, background, co-exposures, and pre-existing conditions) that may modulate susceptibility to TCE carcinogenicity and noncancer effects. However, the Panel disagreed with EPA's conclusion that toxicokinetic

variability can be adequately quantified using existing data. The Panel recommended that exposure to solvent mixtures should be considered for potential co-exposures, since exposure to more than one chemical with the same target organ likely increases risk.

I.7.2. Major SAB Recommendations and EPA Response:

- The Panel disagreed with the statement that "toxicokinetic variability in adults can be quantified given the existing data," as the main study characterizing toxicokinetic variability in adults was small ($n < 100$) and was composed of subjects selected non-randomly. The Hazard Assessment document should note the limitations of the adult data for toxicokinetic modeling in terms of uncertainty and possible bias in Section 4.10.3, and elsewhere in the document where these data are used for hazard characterization modeling.

EPA response: EPA accepts this recommendation and has added a statement in Section 4.10.3 noting the limitations of the toxicokinetic database.

- Section 4.10 of the Hazard Assessment should discuss explicitly the lack of data demonstrating modulation of health effects from TCE by the identified factors (genetics, lifestage, background, co-exposures, and pre-existing conditions), and the need for such data in risk assessment.

EPA response: A statement has been added to the introduction of Section 4.10 noting the lack of data on susceptible populations and the need for such data. A statement on the need for additional data to address uncertainties regarding susceptible populations has been added to Section 4.10.3. The title of Section 4.10.3 has been amended to now read "Uncertainty of Database and Research Needs for Susceptible Populations."

- EPA should make specific recommendations for studies that would fill the data gap for susceptible groups. For example, epidemiologic studies in which TCE exposure is well-characterized and in which internal comparisons can be made to determine whether there is effect modification, and animal studies comparing subgroups (e.g., based on genetics, obesity, multiple solvent exposures).

EPA response: Where appropriate, statements on the need for additional research to fill data gaps regarding susceptible populations have been added where appropriate throughout Section 4.10.

- Modulation of TCE exposure-related hypersensitivity dermatitis by genetic variation may be relevant for future study, given results of the study of hypersensitivity dermatitis in Asian workers reported in Li et al. (2007) and increasing industrial chemical exposures in China.

EPA response: The need for future research on the relationship between genetic variation and generalized hypersensitivity skin diseases is now highlighted in Section 4.10.3.

- The wording in Section 4.10 was often not clear about whether it was describing results for a study that looked at effect modification of the TCE effect or not, as opposed to direct effects of age, gender, etc. Also, the draft document needs to state explicitly where effects of TCE within one subgroup were stated, whether the other subgroup was also examined in the same study.

EPA response: Additional clarification was added throughout Section 4.10 where necessary to address when the results were unrelated to TCE exposure or related to TCE exposure. Additional information was also added regarding the comparison group.

- The Panel recommended that exposure to solvent mixtures should be added as a potential susceptibility factor (co-exposures) to Section 4.10, since exposure to more than one chemical to the same target organ likely increases risk.

EPA response: A new Section 4.10.2.6 has been added on mixtures. This text is broader than solvent mixtures, as there are available studies that address exposure to TCE together with non-solvents.

- Section 4.10.2.4.1 (page 4-585) should be more accurately titled 'Obesity', rather than 'Obesity and metabolic syndrome'. As presently written, Section 4.10.2.4.1 gives no clear message as to how obesity affected the kinetics of TCE, and the section should be revised to provide clarification.

EPA response: As recommended, Section 4.10.2.4.1 has been retitled as "Obesity," and the text has been amended to more clearly present the data on toxicokinetics of TCE as it relates to obesity.

I.7.3. Summary of Major Public Comments and EPA Responses:

- Some comments noted that there is widespread exposure to TCE, including potentially vulnerable subpopulations.

EPA response: No response needed.

- Some comments questioned why EPA was not basing its assessment on in utero exposures.

EPA response: For noncancer effects, studies with in utero exposures were considered and, in one case used, for the basis of the RfC or RfD. No data on in utero exposures and cancer effects were located that were adequate for dose-response analysis.

I.8. NONCANCER DOSE-RESPONSE ASSESSMENT (SAB REPORT SECTION 8): COMMENTS AND EPA RESPONSE

I.8.1. SAB Overall Comments

I.8.1.1. Selection of Critical Studies and Effects

The Panel supported the selection of an RfC and RfD based on multiple candidate reference values that lie within a narrow range at the low end of the full range of candidate reference values developed, rather than basing these values on the single most sensitive critical endpoint. The Panel expressed concerns about the use of several candidate critical studies and effects, specifically NTP (1988) (toxic nephropathy), NCI (1976) (toxic nephrosis), and Woolhiser et al. (2006) (increased kidney weights). However, the Panel noted that uncertainties about the quantitative risk assessment based on kidney effects in NTP (1988), NCI (1976), and Woolhiser et al. (2006) did not indicate that there was uncertainty that TCE caused renal toxicity. As discussed previously, the three PBPK model-based candidate RfCs/RfDs (p-cRfCs/RfDs) for renal endpoints were based on an uncertain dose-metric, especially in regard to the relative rate of formation of the toxic metabolite in humans and animals. Additional issues related to choice of toxic nephropathy in female Marshall rats from NTP (1988) included excessive mortality due to dosing errors and possibly other causes, and a high level of uncertainty in the extrapolation to the BMD due to the use of very high doses and a high incidence (>60%) of toxic nephropathy at both dose levels used. With respect to toxic nephrosis in mice from NCI (1976), the BMD analysis was not supported because the effect occurred in nearly 100% of animals in both dose groups, and because a high level of uncertainty is associated with extrapolation from the LOAEL at which nearly 100% animals were affected. Renal cytomegaly and toxic nephropathy, which were not selected as critical effects, occurred at high frequency in all treated groups.

The Panel recommended that the two endpoints for immune effects from Keil et al. (2009) and the cardiac malformations from Johnson et al. (2003) be considered the principal studies supporting the RfC. The Panel also recommended that the endpoints for immune effects from Keil et al. (2009) and Peden-Adams et al. (2008) and the cardiac malformations from Johnson et al. (2003) be considered as the principal studies supporting the RfD.

I.8.1.2. Derivation of RfD and RfC

The screening, evaluation, and selection of candidate critical studies and effects used for the development of the RfC and RfD were sound. The derivation of the PODs was generally appropriate. However, the BMD modeling results were uncertain for some datasets. For example, the log-logistic BMD analysis for toxic nephropathy in female Marshall rats in NTP (1988), shown in Figure F-10 in Appendix F, may greatly overestimate the risks at low doses. As discussed above, this modeling involved extrapolation from a high LOAEL at which a high percentage of the animals were affected.

EPA used PBPK-based dose-metrics for interspecies, intraspecies, and route-to-route extrapolation. The Panel supported this approach for development of the RfC and RfD. The Panel noted that the candidate RfDs/RfCs for kidney endpoints were highly sensitive to the rate of renal bioactivation of the cysteine conjugate, DCVC, in humans relative to rodents. Candidate RfDs/RfCs developed using this dose-metric were several hundred-fold lower than RfD/RfCs for the same endpoints based on applied dose with standard UFs. The Panel noted that the uncertainties about the in vitro and in vivo data used to estimate the rate of renal bioactivation of DCVC were much greater than for other dose-metrics (e.g. there are large discrepancies in the rates of human GSH conjugation reported by Lash et al. (1999a) and Green et al. (1997a)]). These uncertainties should be clarified and should be the basis of a sensitivity analysis in the next update of the TCE draft risk assessment. The Panel also recommended that the rationale for scaling the dose-metric to body weight$^{3/4}$, in conjunction with the interspecies extrapolation based on PBPK modeling, should be presented in a clearer and more transparent way.

I.8.1.3. UFs

The Panel agreed that, in general, the selection of UFs was clearly and transparently described and appropriate. EPA developed equivalent doses and concentrations for sensitive humans to replace standard UFs for inter- and intra-species toxicokinetics. The Panel concluded that the approach used, including the selections of PODs and the extrapolations from rodent to human, followed by consideration of the 99th percentile human estimates, was acceptable to address the sensitive population. In future work, the variability and uncertainty could be better characterized by considering other quantiles of the distribution.

I.8.2. Major SAB Recommendations and EPA Response:

I.8.2.1. The Screening, Evaluation, and Selection of Candidate Critical Studies and Effects (SAB Report Section 8a)

- Chapter 5 should include a list of all noncancer health effects and studies discussed in Chapter 4, noting those that were considered candidate critical effects and studies.

EPA Response: EPA considered this recommendation and concluded that a list of *all* of the noncancer health effects and studies discussed in Chapter 4 would be overly long and redundant. As an alternative, first, EPA has ensured that each section of Chapter 4 includes tables of the relevant noncancer health effects and studies discussed, with studies and effects in **bold** designating those considered in Chapter 5. Second, EPA has added to Chapter 5 tables with the experimental details (e.g., which species, doses, effects) of the candidate studies for each endpoint type, with cross-references back to the tables in Chapter 4 that contain all of the studies for each type of effect. Therefore, there is now a transparent trace-back from the PODs used in Chapter 5 (tables in the external review draft), to the experimental details from which the POD

was derived (new tables in Chapter 5), to the larger set of studies considered for each effect type (tables in Chapter 4).

- Tables 5-1–5-5 should provide cross-references to the table or page in Chapter 4 and/or to the Appendices (such as Appendix E for hepatic studies) where the listed study was discussed, and should include more details (e.g., gender, strain, duration) of the studies selected as the basis for cRfDs and cRfCs when these details were needed to prevent ambiguity.

EPA Response: EPA accepts this recommendation and has addressed it as part of its response to the previous recommendation for a table in Chapter 5 listing all of the studies.

- Consistent dose units should be used in discussing the same study in different places in the document.

EPA Response: EPA accepts this recommendation and has checked the dose units used as it developed the new tables for Chapter 5.

I.8.2.2. The PODs, Including those Derived from BMD Modeling (e.g., Selection of Dose-Response Models, BMR Levels) (SAB Report Section 8b)

- Chapter 5 should include the information on POD derivation from Table F-13 of Appendix F, including approach, selection criterion and decision points.

EPA Response: After reviewing Chapter 5, EPA did not implement this suggestion. Chapter 5 describes the modeling approaches and selection criteria and important decisions in sufficient detail, and on page 5-3, the reader is directed to Appendix F for further details. The succeeding pages of Chapter 5 describe studies and effects by effect domain quite extensively, and the tables and footnotes contain sufficient detail on BMRs, PODs, and reasons for study selection. We think that it is appropriate to provide the mass of numerical modeling details in Appendix F, and that the modeling decisions are well described therein. Integrating this material into Chapter 5 would greatly increase the length of Chapter 5 and make it unwieldy for the reader.

I.8.2.3. The Selected PBPK-Based Dose-Metrics for Inter-Species, Intra-Species, and Route-to-Route Extrapolation, Including the Use of Body Weight to the ¾ Power Scaling for Some Dose-Metrics (SAB Report Section 8c)

- The uncertainty about the rate of human GSH conjugation found in Lash et al. (1999a) vs. Green et al. (1997a) should be highlighted in the current assessment.

EPA Response: EPA accepts this recommendation and has added discussion and/or mention of the quantitative uncertainties with respect to GSH conjugation wherever relevant throughout the document.

- The basis for the renal bioactivation dose-metric should be more clearly and transparently presented and discussed in Chapter 3 and other appropriate sections. If this dose-metric was derived indirectly from data on other metabolic pathways leading to and/or competing with bioactivation, this should be more clearly discussed.

EPA Response: EPA accepts this recommendation and has revised Section 3.5.7.3.1 to more clearly discuss the basis of the renal bioactivation dose-metric. In other sections of the document where the dose-metric is discussed, reference is made to Section 3.5.7.3.1.

- The rationale for scaling the dose-metric to body weight$^{3/4}$, in conjunction with the interspecies extrapolation based on PBPK modeling, should be presented in a clearer and more transparent way (e.g., on pp. 5-33–5-36).

EPA Response: EPA accepts this recommendation and has revised the discussion of this rationale substantially.

- The discussion of "empirical dosimetry" vs. "concentration equivalence dosimetry" should be made clearer and more transparent (pp. 5-33–5-36).

EPA Response: As noted by the SAB in the narrative preceding this recommendation, it is not necessary to include an extensive discussion of the two dosimetry approaches in these sections. EPA accepts this recommendation and has replaced this discussion with a clearer and more transparent rationale for the body weight$^{3/4}$ scaling.

I.8.2.4. UFs (SAB Report Section 8d)

- The definitions of chronic and subchronic studies should be provided in the document and a citation given.

EPA Response: EPA accepts this recommendation and has added a footnote with this information on page 5-6 in the paragraph describing UFs for subchronic-to-chronic extrapolation.

- The discussion of the subchronic to chronic UF on p. 5-6 should be clarified as far as durations of studies considered suitable as the basis of a chronic risk assessment.

EPA Response: There is no hard and fast rule in this area. Longer studies are generally preferred as the basis for a chronic risk assessment; however, in any given case, the basis of an RfC or RfD, or whether one is derived at all, will depend on the studies available and an assessment of their relevance for extrapolation to longer durations.

- The draft document should include discussion of whether studies in the lower end of the range defined as subchronic (e.g., 4 weeks) are of sufficient duration to be used as the basis for a chronic (lifetime) risk assessment.

EPA Response: EPA notes that studies of this duration have been evaluated for other risk assessments. For any study and endpoint that is used as a basis for a POD in this and previous

assessments, EPA has explained its applicability in the light of alternative studies of the same endpoint having longer and shorter duration and alternative studies and endpoints within the same domain having various durations.

- Studies only slightly longer than the minimum needed to be considered chronic should be noted as such, and the use of an UF to account for less than lifetime exposure (of less than the full UF of 10) could be considered for studies of such durations, especially for endpoints thought to progress in incidence or severity with time.

EPA Response: If there is evidence suggesting there might be further progression with increased exposure duration, a subchronic-to-chronic UF of 3 might be considered for a nominally chronic study. The example given by the panel could merit special justification of an UF of 3 if there were evidence that the response continued to increase with exposure durations longer than 18 weeks. No such evidence was found. For the study of Kulig et al. (1987), severity didn't progress beyond week 9 for the two-choice response, and in the 1,000 ppm exposure group, it didn't progress much in those first 9 weeks; thus, it is not anticipated that the 500 ppm response, which was flat over the 18 weeks, would become significant over an extended duration of exposure.

I.8.2.5. The Equivalent Doses and Concentrations for Sensitive Humans Developed from PBPK Modeling to Replace Standard Ufs for Inter- and Intra-Species Toxicokinetics, Including Selection of the 99th Percentile for Overall Uncertainty and Variability to Represent the Toxicokinetically-Sensitive Individual (SAB Report Section 8e)

- The Panel noted variability/uncertainty for the toxicokinetically-sensitive individual could be quantified in future work by considering distributions in addition to the distribution of the 99th percentile, such as the 95th percentile.

EPA Response: EPA agrees that this could be the subject of future work.

- A quantile regression looking simultaneously at several quantiles could be developed in the future and presented in future refinements of this assessment.

EPA Response: EPA agrees that this could be developed in the future and presented in future refinements of this assessment.

I.8.2.6. The Qualitative and Quantitative Characterization of Uncertainty and Variability (SAB Report Section 8f)

- The quantitative uncertainty analysis of PBPK model-based dose-metrics for LOAEL or NOAEL based PODs (Section 5.1.4.2) should be revised to clarify the objective of this 2-D type analysis, as well as the methodology used.

EPA Response: EPA accepts this recommendation and has revised the discussion, clarifying its objective and methodology.

- In future work, EPA could develop an approach using distribution to characterize uncertainty in a Bayesian framework.

EPA Response: EPA agrees that such an approach could be developed in future work.

I.8.2.7. The Selection of NTP (1988) [Toxic Nephropathy], NCI (1976) [Toxic Nephrosis], Woolhiser et al. (2006) [Increased Kidney Weights], Keil et al. (2009) [Decreased Thymus Weights and Increased Anti-dsDNA and Anti-ssDNA Antibodies], Peden-Adams et al. (2008) [Developmental Immunotoxicity], and Johnson et al. (2003) [Fetal Heart Malformations] as the Critical Studies and Effects for Noncancer Dose-Response Assessment (SAB Report Section 8g)

EPA Response: See recommendation in Section I.8.2.8, below.

I.8.2.8. The Selection of the Draft RfC and RfD on the Basis of Multiple Critical Effects for Which Candidate Reference Values are in a Narrow Range at the Low End of the Full Range of Candidate Critical Effects, Rather than on the Basis of the Single Most Sensitive Critical Effect (SAB Report Section 8h)

- The two endpoints for immune effects from Keil et al. (2009) and the cardiac malformations from Johnson et al. (2003) should be considered the principal studies supporting the RfC.

EPA Response: EPA accepts this recommendation and has revised Chapter 5 accordingly.

- The endpoints for immune effects from Keil et al. (2009) and Peden-Adams et al. (2008) and the cardiac malformations from Johnson et al. (2003) should be considered as the principal studies supporting the RfD.

EPA Response: EPA accepts this recommendation and has revised Chapter 5 accordingly.

I.8.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with the choices of critical studies for dose-response analyses of noncancer endpoints.

EPA response: In accordance with SAB recommendations (see Section I.8.2.8), EPA has selected the immune effects from Keil et al. (2009) and the cardiac malformations from Johnson et al. (2003) as the principal studies supporting the RfC, and the immune effects from Keil et al. (2009) and Peden-Adams et al. (2008) and the cardiac malformations from Johnson et al. (2003) as the principal studies supporting the RfD.

- Some public commenters recommended that EPA not rely on PBPK model-based estimates of DCVC bioactivation in conducting dose-response analysis for kidney endpoints.

EPA response: In accordance with SAB recommendations (see Section I.8.2.3), EPA has noted the uncertainties in the PBPK model-based DCVC bioactivation dose-metrics and considers the kidney effects as supporting, rather than as principal bases for, the RfC and RfD.

- Some public commenters recommended that EPA provide a more concise and consolidated characterization of the RfC and RfD determination, particularly in the context of kidney effects.

EPA response: A concise and consolidated characterization of the RfC and RfD determination appears in Sections 5.1.5.2 and 5.1.5.3. EPA has added discussion of the uncertainties related in kidney effects to these summary characterizations.

- Some public commenters recommended that EPA provide more discussion of the proportionality between applied and internal dose and its impact on the quantitative analysis.

EPA response: The impact of the proportionality of applied and internal dose, as well as its impact both dose-response analysis, is discussed in Section 5.1.3.3 and shown graphically in Appendix F.

- Some public commenters viewed the use of PBPK modeling as "double counting" variability, based on the idea that the observed dose-response is in part due to pharmacokinetic variability.

EPA response: In accordance with the SAB review, the methodology that EPA used is consistent with existing practice in the derivation of RfDs and RfCs. The methodology used is also consistent with previous applications of PBPK modeling in noncancer risk assessment. The comments highlight some uncertainties and ambiguities inherent in the RfD/RfC methodology, but disaggregating the multiple contributions to dose-response assessment—including effects of TK variation, TD variation, experimental error, stochasticity, and other factors in both the experimental animal and human population—requires development of new approaches that are beyond the scope of the assessment. While some published literature have addressed some of these issues, further research and development are needed, as no alternative approach has been generally accepted at the current time. EPA agrees with the SAB (see Section I.8.2.6, above) that a future research need is the development of an approach using distributions to characterize uncertainty in a Bayesian framework. Such an approach, when developed, could also help address the commenters' concerns.

I.9. CANCER DOSE-RESPONSE ASSESSMENT (INHALATION UNIT RISK AND ORAL UNIT RISK) (SAB REPORT SECTION 9): COMMENTS AND EPA RESPONSE

I.9.1. SAB Overall Comment:

In this assessment, EPA developed an inhalation unit risk and oral unit risk for the carcinogenic potency of TCE in accordance with the approach outlined in the U.S. EPA Cancer Guidelines (2005e, b). The unit risks for RCC were based on a case-control study published by Charbotel et al. (2006). The Panel found that the analysis of the Charbotel et al. (2006) data was well described and that the selection of this study to estimate unit risks was appropriate. However, more discussion is needed on whether or not it is necessary to adjust for exposure to cutting oils when computing an OR or RR relating TCE exposure to kidney cancer. The Panel recommended that EPA take a closer look at the literature to determine if there are other studies that suggest that exposure to cutting oils is a risk factor for kidney cancer. EPA should also provide a more detailed discussion on the implication of assumptions made in their analysis. In addition, background kidney cancer rates in the United States were used in constructing the life table, although the Charbotel et al. (2006) data were based on a French cohort. A comparison of background cancer rates in France and the United States would be helpful in supporting their conclusions. The Panel supported the adjustment of the RCC unit risks to account for the added risk of other cancers, using the meta-analysis results and Raaschou-Nielsen et al. (2003).

The Panel agreed that human data, when available, should be preferred over rodent data when estimating unit risk since within species uncertainty is easier to address than between species uncertainty. The Panel supported the use of linear extrapolation from the POD for cancer dose-response assessment of TCE as a default approach. The Panel agreed that characterization of uncertainty and variability was appropriate, and was exceptionally strong in the PBPK models.

I.9.2. Major SAB Recommendations and EPA Response:

I.9.2.1. Estimation of Unit Risks for RCC (SAB Report Section 9a)

- The Panel believed that more discussion was needed on whether or not it is necessary to adjust for exposure to cutting oils when computing an OR or RR relating TCE exposure to kidney cancer. The Panel recommended that EPA take a closer look at the literature to determine if there are other studies that suggest that exposure to cutting oils is a risk factor for kidney cancer.

EPA Response: EPA accepts this recommendation and has discussed other studies examining cutting fluids (Section 4.4.2.3). These studies suggest that potential confounding by cutting fluids is of minimal concern, and thus, including these exposures in the logistic regression may over-adjust because of the correlation with TCE exposure. Nonetheless, EPA has included, as a sensitivity analysis, the derivation of a unit risk estimate based on the Charbotel et al. (2006)

RCC ORs further adjusted for cutting fluids and petroleum oils, and this estimate is essentially the same as the original estimate (Section 5.2.2.1.3).

- The Panel believed that the EPA should provide a more detailed discussion of the limitations of their analysis. In particular, the model described on p. 5-131 made some very restrictive assumptions: linear dose-response and exposure was measured without error. In addition, the life table analysis applied the same estimated RR to each age interval; another restrictive assumption. While the Panel understood that these assumptions were necessary due to limited data, there was inadequate discussion of how violations of these assumptions may affect the results.

EPA Response: EPA accepts the recommendation and has added text pertaining to these assumptions. Note, too, that the uncertainties in the unit risk estimate, including uncertainties about the exposure assessment, are discussed in some detail in the uncertainty section (Section 5.2.2.1.3).

- Finally, in constructing the life table, the EPA used background kidney cancer rates in the United States though the Charbotel et al. (2006) data were based on a French cohort. Hence, a comparison of background cancer rates in France and the United States would be helpful in supporting their conclusions.

EPA Response: EPA accepts this recommendation, and has added additional information to Section 5.2.2.1.2. In particular, this section now notes that the usual assumption is that RR transfers across populations independent of background rates. In addition, this section now contains information comparing background kidney cancer rates in France and the United States.

I.9.2.2. Adjustment of RCC Unit Risks (SAB Report Section 9b)

- No major recommendations in this section.

I.9.2.3. Estimation of Human Unit Risks from Rodent Bioassays (SAB Report Section 9c)

- The Panel agreed that the analysis and results were appropriate but recommended that the EPA provide more details about their implementation and potential biases. For instance, in bioassays in which mortality occurred before time to first tumor, the authors simply adjusted their denominators to equal the number alive at time to first tumor. This approach assumed that drop-out prior to time to first tumor was unrelated to future risk of a tumor which could result in biased estimates.

EPA Response: EPA accepts this recommendation and has added a paragraph discussing the potential biases of this approach, along with citations to relevant literature, to Section G.1.1.

- In addition, more information was needed on the priors used in their Bayesian analysis of combined risk across tumor types.

EPA Response: EPA accepts this recommendation and has added this information to Section G.8.1.2.

I.9.2.4. Use of Linear Extrapolation for Cancer Dose-Response Assessment (SAB Report Section 9d)

- No major recommendations in this section.

I.9.2.5. Application of PBPK Modeling (SAB Report Section 9e)

- No major recommendations in this section.

I.9.2.6. Qualitative and Quantitative Characterization of Uncertainty and Variability (SAB Report Section 9f)

- No major recommendations in this section.

I.9.2.7. Conclusion on the Consistency of Unit Risk Estimates Based on Human Epidemiologic Data and Rodent Bioassay Data (SAB Report Section 9g)

- No major recommendations in this section.

I.9.2.8. Preference for the Unit Risk Estimates based on Human Epidemiologic Data (SAB Report Section 9h)

- No major recommendations in this section.

I.9.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters stated that the time courses of kidney cancer, liver and biliary cancer, and NHL do not support the hypothesis that TCE poses a great risk of cancer in the human population. These comments recommended that EPA perform a "validation" exercise to determine if the draft cancer classification and quantitative risk estimates are consistent with the observable facts concerning human cancer rates and other known risk factors for the tumor types listed.

EPA response: The analysis suggested by this comment is beyond the scope of the Toxicological Review. Moreover, such an analysis would require data that do not currently exist, including detailed historical population estimates not only of TCE exposure, but also of all other exposures and risk factors associated with each cancer, as well as quantitative estimates as to how each risk factor modulates the risk of cancer. It is noted, however, that limited

"validation" was performed by comparing qualitative and quantitative inferences based on epidemiologic data to those based on animal bioassay data. Further quantitative "validation" may be possible in the future if epidemiologic studies with quantitative exposure information are conducted.

- Some public commenters disagreed with the use of epidemiologic data as the primary basis for the cancer dose-response analysis.

EPA response: EPA maintains its conclusion in accordance with the SAB review (see Section I.9.1, above), that the epidemiological data are appropriate to use for estimating cancer risks. In response to recommendations by the SAB, EPA has provided more detailed discussions as to the limitations of the analysis.

- Some public commenters disagreed with the use of linear low-dose extrapolation for estimating cancer risks at levels below the POD, recommending instead the use of nonlinear extrapolation.

EPA response: EPA maintains its conclusion in accordance with the SAB review (see Section I.9.1, above), that the linear low-dose extrapolation is appropriate to use given the available data.

I.10. ADAFs (SAB REPORT SECTION 10): COMMENTS AND EPA RESPONSE

I.10.1. SAB Overall Comment:

The Panel agreed that application of ADAFs in the TCE analysis consistently followed recommendations in the U.S. EPA Cancer Guidelines (2005b). All of the steps were clearly presented for inhalation exposure. However, the discussion for the oral exposure route was shortened and referred back to the inhalation section, making understanding of the example difficult to follow. Currently, EPA's IRIS assessment provides lifetime cancer risk drinking water concentrations for adults only. The Panel recommended that drinking water concentrations for specified cancer risk levels should also be derived for various age groups.

I.10.2. Major SAB Recommendations and EPA Response:

- The Panel recommended that the statement on page 5-151, lines 14–18, be expanded to better explain why ADAFs were used for <16 years of age, but not for the elderly, and why EPA did not directly produce age dependent unit risks per mg/kg-day.

EPA Response: EPA accepts these recommendations. Section 5.2.3.3 notes that due to lack of appropriate data, no ADAFs are used for other life-stages, such as the elderly. ADAF-adjusted unit risks per ppm and per mg/kg-day are now presented in each sample calculation table in Sections 5.2.3.3.1 and 5.2.3.3.2.

- Include all details presented for the inhalation sample calculations as was done for the oral exposure sample calculations.

EPA Response: EPA accepts this recommendation and has revised Sections 5.2.3.3.1 and 5.2.3.3.2 to include all of the details for each sample calculation.

- IRIS assessments in which ADAFs are applied, such as TCE, should include estimated drinking water concentrations for specified lifetime cancer risk levels (10^{-4}, 10^{-5}, 10^{-6}), using representative drinking water intakes for various age groups, while noting that other drinking water estimates may be used if preferred.

EPA Response: EPA accepts this recommendation and has added drinking water concentrations for specified lifetime cancer risks under the assumptions used in the drinking water example calculation to Section 5.1.3.3.2. Similarly, EPA has added air concentrations for specified lifetime cancer risks under the assumptions used in the inhalation example calculation to Section 5.1.3.3.1.

- Include in the documentation a discussion of the perceived conflict between the use of ADAFs and the assumptions underlying the life table analysis of the Charbotel et al. (2006) data.

EPA Response: EPA accepts this recommendation and has added a discussion addressing the use of the ADAFs and the assumptions underlying the life table analysis.

I.10.3. Summary of Major Public Comments and EPA Responses:

- None

I.11. ADDITIONAL KEY STUDIES (SAB REPORT SECTION 11) AND EDITORIAL COMMENTS: COMMENTS AND EPA RESPONSE

- The Panel has identified additional studies to be considered in the assessment, as well as a number of editorial comments.

EPA Response: EPA has incorporated the additional studies in the appropriate sections, and addressed the editorial comments.