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TOXICOLOGICAL REVIEW

OF

DICHLOROMETHANE (METHYLENE CHLORIDE)

(CAS No. 75-09-2)

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

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LIST OF ABBREVIATIONS AND ACRONYMS

A1	ratio of lung V_{maxC} to liver V_{maxC}
A2	ratio of lung k_{fC} to liver k_{fC}
AB_{COC}	background amount of CO
ADAF	age-dependent adjustment factor
AIC	Akaike's Information Criterion
ALT	alanine aminotransferase
AP	alkaline phosphatase
AST	aspartate aminotransferase
AUC	area under the curve
BAER	brainstem-auditory evoked response
BMD	benchmark dose
$BMDL_{10}$	95% lower bound on the BMD
BMDS	benchmark dose software
BMR	benchmark response
BW	body weight
CAEP	cortical-auditory-evoked potential
CASRN	Chemical Abstracts Service Registry Number
CHO	Chinese hamster ovary
CI	confidence interval
CNS	central nervous system
CO	carbon monoxide
COHb	carboxyhemoglobin
CV	coefficient of variation
CYP	cytochrome P450
DAF	dosimetric adjustment factor
DNA	deoxyribonucleic acid
FEP	flash-evoked potential
FOB	functional observational battery
FracR	fraction of V_{maxC} in rapidly perfused tissues
GD	gestational day
GM	geometric mean
GSD	geometric standard deviation
GSH	reduced glutathione
GST	glutathione S-transferase
GST-T1	GST-theta1-1
HEC	human equivalent concentration
HED	human equivalent dose
hprt	hypoxanthine-guanine phosphoribosyl transferase
ICD-9	International Classification of Diseases 9th ed.
IgM	immunoglobulin M
IRIS	Integrated Risk Information System
k_a	first-order oral absorption rate constant
K_m	Michaelis-Menten kinetic constant
k_{fC}	first-order GST metabolic rate constant
LLF	log-likelihood function
LOAEL	lowest-observed-adverse-effect level

LOH	loss of heterozygosity
MCHC	mean corpuscular hemoglobin concentration
MCMC	Markov Chain Monte Carlo
mRNA	messenger ribonucleic acid
NADPH	nicotinamide adenine dinucleotide phosphate
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute of Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
P_{50}	partial oxygen pressure
PB	blood:air partition coefficient
PBPK	physiologically based pharmacokinetic
POD	point of departure
PND	postnatal day
QAlvC	allometric alveolar ventilation constant
QCC	cardiac output
QSC	fractional flow rate of slowly perfused tissues (fraction of QCC)
REnCOC	endogenous rate of CO production
RfC	reference concentration
RfD	reference dose
SD	standard deviation
SEM	standard error of the mean
SEP	somatosensory-evoked potential
SMR	standardized mortality ratio
SSB	single strand break
TWA	time-weighted average
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
VFC	fractional tissue volume of fat (fraction of BW)
VLC	fractional tissue volume of liver (fraction of BW)
V_{maxC}	CYP maximum velocity
VPR	ventilation:perfusion ratio
VSC	fractional tissue volume of slowly perfused tissues (fraction of BW)

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 exposure to dichloromethane. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of dichloromethane.

The intent of Section 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 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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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 dichloromethane. 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 mg/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 ($\leq$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 $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for dichloromethane has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). 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, 1988b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for*

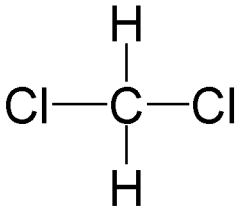
Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies (Whalan and Redden, 1994), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995),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

The literature search strategy employed for dichloromethane 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-literature identified through September 2011 was included where that literature was determined to be critical to the assessment. The relevant literature included publications on dichloromethane that were identified through Toxicology Literature Online (TOXLINE), PubMed, 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 Hazardous Substances Data Bank (HSDB), the Genetic Toxicology Data Bank (GENE-TOX), Chemical abstracts, and Current Contents. Other peer-reviewed 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 reviewers' comments and for the sake of completeness. These references have not changed the overall qualitative and quantitative conclusions. See Section 7 for a list of references added after peer review.

2. CHEMICAL AND PHYSICAL INFORMATION

Dichloromethane is a colorless liquid with a penetrating, ether-like odor (Lewis, 1997).[1] Selected chemical and physical properties of dichloromethane are listed in Table 2-1.

Table 2-1. Physical properties and chemical identity of dichloromethane

	Physical property/chemical identity	Reference
CAS number	75-09-2	Lide (2000)
Synonyms	Methylene chloride, methylene dichloride, methyl bichloride	O'Neil et al. (2001)
Molecular weight	84.93	O'Neil et al. (2001)
Chemical formula	CH_2Cl_2	O'Neil et al. (2001)
Boiling point	40°C	Lide (2000)
Melting point	-95.1°C	Lide (2000)
Vapor pressure	1.15×10^2 mm Hg at 25°C	Boublik et al. (1984)
Density	1.3266 g/mL at 20°C	Lide (2000)
Vapor density	2.93 (air = 1.02)	Holbrook (2003)
Water solubility	1.30×10^4 mg/L at 25°C	Horvath (1982)
Other solubility	Miscible in ethanol, ether, and dimethylformamide; soluble in carbon tetrachloride	IARC (1999)
Partition coefficient	$\log K_{ow} = 1.25$	Hansch et al. (1995)
Flash point	Not flammable	U.S. Coast Guard (1999)
Auto ignition temperature	640°C	Holbrook (2003)
Latent heat of vaporization	3.30×10^5 J/kg	U.S. Coast Guard (1999)
Heat of fusion	16.89 cal/g	U.S. Coast Guard (1999)
Critical temperature	245.0°C	Holbrook (2003)
Critical pressure	6.171×10^6 Pa	Holbrook (2003)
Viscosity	0.430 cP at 20°C	Lewis (1997)
Henry's law constant	3.25×10^{-3} atm m^3/mol at 25°C	Leighton and Calo (1981)
OH reaction rate constant	1.42×10^{-13} cm^3/molecule sec at 25°C	Atkinson (1989)
Chemical structure		

Dichloromethane is produced by two methods of manufacturing (IARC, 1999). The older method involves the direct reaction of methane with chlorine either at high temperatures or at lower temperatures under catalytic or photolytic conditions (Holbrook, 2003). The more

[1] "Dichloromethane" is used throughout this summary even if a specific paper used the term "methylene chloride."

common method used today involves an initial reaction of hydrochloric acid with methanol to yield methyl chloride. Excess methyl chloride is then reacted in the gas phase thermally with chlorine to produce dichloromethane (Holbrook, 2003). This process can also be carried out catalytically or photolytically.

Dichloromethane became an important industrial chemical in the United States during World War II (Hardie, 1964). Dichloromethane has been used in paint strippers and removers, as a propellant in aerosols, in the manufacture of drugs, pharmaceuticals, film coatings, electronics, and polyurethane foam, and as a metal-cleaning solvent. Dichloromethane can also be used in the decaffeination process of coffee and tea (ATSDR, 2000). The U.S. production was 3.8 million pounds in 1941 and 8.3 million pounds in 1944 (Searles and McPhail, 1949). Dichloromethane production rose sharply in the decades following the war due to the increased demand for this substance for use mainly in paint strippers (Hardie, 1964; Searles and McPhail, 1949). U.S. production in 1947, 1955, 1960, and 1962 was approximately 19, 74, 113, and 144 million pounds, respectively (Hardie, 1964; Searles and McPhail, 1949). As other solvent uses and its use in aerosol propellants became important, demand for this substance increased further (Anthony, 1979). Dichloromethane production continued to rise dramatically through the 1970s; production capacities were 520 million pounds in 1973 and 830 million pounds in 1979 (CMR, 1979, 1973).

After 1980, production of dichloromethane began to decline. Production capacities fell from 722 million pounds in 1982 to 465 million pounds in 1997 (CMR, 1997, 1982). The total U.S. production capacity for dichloromethane in 2000 was 535 million pounds (CMR, 2000). The demand for dichloromethane decreased from 600 million pounds in 1979 to 200 million pounds in 1999 (CMR, 2000, 1979). The decline in production of and demand for dichloromethane over the past 2 decades has been attributed to increased regulation, the use of alternative chemicals in aerosol spray cans, and concern over dichloromethane carcinogenicity (Holbrook, 2003; ATSDR, 2000).

Dichloromethane in the environment will partition mainly to air (HSDB, 2003). In air, dichloromethane exists as a vapor. Some of the dichloromethane released to soil or water is expected to volatilize to air. In soil, dichloromethane is expected to be highly mobile and may migrate to groundwater. The potential for dichloromethane to bioconcentrate in aquatic or marine organisms is low. Dichloromethane may biodegrade in soil or water under both aerobic and anaerobic conditions.

3. TOXICOKINETICS

3.1. ABSORPTION

3.1.1. Oral—Gastrointestinal Tract Absorption

There are currently no data available on absorption of dichloromethane following oral intake in humans. However, after oral administration in animals, dichloromethane is rapidly and nearly completely absorbed in the gastrointestinal tract (Angelo et al., 1986a, b; McKenna and Zempel, 1981). Angelo et al. (1986b) reported that, following administration of single radiolabeled oral doses (10, 50, or 200 mg/kg) to mature male F344 rats, 97% of the label was detected in the exhaled air within 24 hours, indicating near complete absorption. At several time points within 40 minutes of dose administration, <2% of the dose was found in the lower part of the gastrointestinal tract, indicating that the majority of dichloromethane absorption occurs in the upper gastrointestinal tract (Angelo et al., 1986b). Similar results were reported in mature male $B6C3F_1$ mice exposed to up to 50 mg/kg (Angelo et al., 1986a). In mature male Sprague-Dawley rats administered a single dose (1 or 50 mg/kg) of radiolabeled dichloromethane, <1% of the label was found in feces collected for 48 hours after dose administration (McKenna and Zempel, 1981). Absorption of dichloromethane generally follows first-order kinetics (Angelo et al., 1986a) and freely passes through phospholipid cell membranes by passive diffusion. The vehicle appears to affect the rate but not the extent of gastrointestinal absorption, with an aqueous vehicle resulting in a more rapid absorption of dichloromethane than an oil-based vehicle (Angelo et al., 1986a).

3.1.2. Inhalation—Respiratory Tract Absorption

Several studies in humans have demonstrated absorption of dichloromethane following inhalation exposure. In a study by Astrand et al. (1975), 14 male volunteers (ages 19–29) were exposed to about 870 mg/m^3 (250 ppm) or 1,740 mg/m^3 (500 ppm) for 30 minutes while resting or exercising on a bicycle ergometer. There was a pause of about 20 minutes without exposure between rest and exercise periods. Uptake of dichloromethane was estimated at about 55% while resting and about 40, 30, and 35% at respective workloads of 50, 100, and 150 watts[2]. Blood levels of dichloromethane correlated directly with exposure concentrations, and did not appear to increase when a workload was applied (Astrand et al., 1975). Similar reports of rapid uptake and a direct correlation between dichloromethane exposure level and blood levels in humans have been presented by other groups (DiVincenzo and Kaplan, 1981; DiVincenzo et al., 1971).

[2]A watt is the International System Unit of power and is equal to 1 joule of energy per second. It is a measure of the rate of energy use or production (i.e., the exercise effort that was exerted by the individuals in the study).

With extended (≥ 1–2 hours) exposure, uptake tends to reach a steady-state level, at which point blood dichloromethane levels remain more or less constant (DiVincenzo and Kaplan, 1981; DiVincenzo et al., 1972; Riley et al., 1966). DiVincenzo et al. (1972) reported that in humans exposed to 100 or 200 ppm dichloromethane for 2 hours (without physical exercise), dichloromethane was rapidly absorbed, reaching an approximate steady state as assessed by levels of unchanged dichloromethane in the expired air within the first 15–30 minutes of exposure. A later study by the same group (DiVincenzo and Kaplan, 1981) similarly reported a rapid absorption of dichloromethane in volunteers exposed to 50–200 ppm for 7.5 hours on each of 5 consecutive days. A steady-state level, as assessed by levels of unchanged dichloromethane in the expired air, was reached quickly (1–2 hours), with exhaled dichloromethane levels increasing with increasing exposure level. A similar pattern was seen with blood dichloromethane levels. Estimated pulmonary uptake was 69–75% and did not vary appreciably with exposure concentration. In another experiment in which one of the investigators was seated during exposure to 100 ppm dichloromethane for 2 hours, concentrations of dichloromethane in expired air reached an apparent plateau of about 70 ppm within the first hour of exposure (Riley et al., 1966).

Body fat may influence absorption of dichloromethane, as evidenced by data from an experiment involving 12 men, ages 21–35, divided into two groups (n = 6 per group) based on percent body fat (Engström and Bjurström, 1977). The mean percent body fat in the leaner group was 7.8% (standard error of the mean [SEM] 1.9), range 2.3–13.6%, compared with 25.1% (SEM 2.8), range 18.3–36.2%, in the more overweight group. Total uptake of dichloromethane during a light exercise period (50 watts) for 1 hour with an exposure level of 750 ppm was positively correlated with percent body fat (r = 0.81), and the estimated amount of dichloromethane in fat storage was also correlated with percent body fat (r = 0.84).

A pattern of absorption similar to that seen in humans has been seen in animals. Initially, dichloromethane is readily absorbed following inhalation exposure, as evidenced by rapid appearance of dichloromethane in blood, tissues, and expired air (Withey and Karpinski, 1985; Stott and McKenna, 1984; Anders and Sunram, 1982; Carlsson and Hultengren, 1975; Roth et al., 1975). For example, absorption of inhaled 500 ppm dichloromethane in anesthetized, mature male F344 rats reached an apparent plateau within 10–20 minutes and was relatively constant for up to 2 hours (Stott and McKenna, 1984). In these experiments, absorption was calculated from measurements of exposure (nose only) and effluent concentrations and ventilation flow rate in intact animals; double tracheostomized rats were used to measure absorption in the isolated upper respiratory tract and the lower respiratory tract. At a ventilation rate of 53 mL/minute, absorption expressed as mean percentage of dichloromethane available for absorption was 44% (standard deviation [SD] 10) in rats, 13.2% (SD 3.6) in the upper respiratory tract, and 37% (SD 4.1) in the lower respiratory tract.

3.2. DISTRIBUTION

Results from studies of animals show that, following absorption, dichloromethane is rapidly distributed throughout the body and has been detected in all tissues that have been evaluated. Twenty minutes after a single intravenous dose of 10 mg [^{14}C]-dichloromethane/kg to mature male B6C3F$_1$ mice (Angelo et al., 1986a), total label was greatest in the liver (6.72 µg-equivalents/g tissue), with lower levels reported in the lung (1.82 µg-equivalents/g tissue), kidney (1.84 µg-equivalents/g tissue), and the remainder of the carcass (1.90 µg-equivalents/g tissue). By 4 hours post administration, levels in the liver had fallen to 3.08 µg-equivalents/g tissue, lung levels were 0.64 µg-equivalents/g tissue, and carcass levels were 0.23 µg-equivalents/g tissue. The levels in the kidney rose sharply in the first hour postexposure but then fell and remained steady at ~1.60 µg-equivalents/g tissue for the remaining 3 hours of the study (Angelo et al., 1986a). McKenna et al. (1982) exposed groups of mature male Sprague-Dawley rats to 50, 500, or 1,500 ppm [^{14}C]-labeled dichloromethane for 6 hours and examined tissues at 48 hours for presence of radiolabel; results are shown in Table 3-1. The greatest concentration of label was found in the liver, followed by the kidney and lung.

Table 3-1. Distribution of radioactivity in tissues 48 hours after inhalation exposure of mature male Sprague-Dawley rats (n = 3) for 6 hours

Tissue	Mean ± SD, µg-equivalent dichloromethane/g tissue, by exposure level		
	50 ppm	500 ppm	1,500 ppm
Liver	8.4 ± 1.5	35.6 ± 7.5	44.2 ± 3.5
Kidney	3.3 ± 0.1	16.2 ± 2.4	30.5 ± 0.2
Lung	1.9 ± 0.2	11.0 ± 1.3	16.5 ± 1.6
Brain	0.8 ± 0.3	4.2 ± 1.3	6.7 ± 0.2
Epididymal fat	0.5 ± 0.2	6.5 ± 0.5	4.1 ± 0.9
Skeletal muscle	1.1 ± 0.1	4.4 ± 1.9	7.7 ± 0.7
Testes	1.1 ± 0.2	5.5 ± 1.3	8.1 ± 0.5
Whole blood	1.1 ± 0.2	8.1 ± 1.9	8.9 ± 1.7
Remaining carcass	1.3 ± 0.2	5.9 ± 0.9	8.6 ± 1.4

Source: McKenna et al. (1982).

As noted in the preceding section, body fat may affect the uptake of dichloromethane, and there is also evidence of a relationship between adiposity and dichloromethane storage. In the study by Engström and Bjurström (1977) involving 12 men, ages 21–35, exposed to 750 ppm dichloromethane during a 1-hour light exercise (50 watts) period, dichloromethane was measured in body fat biopsy specimens at 1, 2, 3, and 4 hours postexposure. All specimens were taken from the buttocks. The concentration of dichloromethane (per gram tissue) was negatively

correlated with percent body fat, but the total estimated amount of dichloromethane in fat tissue 4 hours postexposure was higher in subjects with a higher amount of fat ($r = 0.84$).

Carlsson and Hultengren (1975) exposed groups of 10 mature male Sprague-Dawley rats to [^{14}C]-dichloromethane for 1 hour at a mean concentration of 1,935 mg/m^3 (557 ppm) and SD of 90 mg/m^3 (26 ppm). The initial levels were highest in the white adipose tissue (approximately 80 µg dichloromethane per gram tissue) compared with approximately 35, 20, and 5 µg-equivalent dichloromethane/g tissue in the liver, kidney and adrenal glands, and brain, respectively. These initial levels in the adipose quickly fell to <10 µg-equivalent dichloromethane/g tissue; more moderate declines were seen in the other tissues.

With acute 6-hour exposure scenarios, peak exposure concentrations during the exposure period may have a greater influence on dichloromethane levels in the brain and perirenal fat than time-weighted average (TWA) concentrations (Savolainen et al., 1981). In rats exposed over a 6-hour period for 5 days/week to a TWA of 1,000 ppm dichloromethane consisting of two 1-hour peak concentrations (2,800 ppm) interspersed with exposure to 100 ppm, levels of dichloromethane in the brain were threefold higher ($p < 0.001$) than corresponding levels in rats exposed to constant levels of 1,000 ppm; a twofold increased risk was seen in the dichloromethane levels in perirenal fat after 1 week of exposure ($p < 0.001$), but this difference was much smaller after 2 weeks of exposure. This difference was not seen with blood carbon monoxide (CO) levels (Table 3-2). With constant exposure concentrations of 500 or 1,000 ppm, perirenal fat levels of dichloromethane approximately doubled following 2 weeks of exposure compared with 1 week of exposure, indicating that some storage of dichloromethane in fat tissue can occur with repeated exposure scenarios (Table 3-2). In contrast, brain levels of dichloromethane in rats exposed for 1 week were higher than brain levels in rats exposed for 2 weeks. One possible explanation of these observations is that there is an induction of enzymes involved in dichloromethane metabolism in liver and other tissues with repeated exposure, and dichloromethane in fat is poorly metabolized.

Table 3-2. Brain and perirenal fat dichloromethane and blood CO concentrations in male Wistar rats exposed by inhalation to dichloromethane at constant exposure concentrations compared with intermittently high exposure concentrations

Exposure level[a] (TWA, ppm)	Exposure wks					
	1	2	1	2	1	2
	Brain (nmol/g)		Perirenal fat (nmol/g)		Blood CO (nmol/g)	
Control	0	0	0	0	40 ± 15	30 ± 10
500, constant	30 ± 7	9 ± 3	436 ± 47	918 ± 215	675 ± 195	781 ± 62
1,000, constant	33 ± 2	14 ± 3	1,316 ± 209	2,171 ± 219	876 ± 80	825 ± 56
1,000, with two 1-hr peaks of 2,800 ppm	111 ± 18[b]	50 ± 15[b]	2,295 ± 147[b]	2,431 ± 146	728 ± 84	873 ± 90

[a]Groups of five rats were exposed to 0, 50, or 1,000 ppm 6 hours/day or 100 ppm interspersed with two 1-hour peaks of 2,800 ppm for 5 days/week for 1 or 2 weeks. Tissue concentration values are mean ± SD.
[b]Difference between 1,000 ppm TWA constant exposure, $p < 0.001$; t-test calculated by EPA using sample size, mean and SD as provided by Savolainen et al. (1981).

Source: Savolainen et al. (1981).

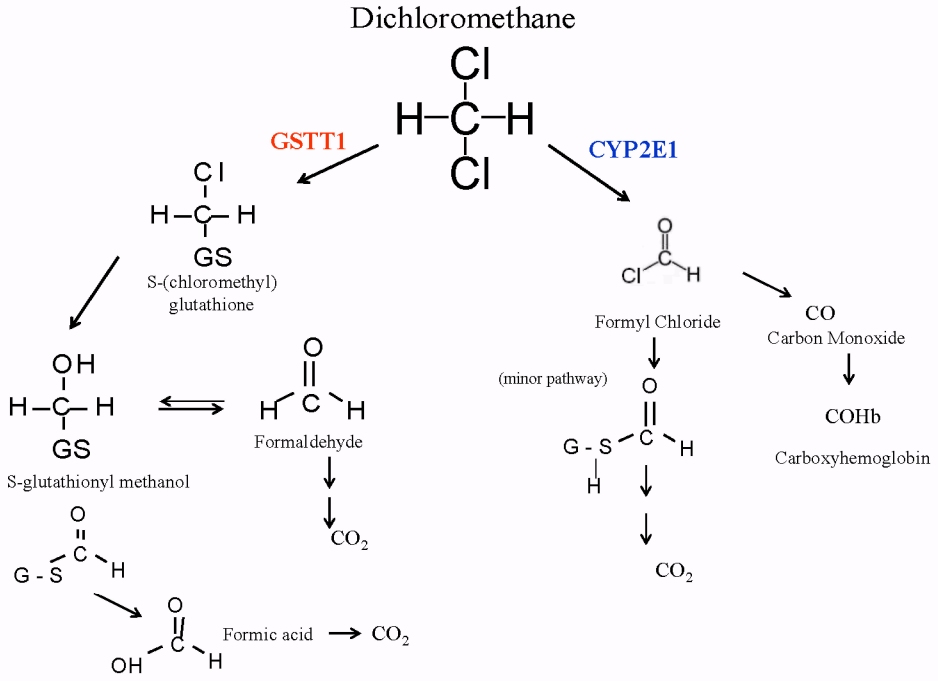
Placental transfer. Dichloromethane is capable of crossing the placental barrier and entering the fetal circulation. Anders and Sunram (1982) reported that when pregnant Sprague-Dawley rats (n = 3) were exposed to 500 ppm dichloromethane for 1 hour on gestational day (GD) 21, mean maternal blood levels were 176 nmol/mL (SEM 50), while fetal levels were 115 nmol/mL (SEM 40). The levels of CO, a metabolite of dichloromethane, were similar in both the maternal blood (167 nmol/mL, SEM 12) and fetal blood (160 nmol/mL, SEM 31). Withey and Karpinski (1985) also reported higher maternal compared with fetal dichloromethane levels based on a study of five pregnant Sprague-Dawley rats exposed to 107–2,961 ppm of dichloromethane. Maternal blood levels of dichloromethane were 2–2.5-fold higher than those found in the fetal circulation.

Blood-brain barrier transfer. Dichloromethane is thought to readily transfer across the blood-brain barrier by passive diffusion, as evidenced by the detection of radioactivity in brain tissue 48 hours after exposures of rats to radiolabeled dichloromethane at concentrations of 50, 500, or 1,500 ppm for 6 hours (McKenna et al., 1982) (see Table 3-1), and by the historical demonstrations that dichloromethane has transient sedative and anesthetic properties in humans [for review of these reports, see Mattsson et al. (1990) and Winneke (1974)]. Dichloromethane is no longer used as an anesthetic gas because the margin between anesthetic and lethal doses is narrow (Winneke, 1974).

3.3. METABOLISM

Metabolism of dichloromethane involves two primary pathways, outlined in Figure 3-1 (ATSDR, 2000; Guengerich, 1997; Hashmi et al., 1994; Gargas et al., 1986). Dichloromethane

is metabolized to CO in a cytochrome P450 (CYP)-dependent oxidative pathway that is predominant at low exposure levels. The CYP-related pathway results in the addition of oxygen, followed by spontaneous rearrangement to formyl chloride and then to CO; each spontaneous rearrangement releases H^+ and Cl^- ions. At higher exposure levels, the CYP pathway becomes saturated and a second pathway begins to predominate. Glutathione S-transferase (GST)-catalyzed addition of glutathione (GSH) is the initial step in this pathway. The replacement of one of the chlorine atoms with the S-glutathione group results in formation of S-(chloromethyl)glutathione and the release of H^+ and Cl^- ions. Hydration of S-(chloromethyl)-glutathione results in an S-glutathionyl methanol molecule, which can spontaneously form formaldehyde or rearrange to form an S-glutathione formaldehyde molecule, and then further rearrange to formate. Both formaldehyde and formate can then be further metabolized to CO_2.



Adapted from: ATSDR (2000); Guengerich (1997); Hashmi et al. (1994); Gargas et al. (1986).

Figure 3-1. Proposed pathways for dichloromethane metabolism.

As described in the following discussion of the CYP- and GST-mediated metabolism, these two pathways effectively compete for the available dichloromethane. Because dichloromethane binds to the CYP reaction site with higher affinity than the GST site, most of the dichloromethane is metabolized by CYP at lower exposure levels. As the available CYP enzyme becomes saturated at higher exposure levels more dichloromethane is left available for binding to the lower-affinity GST metabolic site, and the proportion metabolized by GST

increases. Both pathways are expected to operate, however, even at low exposures. At low substrate concentrations, the rates of enzyme-catalyzed reactions are determined by the probability of molecular collision between the substrate and enzyme active site and the probability of the subsequent reaction step (versus possible release of the unreacted substrate molecule from the site). This joint probability is directly proportional to, or first order in, the concentrations of the substrate(s) and enzyme. In particular, as long as GSH, GST, and dichloromethane are present at non-zero concentrations, the probability of these molecular collisions and transformations will be non-zero, hence the rate of the GST-mediated reaction will be non-zero.

Exposure to other agents may shift the balance between the pathways. For example, pretreatment with compounds that deplete GSH (e.g., buthionine sulfoximine, diethylmaleate, phorone) resulted in an increase in blood carboxyhemoglobin (COHb) levels following a single injection of dichloromethane compared to animals that did not receive GSH depletion, indicating a shift to the CYP pathway (Oh et al., 2002). Similarly, co-exposure to agents that compete for CYP2E1 results in a shift toward the GST pathway and away from CO production (Lehnebach et al., 1995; Pankow and Jagielki, 1993; Pankow et al., 1991a; Pankow et al., 1991b; Glatzel et al., 1987; Roth et al., 1975).

3.3.1. The CYP2E1 Pathway

There is considerable evidence of the importance of the CYP2E1 metabolic pathway in studies in animals (Oh et al., 2002; Wirkner et al., 1997; Kim and Kim, 1996; Lehnebach et al., 1995; Pankow et al., 1991a; Pankow et al., 1991b; Pankow and Hoffmann, 1989; Pankow, 1988; Glatzel et al., 1987; Angelo et al., 1986a, b; Landry et al., 1983; Anders and Sunram, 1982; McKenna et al., 1982; McKenna and Zempel, 1981; Rodkey and Collison, 1977; Carlsson and Hultengren, 1975; Roth et al., 1975; Fodor et al., 1973) and humans (Takeshita et al., 2000; DiVincenzo and Kaplan, 1981; Astrand et al., 1975). These studies demonstrate that exposure to dichloromethane, regardless of exposure route, results in the formation of CO, as assessed by measurement of elevated levels of CO in expired air and increased levels of COHb in the blood.

The first step in the CYP2E1 pathway is the formation of formyl chloride (Figure 3-1). Watanabe and Guengerich (2006) conducted a series of studies to investigate the downstream metabolites of formyl chloride and reported only marginal (3% maximum at pH 9) formation of *S*-formyl GSH from formyl chloride in the presence of GSH. Therefore, most (>97%) of the formyl chloride is metabolized further to CO. Furthermore, CO formation from formyl chloride was independent of GSH presence in the assay.

Results from numerous studies in rats in which CYP2E1 metabolism was blocked or induced indicate that the generation of CO occurs as a result of metabolism of dichloromethane by the CYP2E1 pathway (Figure 3-1). Co-exposure of rats to a high dose of ethanol (174 mmol/kg) and dichloromethane (1.6, 6.2, 15.6 mmol/kg) resulted in no increase in blood

COHb, indicating that the metabolic pathway for CO formation had been either blocked or saturated (Glatzel et al., 1987). Similar results have been seen with coadministration of other known CYP substrates, including diethyldithiocarbamate (Lehnebach et al., 1995), methanol (Pankow and Jagielki, 1993), benzene, toluene, and three xylene isomers (Pankow et al., 1991b). Pretreatment of animals with CYP inducers (e.g., benzene, toluene, xylenes, methanol, isoniazid), particularly those that induce CYP2E1, resulted in an increased level of CO formation as assessed by COHb formation or measurement in expired air following single exposures to dichloromethane (Kim and Kim, 1996; Pankow and Jagielki, 1993; Pankow et al., 1991b; Pankow and Hoffmann, 1989; Pankow, 1988). Pretreatment with disulfiram, a CYP2E1 blocker, resulted in a complete lack of formation of COHb following dichloromethane exposure, indicating that CYP2E1 is the isozyme responsible for metabolism of dichloromethane (Kim and Kim, 1996).

Evidence in hamster and rat studies suggests that the CYP2E1 pathway becomes saturated at high dichloromethane exposure levels; comparable data from studies in mice were not found. In hamsters, mean COHb percentages were elevated to a similar degree (about 28–30%, compared with <1% in controls) in three groups exposed by inhalation to 500, 1,500, or 3,500 ppm dichloromethane for 6 hours (Burek et al., 1984). After 21 months of exposure by this protocol, COHb percentages in the three exposure groups remained similarly elevated, indicative of saturation of the CYP2E1 pathway in hamsters at exposure levels ≥500 ppm and a lack of accumulation of dichloromethane and CYP2E1 metabolites with chronic exposure. McKenna et al. (1982) found that blood COHb levels in rats increased when inhalation exposure concentration was increased from 50 to 500 ppm but that similar levels of COHb were reported following exposure to 1,500 ppm as following exposure to 500 ppm; the peak blood COHb percentages were approximately 10%. In rats exposed to 0, 50, 200, or 500 ppm 6 hours/day, 5 days/week for 2 years, mean COHb percentages were 2.2, 6.5, 12.5, and 13.7%, respectively, suggesting that saturation of the CYP2E1 pathway is approached at 200 ppm (Nitschke et al., 1988a). In male F344 rats exposed for 4 hours to dichloromethane concentrations of about 150, 300, 600, 1,000, and 2,000 ppm, mean COHb percentages (estimated from a figure) were about 4% at 150 ppm and about 8% at each of the four higher exposure concentrations (Gargas et al., 1986). McKenna and Zempel (1981) reported that increasing the oral dose of labeled dichloromethane from 1 to 50 mg/kg in rats resulted in a lower fraction of the total dose being metabolized to CO. Single injections of 3 and 6 mmol/kg of dichloromethane in rats resulted in nearly identical levels of blood COHb (Oh et al., 2002).

In human subjects exposed to dichloromethane in the workplace, saturation of CYP metabolism appears to be approached at the 400–500 ppm range (Ott et al., 1983c). Blood samples were drawn during working hours from 136 fiber production workers who were exposed to dichloromethane, acetone, and methanol. A comparison group of acetate production workers from another plant exposed only to acetone was also included in this study. TWA exposure

concentrations for the workers were determined by personal monitoring techniques, and percent COHb levels in the blood samples were determined. Estimated TWA concentrations in the exposed workers followed a bimodal distribution, with a lower mode of exposure concentrations in the 150–200 ppm range and the higher mode in the range of 450–500 ppm; only 21% (29 out of 136 workers) were in the 200–400 ppm range. In categorical analyses, the workers were divided into three groups with 22, 68, and 46 individuals, respectively, in the <100, 100–299, and ≥300-ppm groups. Plots of percent COHb against TWA exposure concentrations showed the appearance of saturation at around 400 ppm, with the beginning of the plateauing occurring around 300 ppm.

The liver is the tissue most enriched in CYP2E1 catalytic activity, but CYP2E1 protein and messenger ribonucleic acid (mRNA) have been detected in other human tissues, including the lung, brain, kidney, pancreas, bladder, small intestine, and blood lymphocytes (Nishimura et al., 2003). As such, the liver is expected to be the main site of CYP metabolism of dichloromethane, but other tissues are also expected to metabolize dichloromethane via this pathway. Of particular relevance. given the neurologic effects seen with dichloromethane exposure, are the distribution and inducibility of CYP2E1 in different areas of the brain (Miksys and Tyndale, 2004). Individuals with decreased CYP2E1 activity may experience decreased generation of CO and an increased level of GST-related metabolites following exposure to dichloromethane. As a result, these individuals may be more susceptible to the chronic effects of dichloromethane from GST-related metabolites than individuals with higher levels of CYP2E1 activity. Conversely, individuals with higher CYP2E1 activity may experience relatively increased generation of CO at a given dichloromethane exposure level and, therefore, may be more susceptible to the acute toxicity of dichloromethane (e.g., from CO). However, there are currently no studies that have evaluated dichloromethane and CO effects with variable levels CYP2E1 activity.

Results from studies examining human interindividual variation in CYP2E1 activities (e.g., catalytic activities, protein levels, or mRNA levels) indicate that individuals may vary in their ability to metabolize dichloromethane through the CYP2E1 pathway. In a study of liver samples from 30 Japanese and 30 Caucasian individuals, two- to threefold variation was found in the levels of CYP2E1 protein, whereas catalytic activity toward substrates associated with CYP2E1 (e.g., 7-ethoxycoumarin) displayed a wider range of values, approximately 25-fold; no clear gender-specific or ethnic differences were found in hepatic levels of CYP2E1 protein or enzymatic activities associated with CYP2E1 (Shimada et al., 1994). In a study of interindividual variation in 70 healthy human subjects (40 men and 30 women) given an oral dose of chlorzoxazone, a therapeutic agent whose metabolism and blood clearance has been related to CYP2E1 levels, a three- to fourfold range in plasma half-life and clearance values was observed, with no clear or dramatic age- or gender-specific differences (Kim and O'Shea, 1995). A six- to sevenfold range in chlorzoxazone hydroxylation activity was reported for a group of

69 healthy, smoking and nonsmoking male and female volunteers with mixed ethnic backgrounds; the range was markedly increased when a group of 72 alcoholic inpatients was included (Lucas et al., 1999). In studies of human liver microsomes, four- to sixfold ranges in CYP2E1-dependent oxidation of trichloroethylene have been reported (2003; Lipscomb et al., 1997). CYP2E1 protein levels in 50 specimens of human lymphocytes from healthy individuals showed an approximate fivefold range (Bernauer et al., 2000), and a 3.7-fold range in liver CYP2E1 mRNA levels was reported for a group of 24 patients with chronic hepatitis (Haufroid et al., 2003). More recently, a threefold range was reported for maximal rates of hepatic CYP2E1-catalyzed metabolism of dichloromethane, estimated with a modified physiologically based pharmacokinetic (PBPK) model originally developed by Andersen et al. (1987) and kinetic data (e.g., dichloromethane breath and blood concentrations) for 13 volunteers (10 males and 3 females) exposed to one or more concentrations of dichloromethane by inhalation for 7.5 hours (Sweeney et al., 2004). In summary, most studies indicate a three- to sevenfold variability in CYP2E1 activity among "healthy volunteers" as assessed by various types of measurements. However, various clinical factors (i.e., obesity, alcoholism, use of specific medications) or co-exposures (i.e., to various solvents) (Lucas et al., 1999) may result in greater variation and thus the potential for saturation at lower exposures within the general population.

Several genetic polymorphisms for the human CYP2E1 gene have been described, but clear and consistent correlations with interindividual variation in CYP2E1 protein levels or associated enzyme activities have not been identified (Ingelman-Sundberg, 2004; Lucas et al., 1999; Kim and O'Shea, 1995; Shimada et al., 1994). The most frequently studied CYP2E1 polymorphisms, *RsaI/PstI*, are located in the 5′-flanking region of the gene, and mutations are thought to lead to increased CYP2E1 protein expression via transcription (Lucas et al., 2001). Available data indicate that the frequency of this polymorphism, as well as other CYP2E1 polymorphisms, varies among ethnic groups. For example, Stephens et al. (1994) examined blood samples from 126 African-Americans, 449 European Americans, and 120 Taiwanese subjects and found frequencies for a rare *RsaI* allele (C2) of 0.01 in African-Americans, 0.04 in European Americans, and 0.28 in Taiwanese subjects. In a study of 102 Mexicans, the reported mutation frequency at the *RsaI* C2 allele was 0.30 (Mendoza-Cantú et al., 2004).

3.3.2. The GST Pathway

The other major pathway for dichloromethane metabolism involves the conjugation of dichloromethane to GSH, catalyzed by GST. This results in the formation of a GSH conjugate that is eventually metabolized to CO_2 (Figure 3-1). The conjugation of dichloromethane to GSH results in formation of two reactive intermediates that have been proposed to be involved in dichloromethane toxicity, S-(chloromethyl)glutathione and formaldehyde. In studies with rat, mouse, and human liver cytosol preparations in the presence of GSH, examination of metabolites with [^{13}C]-NMR indicated that S-(chloromethyl)glutathione was an intermediate in the pathway

to formaldehyde (Hashmi et al., 1994). Formaldehyde formation from dichloromethane has been noted in human (Bruhn et al., 1998; Hallier et al., 1994; Hashmi et al., 1994), rat, and mouse (Casanova et al., 1997; Hashmi et al., 1994) cells in vitro. Formation of a free hydrogen ion is also hypothesized, although no direct evidence supporting this has been presented.

The GST pathway has approximately a 10-fold lower affinity for dichloromethane than the CYP pathway (Reitz et al., 1989a; Andersen et al., 1987). Although both pathways are assumed to be operating at all exposures, the CYP pathway is expected to predominate at lower exposure concentrations, and as exposure concentrations increase, the GST pathway is expected to gain in relative importance as a dispositional pathway for absorbed dichloromethane. Based on in vitro studies with liver preparations, the estimated Michaelis-Menten kinetic constant (K_m) values in GST assays with dichloromethane were about 137 mM in a $B6C3F_1$ mouse preparation and about 44 mM in two human preparations (Reitz et al., 1989a). In contrast, estimated K_m values in CYP assays were about 1.8, 1.4, and 2.0 mM in $B6C3F_1$ mouse, F344 rat, and Syrian golden hamster preparations, respectively. In four human liver preparations, estimated CYP K_m values were about 2.6, 2.0, 0.9, and 2.8 mM (Reitz et al., 1989a). These values estimated for CYP K_m are quite inconsistent, however, with those estimated by fitting the PBPK model to in vivo data: 7, 6, and 5 μM for the mouse, rat, and human, respectively. A possible resolution of these apparent in vitro versus in vivo discrepancies in K_m values is discussed in Section 3.5.5 (in particular, see Figure 3-6).

Early investigations indicated that in humans, GSTs of the α-, μ-, and π-classes were not responsible for the metabolism of dichloromethane (Bogaards et al., 1993). Tissue samples that metabolized substrates specific to those GST classes did not conjugate dichloromethane to GSH. Later investigations identified the recently-characterized GST theta class (Meyer et al., 1991), specifically GST-theta1-1 (GST-T1), as the GST isoenzyme responsible for the metabolism of dichloromethane (Mainwaring et al., 1996; Blocki et al., 1994). In the absence of the GST-T1 gene, no deoxyribonucleic acid (DNA)-protein cross-links were formed by human liver cells exposed to dichloromethane (Casanova et al., 1997), and formaldehyde production was not detected in human erythrocytes (Hallier et al., 1994). In a mouse model with a disrupted GST-T1 gene, GST activity with dichloromethane in liver and kidney cytosol samples was substantially lower compared with wild-type GST mice (Fujimoto et al., 2007).

A polymorphism of the GST-T1 gene has been demonstrated in humans. People with two functional copies of the gene (+/+) readily conjugate GSH to dichloromethane. Individuals having only one working copy of the gene (+/-) display relatively decreased conjugation ability. Individuals with no functional copy of the gene (-/-) do not express active GST-T1 protein and do not metabolize dichloromethane via a GST-related pathway (Thier et al., 1998b). Results from studies of GST-T1 genotypes in human blood samples indicate that average prevalences of the GST-T1 null (-/-) genotype are higher in Asian ethnic groups (47–64%) than in other groups, including Caucasians (19–20%), African-Americans (22%), and mixed groups (19%) (Raimondi

et al., 2006; Garte et al., 2001; Nelson et al., 1995) (see Table 3-3). Based on data collected by Nelson et al. (1995) and ethnicity data from U.S. 2000 census data (and assuming Hardy-Weinberg equilibrium), Haber et al. (2002) calculated U.S. average distributions of GST-T1 genotypes as follows: 32% +/+; 48% +/-; and 20% -/-.

Table 3-3. Mean prevalences of the GST-T1 null (-/-) genotype in human ethnic groups

Ethnic group	Reference		
	Nelson et al. (1995)[a]	Garte et al. (2001)[b]	Raimondi et al. (2006)[c]
Chinese	64.4% (n = 45)	Not reported	Not reported
Korean	60.2% (n = 103)	Not reported	Not reported
Caucasian	20.4% (n = 442)	19.7% (n = 5,577)	19.0% (n = 6,875)
Asian	Not reported	47.0% (n = 575)	53.6% (n = 1,727)
African-American	21.8% (n = 119)	Not reported	Not reported
Mexican American	9.7% (n = 73)	Not reported	Not reported
Other	Not reported	Not reported	19.4% (n = 1,485)

[a]Nelson et al. (1995) examined prevalence of the null GST-T1 genotype from analysis of blood samples from subjects of various ethnicities as noted above.
[b]Garte et al. (2001) collected GST-T1 genotype data in Caucasian (29 studies; 5,577 subjects) and Asian (3 studies, 575 subjects) ethnic groups; subjects were controls in case-control studies of cancer and various polymorphisms in genes for bioactivating enzymes.
[c]Raimondi et al. (2006) collected GST-T1 genotype data from 35 case-control studies of cancer and GST-T1 genotype; data in this table are for control subjects. The "other" group in this study is defined as Latino, African-American, and mixed ethnicities.

Results from a study of the distribution of activity levels for in vitro conjugation of dichloromethane with GSH in 22 human liver samples are roughly reflective of these estimates of the distribution of this polymorphism (Bogaards et al., 1993). No activity was found in 3 of the 22 liver samples. Eleven of the samples showed low activity levels (0.21–0.41 nmol product/minute/mg protein), and eight samples showed high activity levels ranging from 0.82 to 1.23 nmol/minute/mg protein. In another study of seven human subjects, lysates of erythrocytes showed high activities for producing formaldehyde from dichloromethane (presumably via GST-T1) in three subjects (15.4, 17.7, and 17.8 pmol product/minute/mg hemoglobin) and lower activity in the other four subjects (4.3, 6.0, 7.2, and 7.6 pmol product/minute/mg hemoglobin) (Hallier et al., 1994).

Comparisons of mice, rats, humans, and hamsters for the ability to metabolize dichloromethane via the GST pathway in liver and lung tissues indicate that mice appear to be the most active at metabolizing dichloromethane (Sherratt et al., 2002; Thier et al., 1998b) (Casanova et al., 1997; Casanova et al., 1996; Hashmi et al., 1994; Reitz et al., 1989a). Reitz et al. (1989a) reported mean (± SD) GST enzymatic activity levels with dichloromethane as substrate (in units of nmol product formed/minute/mg protein) in liver cytosol preparations to be:

25.9 ± 4.2 units in $B6C3F_1$ mice (n = 15 determinations per preparation); 7.05 ± 1.7 nmol/minute/mg in F344 rats (n = 6); and 1.27 ± 0.21 nmol/minute/mg in Syrian golden hamsters (n = 6). Mean GST activity levels in liver preparations from four human subjects (accident victims screened for human immunodeficiency virus and hepatitis B and C and obtained through a transplant center) were 2.62 ± 0.44 (n = 10), –0.01 ± 0.04 (n = 6), 2.71 ± 0.45 (n = 6), and 3.03 ± 0.44 nmol/minute/mg (n = 6) (Reitz et al., 1989a). The finding that one of the four individuals was unable to conjugate dichloromethane with GST was reflective of the estimated frequency of the GST-T1 null genotype in the U.S. population [approximately 20% in Caucasians and African-Americans; see Table 3-3 and Haber et al. (2002)]. Mean GST activity levels in lung cytosol preparations showed a similar rank order among species: 7.3 ± 1.4 nmol/minute/mg in mice (n = 4), 1.0 ± 0.1 nmol/minute/mg in rats (n = 4), 0.0 ± 0.2 nmol/minute/mg in hamsters (n = 4), and 0.37 ± 0.25 nmol/minute/mg in a pooled lung preparation from humans (n = 2).

Thier et al. (1998b) conducted a study evaluating the activity of GST-T1 after treatment of dichloromethane in the cytosol of liver and kidney homogenates from hamsters (pooled male and females), rats (pooled male and female), male mice, and female mice and for humans classified as nonconjugators, low conjugators, or high conjugators of GST to dichloromethane. Little information is provided about the human samples other than that 13 kidney cancer patients were the source of the kidney samples; normal tissue identified by pathological exam was used. Blood samples from 10 of these patients were collected, and enzyme activities measured in erythrocytes from 9 of these samples were reported. Results of conjugation of dichloromethane to GSH from these studies are presented in Table 3-4. As can be seen from the table, activity levels (expressed as nmol/minute per mg of cytosolic protein) of humans varied considerably, with nonconjugators (presumed to be GST-$T1^{-/-}$) having no detectable activity, low conjugators (presumed to be GST-$T1^{+/-}$) having moderate activity, and high conjugators (presumed to be GST-$T1^{+/+}$) having approximately twice the activity seen in low conjugators. In the liver, the activity of rat GST conjugation was over twofold that seen in human high conjugators, while levels in mice were >11-fold (males) or 18-fold (females) greater than those of human high conjugators. In the kidney, the activity of high-conjugator humans was approximately 1.8-fold that of rats and was comparable to the activity of both male and female mice. The data in Table 3-4 show the following order for GST-T1 activities with dichloromethane as substrate: in liver preparations, mouse >> rat > human high conjugators > human low conjugators > hamster > human nonconjugators and, in kidney preparations, female mouse ≈ male mouse ≈ human high conjugators > rat ≈ human low conjugators > hamster > human nonconjugators. In addition, the data indicate that activity levels in liver, kidney, and erythrocytes of human subjects are in correspondence with the nonconjugator, low conjugator, and high conjugator designations.

Table 3-4. GST-T1 enzyme activities toward dichloromethane in human, rat, mouse, and hamster tissues (liver, kidney, and erythrocytes)

	Activity (nmol/min per mg protein)[a]		Activity (nmol/min per mL)[a]
	Liver	**Kidney**	**Erythrocytes**
Human, nonconjugators	Not detectable (2)	Not detectable (1)	Not detectable (1)
Human, low conjugators	0.62 ± 0.30 (11)	1.38 ± 0.52 (8)	9.67 ± 2.49 (5)
Human, high conjugators	1.60 ± 0.48 (12)	3.05 ± 0.72 (4)	18.28 ± 0.46 (3)
Rat	3.71 ± 0.28 (8)	1.71 ± 0.28 (8)	Not measured
Mouse, male	18.2 ± 2.22 (5)	3.19 ± 0.46 (5)	Not measured
Mouse, female	29.7 ± 6.31 (5)	3.88 ± 0.90 (5)	Not measured
Hamster	0.27 ± 0.20 (6)	0.25 ± 0.21 (6)	Not measured

[a]Mean ± SD with number of samples noted in parentheses.

Source: Adapted from Thier et al. (1998b).

Sherratt et al. (2002) reported that, on a per mg basis, native recombinant mouse GST-T1 (purified after expression in *Escherichia coli*) was approximately twofold more active toward dichloromethane than native recombinant human enzyme, as well as being approximately fivefold more efficient (as assessed by the ratio of k_{cat}/K_m), where k_{cat} is the maximum rate of the reaction catalyzed by the enzyme *per enzyme molecule*; i.e., V_{max}/E_t where E_t is the total enzyme concentration).

The distribution of GST-T1 in human tissues has been examined with antibodies raised against recombinant human GST-T1 (Sherratt et al., 2002; Sherratt et al., 1997). Immunoblotting of sodium dodecyl sulfate polyacrylamide gel electrophoresis gels loaded with tissue extracts from a 73-year-old man who had died with bronchopneumonia and atherosclerosis indicated the following order of expression of GST-T1: liver ≈ kidney > prostate ≈ small intestine > cerebrum ≈ pancreas ≈ skeletal muscle > lung ≈ spleen ≈ heart ≈ testis (Sherratt et al., 1997). It was estimated that the levels of cross-reacting materials in the cerebrum, pancreas, or skeletal muscle extracts were about 10% of those in the liver, whereas levels in the lung, spleen, heart, and testis were <5% of the levels in the liver. Comparison of the amounts of cross-reacting material in soluble liver extracts from a $B6C3F_1$ mouse and five human subjects (i.e., normal liver tissue samples from biopsies of secondary liver tumors) found that levels of GST-T1 protein were higher in the mouse extracts than in any of the human liver extracts (Sherratt et al., 2002). Densitometer analysis indicated that the GST-T1 level in the mouse liver extract was about fivefold higher than those in human liver extracts displaying the highest level. Cross-reacting material was not detectable in liver extracts from one of the five human subjects, indicating that this individual may have been GST-T1 null (Sherratt et al., 2002).

Results from in situ hybridization with oligonucleotide antisense probes for GST-T1 mRNA levels and immunohistochemical studies with antibodies to GST-T1 have indicated that

there may be subtle differences between mice, rats, and humans in the intracellular localization of GST-T1 in the liver. Mainwaring et al. (1996) reported that staining for GST-T1 mRNA was higher in liver slices from $B6C3F_1$ mice than in liver slices from F344 rats and that staining in human liver samples was very low. Although the number of mouse and rat liver samples examined in this study was not indicated in the available report, it was reported that slices from five human liver samples were examined. No information was provided regarding the clinical history of the sources of the human samples. In mouse liver, staining for GST-T1 mRNA was enhanced in the limiting plate hepatocytes, in nuclei, in bile-duct epithelial cells, and in lesser amounts in the centrilobular cells in general. In rat liver, a similar pattern was observed, except no enhanced staining was observed in the limiting plate hepatocytes or in nuclei. Staining for GST-T1 mRNA in the human liver samples showed an even distribution throughout the liver lobule, and no mention of a specific nuclear localization was made (Mainwaring et al., 1996). Quondamatteo et al. (1998), using antibodies to GST-T1, subsequently reported a similar localization of GST-T1 protein in nuclei of cells in mouse liver slices. In another study using antibodies raised against recombinant human GST-T1 or a peptide derived from the deduced mouse GST-T1 primary sequence, Sherratt et al. (2002) reported that nuclear staining was observed in all cells in mouse liver slices (from five individual $B6C3F_1$ mice) showing the presence of mouse GST-T1; staining in the cytoplasm was only detected in cells with very high levels of GST-T1. In liver slices obtained from two human subjects (males, ages 60 and 61 years, with a secondary liver tumor and what was described as a "cavernous hemangioma" without malignancy, respectively), the most intense nuclear staining was associated with bile duct epithelial cells, but there was heterogeneity of staining within hepatocytes; some cells showed nuclear staining, but others only exhibited cytoplasmic staining (Sherratt et al., 2002).

In summary, the relative amount of dichloromethane metabolized via the GST pathway increases with increasing exposure concentrations. As the high affinity CYP pathway becomes saturated (either from high exposure levels or from genetic or other factors that decrease CYP2E1 activity), the GST pathway increases in relative importance as a dispositional pathway for dichloromethane. Two reactive metabolites (S-(chloromethyl)glutathione and formaldehyde) resulting from this pathway have been identified. GST-T1 is the GST isozyme that catalyzes conjugation of dichloromethane with GST. Interindividual variation in the ability to metabolize dichloromethane via GST-T1 is associated with genetic polymorphisms in humans. Estimated U.S. population prevalence of nonconjugators (-/- at the GST-T1 locus) is about 20%, but higher prevalences (47–64%) have been reported for Asians (Raimondi et al., 2006; Haber et al., 2002; Garte et al., 2001; Nelson et al., 1995). The prevalences for low (+/- at the GST-T1 locus) and high (+/+) conjugators have been estimated at 48 and 32%, respectively (Haber et al., 2002). The liver and kidney are the most enriched tissues in GST-T1, but evidence is available for the presence of GST-T1 in other tissues at lower levels, including the brain and lung. In humans, GST-T1 expression in the brain is lower than that seen in the liver or kidney but higher than in

the lung. Comparisons of mice, rats, humans, and hamsters for the ability to metabolize dichloromethane via the GST pathway in liver (based on measurement of tissue-specific enzyme activity) indicate the following rank order: mice > rats > or ≈ humans > hamsters. In mouse liver tissue, GST-T1 appears to be localized in the nuclei of hepatocytes and bile-duct epithelium, but rat liver does not show preferential nuclear localization of GST-T1. In human liver tissue, some hepatocytes show nuclear localization of GST-T1 and others show localization in cytoplasm, as well as in bile duct epithelial cells. The apparent species differences in intracellular localization of GST-T1 may play a role in species differences in susceptibility to dichloromethane carcinogenicity if nuclear production of S-(chloromethyl)glutathione is more likely to lead to DNA alkylation than cytoplasmic production.

3.4. ELIMINATION

Dichloromethane is eliminated mainly through exhalation either of the parent compound or as the two primary metabolites, CO_2 and CO (Angelo et al., 1986a, b; McKenna et al., 1982; DiVincenzo and Kaplan, 1981; DiVincenzo et al., 1972, 1971). In human studies, dichloromethane is rapidly eliminated from the body following the cessation of exposure, with much of the parent compound completely removed from the bloodstream and expired air by 5 hours postexposure in experiments using exposure levels of 90, 100, or 210 ppm (DiVincenzo et al., 1972, 1971; Riley et al., 1966). Studies in rats have similarly demonstrated that elimination from the blood is rapid, with elimination half-times in F344 rats on the order of 4–6 minutes following intravenous doses in the range of 10–50 mg/kg (Angelo et al., 1986a). In a study using Sprague-Dawley rats, Carlsson and Hultengren (1975) demonstrated variability in elimination rates between different types of tissues, with the most rapid elimination seen in the adipose and brain tissue, and slower elimination from liver, kidneys, and adrenals.

In a study using human volunteers, DiVincenzo and Kaplan (1981) reported a dose-related increase in CO in the expired breath after inhalation exposure to 50–200 ppm of dichloromethane, with a net elimination as CO on the order of 25–35% of the absorbed dose. Similar results have been reported in animal studies. Following gavage administration of 50 or 200 mg/kg-day doses of [^{14}C]-labeled dichloromethane in water to groups of six mature male F344 rats for up to 14 days, >90% of the label was recovered in the expired air within 24 hours of dose administration (Angelo et al., 1986b). Following administration of the first of 14 daily 50 mg/kg-day doses, radioactivity in parent compound, CO_2, and CO in the 24-hour expired breath accounted for 66, 17, and 16% of the administered radioactivity, respectively; similar patterns were reported for 24-hour periods following administration of the seventh and fourteenth 50 mg/kg-day dose. Following administration of the first 200 mg/kg-day dose, radioactivity in parent compound, CO_2, and CO in the 24-hour expired breath accounted for 77, 9, and 6%, respectively, of the administered radioactivity (Angelo et al., 1986b). In mature, male Sprague-Dawley rats given a smaller dose (1 mg/kg) of [^{14}C]-labeled dichloromethane,

radioactivity in parent compound, CO_2, and CO in 48-hour expired breath accounted for 12, 35, and 31%, respectively; these data indicate that, at lower dose levels, a greater percentage of the administered dose was metabolized by the CYP pathway and eliminated in the expired breath, compared with higher dose levels (McKenna and Zempel, 1981). Similar patterns of radioactivity distribution in parent compound, CO_2, and CO in expired breath were found in mature, male $B6C3F_1$ mice following gavage administration of 50 mg/kg-day (in water) or 500 or 1,000 mg/kg-day (in corn oil) [^{14}C]-labeled dichloromethane (Angelo et al., 1986a). For example, radioactivity in parent compound, CO_2, and CO in 24-hour expired breath accounted for 61, 18, and 11% of the administered radioactivity, following administration of a single 50 mg/kg dose to a group of six mice (Angelo et al., 1986a). Exhalation rates were similarly high following inhalation exposure of mature, male Sprague-Dawley rats (>90%) (McKenna et al., 1982) or following intravenous administration of dichloromethane to mature, male F344 rats (Angelo et al., 1986b).

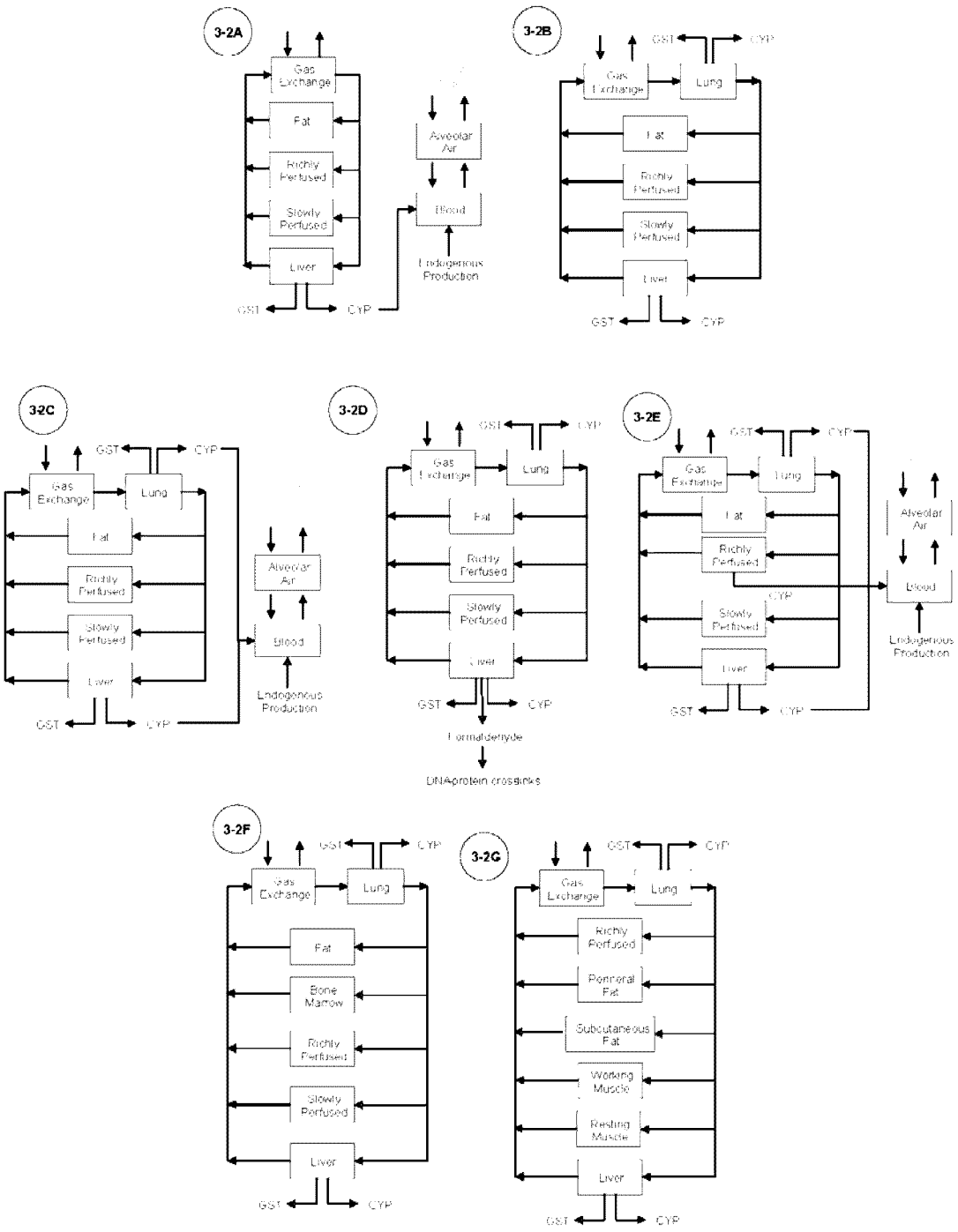
Elimination of dichloromethane in the urine of exposed humans is generally small, with total urinary dichloromethane levels on the order of 20–25 or 65–100 μg in 24 hours following a 2-hour inhalation exposure to 100 or 200 ppm, respectively (DiVincenzo et al., 1972). However, a direct correlation between urinary dichloromethane and dichloromethane exposure levels was found in volunteers, despite the comparatively small urinary elimination (Sakai et al., 2002). Following administration of a labeled dose in animals, regardless of exposure route, generally <5–8% of the label is found in the urine and <2% in the feces (McKenna et al., 1982; McKenna and Zempel, 1981; DiVincenzo et al., 1972, 1971).

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

Several PBPK models for dichloromethane in animals and humans have been developed from 1986 to 2006. These models are mathematical representations of the body and its absorption, distribution, metabolism, and elimination of dichloromethane and select metabolites, based on the structure of the Ramsey and Andersen (1984) model for styrene. The models' equations are designed to mimic actual biological behavior of dichloromethane, incorporating in vitro and in vivo data to define physiological and metabolic equation parameters. As such, the models can simulate animal or human dichloromethane exposures and predict a variety of dichloromethane and metabolite internal dosimeters (i.e., instantaneous blood and tissue concentration, area under the curve [AUC] of concentration versus time plots, rate of metabolite formation), allowing for the extrapolation of toxicity data across species, route of exposure, and high to low exposure levels. The development of dichloromethane PBPK models has resulted in either increased biological detail and functionality or refinement of model parameters with newly available data. The former type of development provides more options for toxicity data extrapolation, while the latter serves to increase confidence in model predictions and decrease uncertainty in risk assessments for which the models were, or will be, applied. This section of

the document describes each of the models reported in the scientific literature and/or used by the regulatory community (i.e., Occupational Safety and Health Administration [OSHA], EPA) and their contribution to the advancement of predictive dosimetry and data extrapolation for dichloromethane. In some instances, model development was accomplished by the addition of new biological compartments (e.g., tissue systems). Diagrams of the compartmental structure of the models are shown in Figure 3-2. Significant statistical advances in parameter estimation also have been incorporated in model development. For this reason, some animal and human PBPK models may be described as deterministic (Sweeney et al., 2004; Casanova et al., 1996; Reitz et al., 1989a; Reitz et al., 1988; U.S. EPA, 1988a; Andersen et al., 1987; U.S. EPA, 1987a, b; Gargas et al., 1986) in which point estimates for each model parameter are used, resulting in point estimates for dosimetry. Others may be described as probabilistic (Jonsson and Johanson, 2001; El-Masri et al., 1999; 1997), in which probability distributions for each parameter were defined, resulting in probability distributions for dosimetry. The latter approach, particularly utilizing a Bayesian hierarchical statistical model structure (described below) (David et al., 2006; Marino et al., 2006) to estimate parameter values, allows for the introduction of intra- and interspecies variability into model predictions and quantitative assessment of model uncertainty. Both deterministic (U.S. EPA, 1988a, 1987a, b) and probabilistic (OSHA, 1997) applications have been used to develop regulatory values. As discussed below, subsequent applications of the developed models for cancer risk assessment have resulted in significantly different estimates of human cancer risk.

The deterministic rat model of Gargas et al. (1986), based on previous work by Ramsey and Andersen (1984) examining inhalation pharmacokinetics of styrene in rats, was the first PBPK model for dichloromethane. It comprised four compartments (fat, liver, richly perfused tissues, and slowly perfused tissues [Figure 3-2A]) and described flows and partitioning of parent material and metabolites through the compartments with differential equations. Metabolism, which was restricted to the liver compartment, was described as two competing pathways: the GST pathway, described with a linear first-order kinetic model, and the CYP pathway, described with a saturable Michaelis-Menten kinetic model. Rate constants for the CYP and GST pathways in rats were determined by optimization of the model with in vivo gas uptake data. COHb production was modeled both endogenously and from CYP-mediated metabolism of dichloromethane. This model demonstrated the dose-dependent flux through the competing CYP and GST metabolic pathways and the effect of CYP inhibition on COHb generation.



Models C–G all build on the structure in model B. Models E and G have been applied in humans; all others have been applied in humans and rodents (mice and/or rats). CYP = CYP pathway metabolites; GST = GST pathway metabolites. Adapted from: Model A—Gargas et al. (1986); B—Andersen et al. (1987); C—Andersen et al. (1991); D—Casanova et al. (1996); E—Sweeney et al. (2004); F—OSHA (1997); G—Jonsson and Johanson (2001).

Figure 3-2. Schematics of PBPK models (1986–2006) used in the development of estimates for dichloromethane internal dosimetry.

Andersen et al. (1987) extended the rat model of Gargas et al. (1986) to include a lung compartment, including CYP and GST metabolism pathways within the lung, in rats, mice, hamsters, and humans (Figure 3-2B). Physiological flow rates were allometrically scaled among species by ¾ power of body weight (BW). Rate constants for the CYP and GST pathways in rodents were determined by optimization of the model with in vivo gas uptake data. CYP rate constants for humans were derived from data on dichloromethane uptake in human subjects (number of subjects not reported). Human GST rate constants were derived by allometric scaling of the animal GST rate constants. Model predictions compared favorably with kinetic data for human subjects exposed by inhalation to dichloromethane (Andersen et al., 1987). Using the mouse cancer bioassay data from the National Toxicology Program (NTP, (1986), Andersen et al. (1987) compared the linear body surface area-derived or the PBPK model-derived human liver and lung dose surrogates associated with tumor development (mg dichloromethane metabolized via GST pathway/volume tissue/day). They reported that PBPK model-extrapolated human liver and lung internal doses were 167- and 144-fold lower for inhalation exposure and 45- and 213-fold lower for drinking water exposure, respectively, than body surface area scaled internal doses. The study authors suggested that the lower model-predicted human internal dose surrogates were due to the need to saturate the CYP pathway before appreciable tumorigenic metabolite levels could be attained, which is not captured by extrapolation based on body surface area.

U.S. EPA (1988a, 1987a, b) slightly modified the Andersen et al. (1987) model for mice by using different alveolar ventilation and cardiac flow rates and used the mouse and human models to derive human cancer risks from animal tumor incidence data. The flow rate parameters in the Andersen et al. (1987) model were based on a human breathing rate of 12.5 m^3/day (reflecting a resting rate), compared with the EPA value of 20 m^3/day (reflecting average daily activity level), and a mouse breathing rate of 0.084 m^3/day (based on allometric scaling of bioassay-specific BWs), compared with the rate commonly used by EPA, 0.043 m^3/day (U.S. EPA, 1987b). The internal dose metric used in the applications of the model to cancer risk assessment was reflective of the amount of dichloromethane metabolized by the GST pathway. In addition to using the mouse and human PBPK models to account for species differences in dosimetry, a body surface area correction factor of 12.7 was applied to low-dose slopes of estimated dose-response relationships for liver and lung tumors in mice to account for presumed higher human responsiveness, relative to mice, to dichloromethane-induced cancer (U.S. EPA, 1987b). The factor of 12.7 is the cube root of the ratio of human to mouse reference BWs; this BW scaling factor was applied to adjust for differences in the lifetime impact in mice and humans resulting from both processes leading to differences in internal doses (e.g., clearance of a given daily amount of dichloromethane metabolically activated/L of tissue) and differences in pharmacodynamics or response (Rhomberg, 1995). A human cancer inhalation unit risk of 4.7×10^{-7} per ($\mu g/m^3$), based on this analysis, was placed on IRIS in September 1990.

The Andersen et al. (1987) models were also modified by addition of submodel structures for estimation of new dosimeters of interest. Andersen et al. (1991) added the capability to specifically describe the kinetics of dichloromethane, CO, and COHb in rats and humans with the addition of the Coburn-Forster-Kane equation to describe CO and COHb kinetics (Figure 3-2C). However, equations were not added for metabolism of dichloromethane to CO in the lung. Casanova et al. (1996) extended the Andersen et al. (1987) mouse model to include a submodel that predicted the formation of formaldehyde and DNA-protein cross-links in the liver (Figure 3-2D).

New in vitro measurements of metabolic rate constants in human and animal tissues were incorporated into the Andersen et al. (1987) models by Reitz and coworkers (1991; 1989a; 1988). Sweeney et al. (2004) modified the Andersen et al. (1987) human PBPK model, adding extrahepatic CYP metabolism in richly perfused tissues (Figure 3-2E) to obtain a better fit of the model to kinetics data for humans. Data for 13 volunteers (10 men and 3 women) who were exposed to one or more concentrations of dichloromethane for 7.5 hours included dichloromethane concentrations in breath and blood, COHb concentrations in blood, and CO concentrations in exhaled breath. Individual CYP maximal velocity (V_{maxC}) values were obtained by optimizing model predictions to match time-course data simultaneously for dichloromethane concentrations in blood and exhaled breath for each individual. Resultant individual values of CYP V_{maxC} ranged from 7.4 to 23.6 mg/hour/$kg^{0.7}$, indicating an approximate threefold range in maximal CYP metabolic activity.

The significance of metabolic variability for the kinetics of dichloromethane in animals and humans was explored by several investigators using PBPK models. Dankovic and Bailer (1994) used the updated human model presented by Reitz et al. (1989a; 1988) to explore the consequences of interindividual variability for in vitro kinetic constants with the CYP and GST pathways (based on data for four human subjects) and reported that predicted GST-metabolized doses to the lung and liver could range from about zero to up to fivefold greater than those predicted with the values of these rate constants used in the Reitz et al. (1989a; 1988) model. El-Masri et al. (1999) replaced parameter estimates in the mouse and human PBPK models presented by Casanova et al. (1996) with probability distributions, including published information on the distribution of GST-T1 polymorphism in human populations, and used Monte Carlo simulations to estimate distributions of cancer potency of dichloromethane in mice, distributions of the amount of DNA-protein cross-links formed in the liver of humans, and distributions of human cancer risks at given exposure levels of dichloromethane. The analysis showed that, at exposure levels of 1, 10, 100, and 1,000 ppm dichloromethane, average and median cancer risk estimates were 23–30% higher when GST-T1 polymorphism was not included in the model.

Given the demonstrated influence of population variability in dichloromethane metabolism on PBPK model-derived cancer risk estimates (El-Masri et al., 1999; Dankovic and

Bailer, 1994), PBPK model development has included a more formal statistical treatment of data for physiological and metabolic variability. Bayesian statistical approaches have been applied to develop probabilistic PBPK models for dichloromethane. Probabilistic models account for variability between individuals in model parameters by replacing point estimates for the model parameters with probability distributions. Calibration or fitting of probabilistic PBPK models to experimental toxicokinetic data is facilitated by a Bayesian technique called Markov Chain Monte Carlo (MCMC) simulation, which quantitatively addresses both variability and uncertainty in PBPK modeling (Jonsson and Johanson, 2003).

OSHA (1997) used MCMC simulation to fit probabilistic versions of the Reitz et al. (1989a; 1988) and Andersen et al. (1991; 1987) mouse and human models, which included probability distributions for all model parameters. GST- and CYP-mediated metabolism occurred in the liver and lung compartments (see Figure 3-2F). The model parameters were modified to focus on occupational exposure scenarios; that is, a parameter distribution for work intensity [using data from Astrand et al. (1975)] was added, which adjusted physiological flow rates as a function of work intensity as measured in watts. In addition, updated measurements of blood:air and tissue:air partition coefficients (Clewell et al., 1993) were used to describe distributions for these parameters. The Clewell et al. (1993) blood:air partition coefficient (PB) of 23 for mice is higher than the value of 8.29 reported by Andersen et al. (1987) and used previously by EPA (1988a, 1987a, b). The newer Clewell et al. (1993) value for mice is the preferred value, since it is much closer to the values for rats (19.4) and hamsters (22.5) rather than humans (9.7), as reported by Andersen et al. (1987). Distributions of metabolic, physiological, and partitioning parameters in the mouse and human models were updated by using Bayesian methods with data for mice and humans in published studies of mouse and human physiology and dichloromethane kinetic behavior.

Jonsson et al. (2001) used additional human kinetics data to expand the PBPK model of Reitz et al. (1989a; 1988) and added new model compartments (Figure 3-2G). These investigators used MCMC simulation to develop a probabilistic model from the Reitz et al. (1989a; 1988) human model by using published in vitro measurements of liver V_{maxC} for the CYP pathway (Reitz et al., 1989a) and kinetic data for five human subjects exposed by inhalation to dichloromethane (Astrand et al., 1975). A working muscle compartment was added to the basic Andersen et al. (1987) and Reitz et al. (1989a; 1988) structure (see Figure 3-2G). Jonsson and Johanson (2001) refined and extended this probabilistic model by including an additional fat compartment (to provide a better description of the experimental data for the time course of dichloromethane in subcutaneous fat), incorporating (with MCMC simulation) kinetic data for dichloromethane in an additional 21 human subjects and including three GST-T1 genotypes/phenotypes (nonconjugators -/-, low conjugators +/-, high conjugators +/+). Monte Carlo simulations were then used with the refined probabilistic model to predict human liver cancer risk estimates at several dichloromethane exposure levels using an algorithm similar to

the one used by El-Masri et al. (1999), using DNA-protein cross-links as the internal dose metric. The mean, 50th, 90th, and 95th percentile human cancer risk values from Jonsson et al. (2001) and El-Masri et al. (1999) were very similar, within onefold of one another for simulated exposure levels up to 100 ppm.

The most statistically rigorous and data-intensive PBPK model development was performed by Marino et al. (2006) for mice and David et al. (2006) for humans. Development of these models used multiple mouse and human data sets in a Bayesian hierarchical statistical structure to quantitatively capture population variability and reduce uncertainty in model dosimetry and the resulting risk values. EPA used these models in the derivation of reference values and cancer risk estimates in the current assessment, and these models are described in more detail below.

3.5.1. Probabilistic Mouse PBPK Dichloromethane Model (Marino et al., 2006)

Marino et al. (2006) used MCMC analysis to develop a probabilistic PBPK model for dichloromethane in mice, using the Andersen et al. (1987) model structure as a starting point (Figure 3-3). Metabolic kinetic parameters (V_{maxC}, K_m, k_{fC}, ratio of lung V_{max} to liver V_{max} [A1], and ratio of lung k_{fC} to liver k_{fC} [A2]) (Table 3-5) were calibrated with this Bayesian methodology by using several experimental data sets. Distribution parameters (i.e., means and coefficients of variation [CVs]) for other physiological parameters (i.e., BW, fractional flow rates, and fractional tissue volumes) and partition coefficients were taken from the general literature as noted by Clewell et al. (1993). Marino et al. (2006) noted that using distributions for these latter parameters from the general literature (based on a large number of animals) was better than updating them based on the relatively smaller number of animals in the available dichloromethane kinetic studies. Clewell et al. (1993) determined blood:air and tissue:air partition coefficients (means and CVs) with tissues from groups of male and female $B6C3F_1$ mice. These partition coefficients were derived by using a vial equilibration method similar to that used by prior investigators (Andersen et al., 1987; Gargas et al., 1986). Tissue:air partition coefficients were approximately 2–3 times lower than previously utilized values with the exception of the liver coefficient, which was similar to previous values (Table 3-5). As noted previously, the PB (23) from Clewell et al. (1993) is higher than the previously reported value of 8.29 (Andersen et al., 1987; Gargas et al., 1986). The higher value is more in line with values measured in rats (19.4) and hamsters (22.5) and, thus, is more reasonable than the older value of 8.3. Table 3-5 shows mean and CVs for physiological parameters and partition coefficients in the Marino et al. (2006) mouse model as well as values used in earlier deterministic PBPK mouse models for dichloromethane.

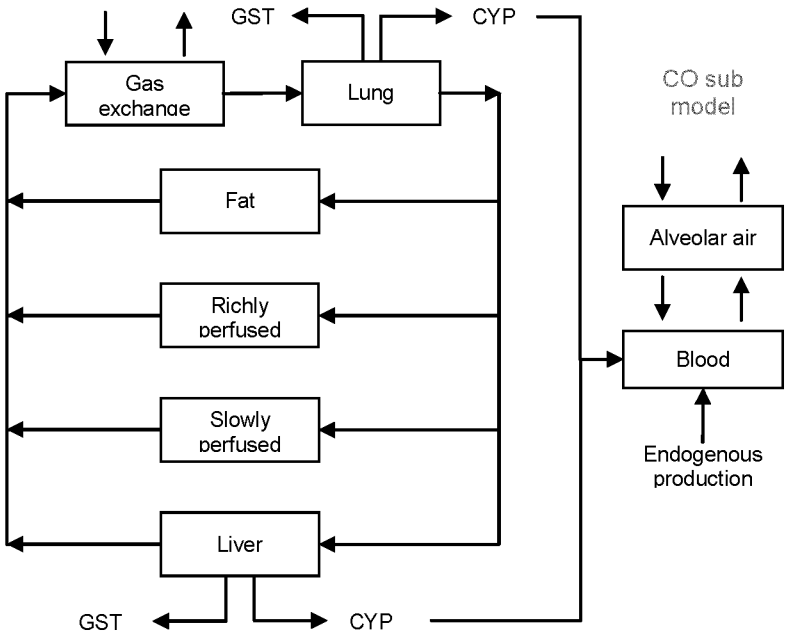


Figure 3-3. Schematic of mouse PBPK model used by Marino et al. (2006).

The Bayesian calibration of the cardiac output (QCC) constant, ventilation:perfusion ratio (VPR), and metabolic parameters was divided into three sequential steps: using kinetic data from closed chamber studies with mice treated with an inhibitor of CYP2E1 (trans-1,2-dichloro-ethylene) to minimize the oxidative pathway and enable a more precise estimate of parameters for the GST pathway, followed by kinetic data for mice given intravenous injections of dichloromethane to estimate metabolism parameters in the absence of pulmonary absorption processes and, finally, kinetic data for naïve mice exposed to dichloromethane in closed chambers (Marino et al., 2006). The initial prior distributions were based on mean values used by Andersen et al. (1987) for the metabolic parameters and by OSHA (1997) for the parameters for VPR, A1, and A2. Posterior distributions from the first Bayesian analysis were used as prior distributions for the second step, and posterior distributions from the second step were used as prior distributions for the final updating. Final results from the Bayesian calibration of the mouse probabilistic model are shown in Table 3-5.

Marino et al. (2006) used the Bayesian-calibrated mouse model to calculate internal dose metrics associated with exposure conditions in the NTP (1986) B6C3F$_1$ mouse cancer inhalation bioassay. The internal dose metric selected was mg dichloromethane metabolized by the GST pathway/L tissue/day. This is the same dose metric used in earlier applications of PBPK models to derive human cancer inhalation unit risk estimates based on cancer responses in mice (1997; Andersen et al., 1987; U.S. EPA, 1987a, b). Its use is consistent with evidence that dichloromethane metabolism via GST-T1 results in the formation of a reactive metabolite that damages DNA and results in the formation of tumors (see Section 4.7). The model was used to calculate values for this internal dose metric in the lung and liver of mice in the NTP (1986)

Table 3-5. Values for parameter distributions in a B6C3F$_1$ mouse probabilistic PBPK model for dichloromethane compared with associated values for point parameters in earlier deterministic B6C3F$_1$ mouse PBPK models for dichloromethane

Parameter		Marino et al. (2006)[a]				EPA (1988a, 1987a, b)	Andersen et al. (1987)
		Prior mean	Prior CV	Final posterior mean	Final posterior CV		
Fractional flow rates (fraction of QCC)[b]				These parameters were taken from an extensive literature database derived from a large number of animals; therefore, further Bayesian updating does not inform on the true mean and variance for these values.			
QFC	Fat	0.05	0.60			0.05	0.05
QLC	Liver	0.24	0.96			0.24	0.24
QRC	Rapidly perfused tissues	0.52	0.50			0.52	0.52
QSC	Slowly perfused tissues	0.19	0.40			0.19	0.19
Fractional tissue volumes (fraction of BW)[b]							
VFC	Fat	0.04	0.30			0.04	0.04
VLC	Liver	0.04	0.06			0.04	0.04
VLuC	Lung	0.0115	0.27			0.0119	0.0119
VRC	Rapidly perfused tissues	0.05	0.30			0.05	0.05
VSC	Slowly perfused tissues	0.78	0.30			0.78	0.78
Partition coefficients[c]							
PB	Blood:air	23	0.15			8.29	8.29
PF	Fat:blood	5.1	0.30			14.5	14.5
PL	Liver:blood	1.6	0.20			1.71	1.71
PLu	Lung:blood	0.46	0.27			1.71	1.71
PR	Rapidly perfused:blood	0.52	0.20			1.71	1.71
PS	Slowly perfused:blood	0.44	0.20			0.96	0.96
Flow rates							
QCC	Cardiac output (L/hr/kg$^{0.74}$)	28.0	0.58	24.2	0.19	14.3[d]	28.0[e]
VPR	ventilation:perfusion ratio	1.52	0.75	1.45	0.20	1.0	1.0
Metabolism parameters							
V_{maxC}	Maximum CYP metabolic rate (mg/hr/kg$^{0.7}$)	11.1	2	9.27	0.21	11.1	11.1
K_m	CYP affinity (mg/L)	0.396	2	0.574	0.42	0.396	0.396
k_{fC}	First-order GST metabolic rate constant (kg$^{0.3}$/hr)	1.46	2	1.41	0.28	1.46	1.46
A1	Ratio of lung V_{maxC} to liver V_{maxC}	0.462	0.55	0.207	0.36	0.416	0.416
A2	Ratio of lung k_{fC} to liver k_{fC}	0.322	0.55	0.196	0.37	0.137	0.137

[a]MCMC analysis was used to update prior distributions (means and CVs) for flow rate and metabolic parameters in a sequential process with three sets of kinetic data from mouse studies, as explained further in the text. Final values for posterior distributions are given in this table.
[b]Source: Andersen et al. (1991; 1987).
[c]Source: Clewell et al. (1993).
[d]Based on a mouse breathing rate of 0.043 m^3/day.
[e]Based on a mouse breathing rate of 0.084 m^3/day.

study, using the mean values of the final distributions for the parameters in the model. Resultant values were three- to fourfold higher than values calculated with the Andersen et al. (1987) and U.S. EPA (1987a, b) versions of the model (Table 3-6). Marino et al. (2006) noted that the difference could be primarily attributed to the changes in the partition coefficients based on Clewell et al. (1993) as well as to the Bayesian updating of the metabolic parameters (see Table 3-5).

Table 3-6. Internal daily doses for B6C3F$_1$ mice exposed to dichloromethane for 2 years (6 hours/day, 5 days/week) calculated with different PBPK models

Target organ	NTP (1986) exposure level[a]	PBPK model		
		Marino et al. (2006)	EPA (1987a, b)	Andersen et al. (1987)
Liver[b]	Control	0	0	0
	2,000 ppm	2,359.99	727.8	851
	4,000 ppm	4,869.85	1,670	1,811
Lung[b]	Control	0	0	0
	2,000 ppm	474.991	111.4	123
	4,000 ppm	973.343	243.7	256

[a]2,000 ppm = 6,947 mg/m^3; 4,000 ppm = 13,894 mg/m^3.
[b]Internal dose expressed as mg dichloromethane metabolized by the GST pathway/L tissue/day.

Marino et al. (2006) noted that inclusion of extrahepatic CYP metabolism in the slowly perfused tissue compartment in the mouse model had little impact on the formation of GST metabolites in the liver and lung, especially at exposure levels used in the mouse NTP (1986) bioassay. To support this contention, the Andersen et al. (1987) model was modified to include 10% of the liver rate of oxidative metabolism in the slowly perfused tissue compartment [as suggested by Sweeney et al. (2004)], and the modified model was used to calculate the formation of GST metabolites. If extrahepatic metabolism was included in the slowly perfused tissue compartment, there was a 5–6% reduction in the formation of GST metabolites in the lung and liver at an exposure level of 50 ppm. At 2,000 or 4,000 ppm, however, there was only a 0.77 or 0.37% reduction, respectively. Marino et al. (2006) did not discuss the impact of including extrahepatic metabolism in the rapidly perfused tissue compartment; the same group of investigators developed a human PBPK model that included CYP metabolism in the richly perfused compartment (David et al., 2006).

3.5.2. Probabilistic Human PBPK Dichloromethane Model (David et al., 2006)

The basic model structure used by David et al. (2006) was that of Andersen et al. (1987) with the addition of the CO submodel of Andersen et al. (1991), refinements from the Marino et al. (2006) mouse model, and an inclusion of CYP metabolism in richly perfused tissue (Figure 3-4). David et al. (2006) used Bayesian analysis to develop and calibrate metabolic

parameters in a human probabilistic PBPK model for dichloromethane, using kinetic data from several studies of volunteers exposed to dichloromethane (n = 13 from DiVincenzo and Kaplan (1981); n = 12 from Engström and Bjurström (1977); n = 14 from Astrand et al. (1975); n = 3 from Stewart et al. (1972b), and group means for metabolism parameters from Andersen et al. (1991)]. Exhaled dichloromethane and CO and blood levels of dichloromethane and COHb were available in the studies by Andersen et al. (1991) and DiVincenzo and Kaplan (1981). The other three studies included two or three of these measures. The only available data for levels of dichloromethane in fat came from the study of Engström and Bjurström (1977) (described in Section 3.2).

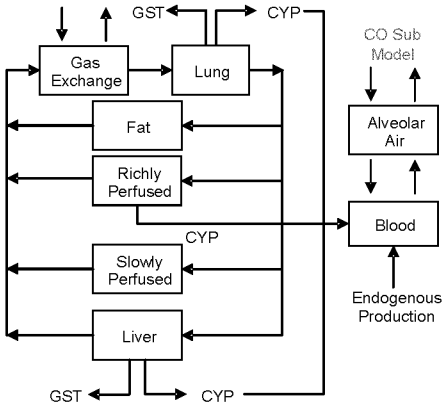


Figure 3-4. Schematic of human PBPK used by David et al. (2006).

Values (means and SDs or CVs) for the model parameter distributions were selected from multiple sources considered to provide the most current scientific evidence for each parameter (David et al., 2006). Mean values for QCC, VPR, and all fractional tissue volumes and blood flow rates were based on mean values used by U.S. EPA (2000d) in a PBPK model for vinyl chloride, as were values for CVs for all physiological parameters, except CVs for VPR and fractional lung volume, which were set to those used by OSHA (1997). Means for the CO submodel parameters were set equal to those in Andersen et al. (1991), except for those for the endogenous rate of CO production (REnCOC) and the background amount of CO (AB_{COC}), which were based on data collected by DiVincenzo and Kaplan (1981). Means for partition coefficients, the A1 ratio, and the A2 ratio were those used by Andersen et al. (1987), whereas prior means for V_{maxC} and K_m were those used by Andersen et al. (1991). The prior mean for the metabolic parameter for CYP metabolism in the rapidly perfused tissue was set at 0.03, slightly lower than the value suggested by Sweeney et al. (2004). Prior CVs for the metabolic parameters were set at 200%.

MCMC analysis was used to calibrate metabolic parameters in the human model in a two-step approach: (1) posterior distributions were estimated separately by using data from each of the five studies with kinetic data for humans exposed to dichloromethane (with durations ranging from 1 to 8 hours and concentrations ranging from 50 to 1,000 ppm), and (2) posterior distributions were estimated with combined data from the 42 individual subjects from the four studies with individual subject data (DiVincenzo and Kaplan, 1981; Engström and Bjurström, 1977; Astrand et al., 1975; Stewart et al., 1972b). Estimates of the population mean values for the fitted parameters from the Bayesian calibration with the combined kinetic data for individual subjects are shown in Table 3-7. This analysis resulted in a narrowing of the distribution for the CYP2E1 metabolism parameters V_{maxC} and K_m from a fairly broad prior distribution, with a CV of 200% for both parameters, to 13.1 and 33.6%, respectively, for V_{maxC} and K_m. It should be noted that the CV values only represent the uncertainties in the corresponding population mean values and do not include the estimated interindividual variability (email from Harvey Clewell to Paul Schlosser, U.S. EPA, dated October 14, 2009). Thus, that narrowing should only be interpreted as indicating a high degree of confidence in the population mean. As will be discussed in detail later, other data which better characterize the variability in CYP2E1 activity among the human population should then be used in conjunction with these uncertainties to characterize the full range of uncertainty and variability.

Table 3-7. Results of calibrating metabolic parameters in a human probabilistic PBPK model for dichloromethane with individual kinetic data for 42 exposed volunteers and MCMC analysis

Parameter	Prior distributions		Posterior distributions	
	Mean (arithmetic)	CV	Mean (arithmetic)	CV
V_{maxC}—maximal CYP metabolic rate (mg/hr/kg$^{0.7}$)	6.25	2	9.42	0.131
K_m—CYP affinity (mg/L)	0.75	2	0.433	0.336
k_{fC}—first-order GST metabolic rate (kg$^{0.3}$/hr)	2	2	0.852	0.711
A1—ratio of lung V_{maxC} to liver V_{maxC}	0.00143	2	0.000993	0.399
A2—ratio of lung k_{fC} to liver k_{fC}	0.0473	2	0.0102	0.728
FracR—fraction of V_{maxC} in rapidly perfused tissues	0.03	2	0.0193	0.786

Source: David et al. (2006).

A component of quantitative uncertainty arises in examining the results of David et al. (2006), specifically for the GST metabolic parameter, k_{fC}. The authors reported Bayesian posterior statistics for the population mean parameters when calibration was performed either with specific published data sets or the entire combined data set. While one would generally expect that the values obtained from the combined data set should be a weighted average of the values from individual data sets, the population mean for the liver GST activity (coefficient), k_{fC},

was 0.852 from the combined data set while the values from the individual data sets ranged from 1.92 to 34.0 $kg^{0.3}$/hour.

A clarification provided by Marino (email from Dale Marino to Glinda Cooper, U.S. EPA, dated April 25, 2007) is that the parameter bounds stated in the text of David et al. (2006) were only applied for the analysis of the DiVincenzo and Kaplan (1981) and the combined data set. But according to the text and distribution prior statistics specified, the upper bound for k_{fC} would have been 12 $kg^{0.3}$/hour (mean + 2.5 SDs, with mean = 2 and SD = mean × CV = 2 × 2 = 4). The data of Andersen et al. (1991) were not used in the combined analysis because only group average values were available from that source, rather than individual data. Since the remaining study-specific mean k_{fC} values were 7.95, 5.87, 34.0, and 1.92, with CVs of <2, it seems unlikely that application of this upper bound would result in a value of k_{fC} = 0.852 $kg^{0.3}$/hour. Given the convergence problems with the combined data set when parameter values were unbounded, it is possible that convergence had not actually been reached after parameter bounds were introduced, and a higher value for k_{fC} would have been obtained had the chain been continued longer. The implications of this parameterization uncertainty are discussed in Section 5.3 for noncancer toxicity modeling and Section 5.4.5 for cancer dose-response modeling.

Setting this uncertainty aside, since the parameter statistics shown in Table 3-7 [values reported by David et al. (2006)] represent population means and the level of uncertainty in those means, their correct interpretation requires further consideration. As noted above, there is a known range of variability in CYP2E1 expression among the human population which should be incorporated when estimating overall population variability. For the remaining parameters except k_{fC} (i.e., for K_m, A1, A2, and FracR), there is not a known equivalent level of variability and it will be assumed that there is, in fact, a single true value for the population which is estimated as the population mean by David et al. (2006). In that case one needs only to include the uncertainty in the mean represented by the CV values in Table 3-7 in a statistical (Monte Carlo) sampling in order to fully characterize model uncertainty and variability. While the analysis of David et al. (2006) may have included variability among individual-specific estimates for those parameters, this treatment effectively assumes that this variability was only an apparent artifact of attributes such as the limited data and measurement noise.

David et al. (2006) further refined the human probabilistic model to reflect polymorphisms in the GST pathway: homozygous positive (+/+) GST-T1, heterozygous (+/-) GST-T1, and homozygous negative (-/-) GST-T1 individuals with no GST activity. Distributions of GST activities for these genotypes in a group of 208 healthy male and female subjects from Sweden were scaled to obtain distributions of k_{fC} for each genotype (Warholm et al., 1994). When weighted by estimated frequencies of the genotypes in the U.S. population and appropriately scaled, these genotype-specific activity distributions would result in an overall population mean equal to the k_{fC} mean for the posterior distribution shown in Table 3-7 (0.852 $kg^{0.3}$/hour). The resultant mean k_{fC} values were 0.676 $kg^{0.3}$/hour (SD 0.123) for

heterozygous individuals and 1.31 $kg^{0.3}$/hour (SD 0.167) for homozygous positive individuals, as indicated in Table 2 of David et al. (2006). The final parameter distributions used by David et al. (2006) are summarized in Table 3-8.

Since the measurements of GST-T1 activity by Warholm et al. (1994) were performed ex vivo using blood samples, it is reasonable to assume that those measurements are accurate and, hence, that the characterization of the distributions for each genotype as being normal with the reported level of variance represents the true level of human variability for each genotype. However, that characterization does not include the uncertainty in the overall population mean for the rate of hepatic GST activity towards dichloromethane, indicated by the CV for k_{fC} in Table 3-7 [from Table 4 of David et al. (2006)]. Thus, to fully account for both the population variability and parameter uncertainty, a Monte Carlo statistical sampling should first sample the population mean from a distribution with mean = 0.852 $kg^{0.3}$/hour and CV = 0.711 (thus accounting for uncertainty) and then reweight the population-specific distributions listed in Table 3-8 to have that sampled population mean, before selecting (sampling) an individual value of k_{fC} from those weighted distributions (thus accounting for variability). EPA incorporated this change in the PBPK modeling used in this assessment.

As described in Appendix B, EPA evaluated the adequacy of the parameter distributions used by David et al. (2006) to characterize variability among the full human (U.S.) population. EPA's conclusion is that the reported distributions for many of the physiological parameters in particular, as well as V_{maxC} (CYP2E1), only represented a narrow set of adults and did not represent the full range of variability. Thus EPA used supplemental data sources to define these distributions to more fully characterize the variability in the human population for individuals between 6 months and 80 years of age. Specifically, while the BW distribution used in the David et al. (2006) PBPK model used ranges from 7 to 130 kg, thus covering 6-month-old children to obese adults, there are age-dependent changes and gender-dependent differences in ventilation rates and body fat that are not explicitly included. To more accurately reflect the distribution of physiological parameters in the entire population, EPA replaced the unstructured distributions of David et al. (2006) with distributions based on available information that specifically account for population variability in age, gender, and age- and gender-specific distributions or functions for BW, QCC, alveolar ventilation, body fat (fraction), and liver fraction (see Appendix B for more details of the evaluation of each of these parameters).

Table 3-8. Parameter distributions used in human Monte Carlo analysis for dichloromethane by David et al. (2006)

Parameter		Distribution		Source
		Mean (arithmetic)	SD	
BW	Body weight (kg)	70.0	21.0	Humans[a]
QCC	Cardiac output (L/hr/kg$^{0.74}$)	16.5	1.49	Humans[a]
VPR	Ventilation:perfusion ratio	1.45	0.203	Humans[a]
QFC	Fat	0.05	0.0150	Humans[a]
QLC	Liver	0.26	0.0910	Humans[a]
QRC	Rapidly perfused tissues	0.50	0.10	Humans[a]
QSC	Slow perfused tissues	0.19	0.0285	Humans[a]
Tissue volumes (fraction BW)				
VFC	Fat	0.19	0.0570	Humans[a]
VLC	Liver	0.026	0.00130	Humans[a]
VLuC	Lung	0.0115	0.00161	Humans[a]
VRC	Rapidly perfused tissues	0.064	0.00640	Humans[a]
VSC	Slowly perfused tissues (muscle)	0.63	0.189	Humans[a]
Partition coefficients				
PB	Blood:air	9.7	0.970	Humans[b]
PF	Fat:blood	12.4	3.72	Rats[b]
PL	Liver:blood	1.46	0.292	Rats[b]
PLu	Lung:arterial blood	1.46	0.292	Rats[b]
PR	Rapidly perfused tissue:blood	1.46	0.292	Rats[b]
PS	Slowly perfused tissue (muscle:blood)	0.82	0.164	Rats[b]
Metabolism parameters				
V_{maxC}	Maximum metabolism rate (mg/hr/kg$^{0.7}$)	9.42	1.23	Calibration[c]
K_m	Affinity (mg/L)	0.433	0.146	Calibration[c]
A1	Ratio of lung V_{max} to liver V_{max}	0.000993	0.000396	Calibration[c]
A2	Ratio of lung KF to liver KF	0.0102	0.00739	Calibration[c]
FracR	Fractional CYP2E1 capacity in rapidly perfused tissue	0.0193	0.0152	Calibration[c]
First order metabolism rate (/hr/kg$^{0.3}$)				
k_{fC}	Homozygous (-/-)	0	0	Hybrid[d]
	Heterozygous (+/-)	0.676	0.123	Hybrid[d]
	Homozygous (+/+)	1.31	0.167	Hybrid[d]

[a]U.S. EPA (2000d). Human PBPK model used for vinyl chloride.
[b]Andersen et al. (1987). Blood:air partition measured using human samples; other partition coefficients based on estimates from tissue measures in rats.
[c]Bayesian calibration based on five data sets (see text for description); posterior distributions presented in this table.
[d]The overall population mean for k_{fC} as determined by Bayesian calibration; the distribution of activity among the three genotypes and variability in activity for each genotype (SD values) were then scaled from the ex vivo data of Warholm et al. (1994).

Source: David et al. (2006).

For V_{maxC} (CYP2E1), EPA also incorporated additional data for the variability in CYP2E1 activity among humans based on Lipscomb et al. (2003). The Lipscomb et al. (2003) study used in vitro analysis of liver samples from 75 human tissue donors (activity towards trichloroethylene and measurements of protein content) to estimate a distribution of activity in the population. These data support a wider distribution in CYP2E1 activity than had been used in the David et al. (2006) dose-metric and unit-risk calculations, with approximately a sixfold range observed for CYP2E1 in Lipscomb et al. (2003) and a twofold range used by David et al. (2006). After sampling the population mean for CYP2E1 from the distribution indicated by the parameters in Table 3-8 to capture the uncertainty in the population mean, EPA assumed a log-normal distribution for human variability around that mean and sampled the individual value using that population mean with geometric standard deviation (GSD) = 1.73. Further, since even the data available to Lipscomb et al. (2003) were limited, and the log-normal distribution is naturally bounded to be greater than zero, EPA used a nontruncated log-normal distribution in the second (variability) sampling step for this parameter. (In sampling the population-mean value for V_{maxC} from its range of uncertainty, EPA did truncate the distribution as indicated by David et al. (2006), since that mean is expected to be bounded away from zero.) Finally, the scaling of CYP2E1 for individuals under the age of 18 was adjusted based on the data of Johnsrud et al. (2003); EPA's analysis of these data indicates that CYP2E1 activity in children is better predicted when assumed to scale with BW raised to the 0.88 power, as compared to the more general power of 0.7, used by David et al. (2006). (Details of EPA's analysis are given in Appendix B, Section B.3.) CYP2E1 activity for individuals over the age of 18 is still assumed to scale as $BW^{0.7}$.

The resulting set of parameter distribution characteristics, including those used as defined by David et al. (2006) are described in Table 3-9. Using this revised set of distributions, including the CYP and GST activity distributions, and other distributions used as defined by David et al. (2006) or revised by EPA, the model as applied should reflect the full variability in the (U.S.) human population.

Table 3-9. Parameter distributions for the human PBPK model for dichloromethane used by EPA

Parameter		Distribution					Section or source
		Shape	(Geometric) mean[a]	SD/GSD[a]	Lower bound	Upper bound	
BW	Body weight (kg)	Normal	*f (age, gender)*		*1st Percentile*	*99th Percentile*	B.4.3; NHANES IV
Flow rates							
QAlvC	Alveolar ventilation (L/hr/kg$^{0.75}$)	Normal	*f (age, gender)*	*f (age)*	*5th Percentile*	*95th Percentile*	B.4.4; mean: Clewell et al. (2004); SD: Arcus-Arth and Blaisdell (2007)
vprv	Variability in ventilation:perfusion ratio	Log-normal	1.00	0.203	0.69	1.42	VPR/VPR$_{mean}$ of David et al. (2006)
QCC	Cardiac output (L/hr/kg$^{0.75}$)	QCC$_{mean}$ = *f*(QAlvC)		QCC = QCC$_{mean}$/vprv			B.4.5; Clewell et al. (2004) (mean)
Fractional flow rates (fraction of QCC)							
QFC	Fat	Normal	0.05	0.0150	0.0050	0.0950	David et al. (2006); *after sampling from these distributions, normalize:* $Qi = \frac{QC \cdot QiC}{\sum QjC}$
QLC	Liver	Normal	0.26	0.0910	0.010	0.533	
QRC	Rapidly perfused tissues	Normal	0.50	0.10	0.20	0.80	
QSC	Slow perfused tissues	Normal	0.19	0.0285	0.105	0.276	
Tissue volumes (fraction BW)							
VFC	Fat	Normal	*f (age, gender)*	0.3 × mean	0.1 × mean	1.9 × mean	B.4.6; fat mean: (Clewell et al., 2004); B.4.7; liver mean: (Clewell et al., 2004); otherwise, David et al. (2006); *after sampling from these distributions, normalize:* $Vi = \frac{0.9215 \cdot BW \cdot ViC}{\sum VjC}$
VLC	Liver	Normal	*f (age)*	0.05 × mean	0.85 × mean	1.15 × mean	
VLuC	Lung	Normal	0.0115	0.00161	0.00667	0.0163	
VRC	Rapidly perfused tissues	Normal	0.064	0.00640	0.0448	0.0832	
VSC	Slowly perfused tissues	Normal	0.63	0.189	0.431	0.829	
Partition coefficients							
PB	Blood:air	Log-normal	9.7	1.1	7.16	13.0	Geometric mean (GM) & GSD values listed here, converted from arithmetic mean and SD values of David et al. (2006)
PF	Fat:blood	Log-normal	11.9	1.34	4.92	28.7	
PL, PLu, & PR	Liver:blood, lung:arterial blood, and rapidly perfused tissue:blood	Log-normal	1.43	1.22	0.790	2.59	
PS	Slowly perfused tissue (muscle):blood	Log-normal	0.80	1.22	0.444	1.46	

(Table 3-9; page 1 of 2)

Table 3-9. Parameter distributions for the human PBPK model for dichloromethane used by EPA

Parameter		Distribution: Shape	(Geometric) mean[a]	SD/GSD[a]	Lower bound	Upper bound	Section or source
Metabolism parameters (based on Monte Carte calibration from five human data sets)							
$V_{maxCmean}$/ V_{maxC}	Population mean / individual maximum metabolism rate (mg/hr/kg^{Xvmax})	Log-normal Log-normal	9.34 $V_{maxCmean}$	1.14 1.73	6.96 (none)	11.88 (none)	B.3 mean: David et al. (2006); Individual GSD: Lipscomb et al. (2003); Xvmax = 0.88 for age <18; Xvmax = 0.70 for age ≥18
K_m	Affinity (mg/L)	Log-normal	0.41	1.39	0.154	1.10	GM and GSD values listed here, converted from arithmetic mean and SD values of David et al. (2006)
A1	Ratio of lung V_{max} to liver V_{max}	Log-normal	0.00092	1.47	0.000291	0.00292	
A2	Ratio of lung KF to liver KF	Log-normal	0.0083	1.92	0.00116	0.0580	
FracR	Fractional MFO capacity in rapidly perfused tissue	Log-normal	0.0152	2.0	0.00190	0.122	
First order metabolism rate ([hr/kg$^{0.3}$]$^{-1}$)							
k_{fCmean}	Population average	Log-normal	0.6944	1.896	0.1932	2.496	Adapted from David et al. (2006); k_{fCmean} is first sampled, then the relative individual value, k_{fC}/k_{fCmean}, given the genotype; k_{fC} is then the product
k_fC/k_fC_{mean}	Homozygous (-/-)	Normal	0	0	0	0	
	Heterozygous (+/-)	Normal	0.8929	0.1622	0	1.704	
	Homozygous (+/+)	Normal	1.786	0.2276	0	2.924	

[a]Arithmetic mean and SD listed for normal distributions; GM and GSD listed for log-normal distributions.

(Table 3-9; page 2 of 2)

3.5.3. Evaluation of Rat PBPK Dichloromethane Models

Several deterministic PBPK rat models have been reported (Sweeney et al., 2004; Andersen et al., 1991; Reitz, 1991; Reitz et al., 1989a; Reitz et al., 1988; U.S. EPA, 1988a; Andersen et al., 1987; U.S. EPA, 1987a, b; Gargas et al., 1986). Unlike the mouse (Marino et al., 2006) and human (David et al., 2006), no hierarchical population model for dichloromethane in the rat exists in which parameter uncertainty is quantitatively integrated into model calibration. Rat data are not available for Bayesian calibration of individual metabolic parameters for the CYP or GST pathways. EPA assessed modified versions of deterministic rat PBPK models to select the most appropriate model for use in extrapolating internal dosimetry from rats to humans for the determination of RfDs and RfCs based on effects seen in the rat. This work is described in detail in Appendix C and is based on evaluation of blood levels of dichloromethane, the percent saturation of hemoglobin as COHb (%COHb), and expired dichloromethane following intravenous injection (Angelo et al., 1986b) and closed chamber gas uptake (Gargas et al., 1986), as well as %COHb blood levels from a 4-hour inhalation exposure (Andersen et al., 1991; Andersen et al., 1987). Based on this work, the basic model structure of Andersen et al. (1991) was chosen, with inclusion of lung dichloromethane metabolism via CYP (0.2% of liver metabolite production) and GST (14.9% of liver metabolite production) pathways [estimated from Reitz et al. [(1989a)] (Figure 3-5) with metabolic parameters recalibrated against data of Andersen et al. (1991), based on prediction agreement of the various parameters with the available rat data sets. Table 3-10 presents the parameter distribution data for this model.

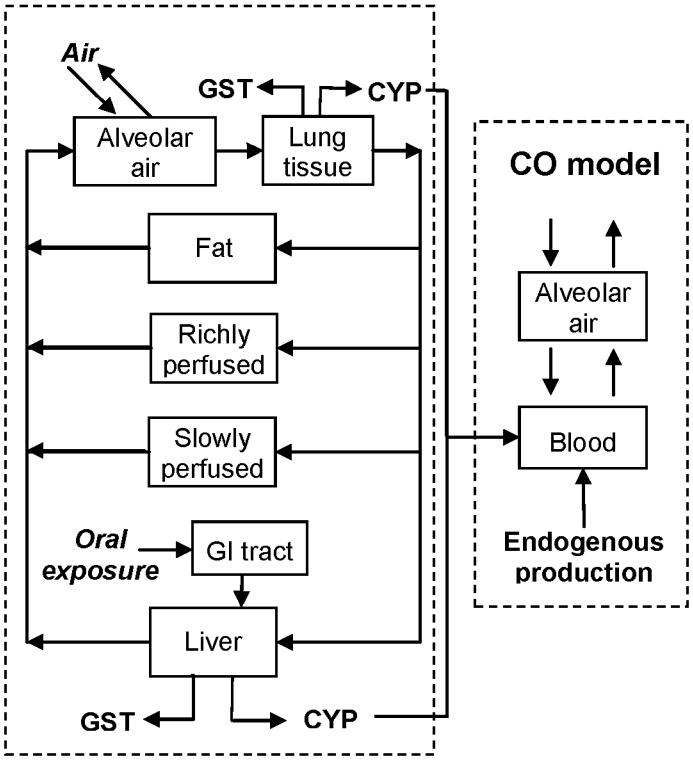


Figure 3-5. Schematic of rat PBPK model used in current assessment.

Table 3-10. Parameter values for the rat PBPK model for dichloromethane used by EPA

Parameter	Mean
Flow rates	
QCC (L/hr/kg$^{0.74}$)	15.9
VPR	0.94
Fractional flow rates (percent of QCC)	
Fat	9
Liver	20
Rapidly perfused tissues	56
Slowly perfused tissues	15
Tissue volumes (percent BW)	
Fat	7
Liver	4
Lung (scaled as BW$^{0.99}$)	1.15
Rapidly perfused tissues	5
Slowly perfused tissues	75
Partition coefficients	
Blood:air	19.4
Fat:blood	6.19
Liver:blood	0.732
Lung:arterial blood	0.46
Rapidly perfused tissue:blood	0.732
Slowly perfused tissue (muscle):blood	0.408
Metabolism parameters	
V_{maxC}: maximum metabolism rate (mg/hr/kg$^{0.7}$)	3.97
K_m: affinity (mg/L)	0.510
$k_f c$: first order GST metabolic rate in liver (kg$^{0.3}$/hr)	2.47
A1: ratio of lung V_{max} to liver V_{max}	0.002
A2: ratio of lung k_f to liver k_f	0.149
P1: yield of CO from CYP metabolism	0.68
Other elimination/absorption constants	
F1: correction factor for CO exhalation rate	1.21
k_a: first-order oral absorption rate constant, k_a (1/hr)	4.31

3.5.4. Comparison of Mouse, Rat, and Human PBPK Models

The comparison of various parameters across species (Table 3-11) primarily shows the modest interspecies differences that are known to occur in physiological parameters, also including the approximately twofold differences in partition coefficients which occur because of differences in rodent versus human blood lipid content. The 2.5-fold lower V_{maxC} (CYP activity) in rats versus mice is also typical. The most striking difference is the variation in A1 and A2. Those values, however, reflect the in vitro differences originally quantified by Lorenz et al.

(1984) and Reitz et al. (1989a), with the mouse and human values being those used in the dichloromethane PBPK modeling of Andersen et al. (1987). In examining the derivation of the rat values, however, it appeared that Andersen et al. (1987) did not adjust the relative activity per mg microsomal protein to account for the difference in total microsome content of the lung versus liver. Once this adjustment was performed, the rat value was found to be 20-fold lower than the value used by Andersen et al. (1987) and about twice that of the human. These interspecies differences in A1 and A2 are based on independent measurements of tissue-specific metabolic capacity; while the specific values for mouse and human were refined through Bayesian analysis, the ultimate (posterior) values used are within a reasonable range of the in vitro measurements and so do not appear to be artifactual. (Since in vivo kinetics often indicate some differences from what would be predicted without adjustment from in vitro, it is not surprising that such differences occur here.) These differences do explain why lung-specific metrics in particular lead to lower internal dose and hence risk predictions in humans compared to whole-body metrics.

3.5.5. Uncertainties in PBPK Model Structure for the Mouse, Rat, and Human

There is uncertainty in the dichloromethane PBPK modeling because there are parts of the entire data set for which the existing model and parameters fit very poorly and where the discrepancies appear to be structural (i.e., cannot be resolved simply by re-fitting model parameters unless fits to other data are degraded). The data used for model parameter estimation are primarily measurements of parent dichloromethane kinetics (e.g., blood or closed-chamber air concentrations over time), rather than measurements of metabolite levels which can be unambiguously attributed to one of the two principal metabolic pathways (GST and CYP). For the mouse model in particular, only parent dichloromethane data were used, although exhaled amounts of CO_2 and CO are available. Because only dichloromethane measurements are used, estimation of the fraction of dichloromethane metabolized by the GST versus CYP pathway depends strongly on the assumed equations describing those rates and how the pharmacokinetic data are interpreted. Specifically, if there is some degree of saturation in the GST kinetics or the CYP kinetics are not accurately described by Michaelis-Menten kinetics (see next paragraph), then the estimation of the fraction of metabolism going down each pathway would shift. Further, while Marino et al. (2006) used data from mice pretreated with trans-1,2-dichloroethylene (tDCE), a specific CYP2E1 inhibitor (mice were exposed to 100 ppm tDCE for 1.5 hours prior to dichloromethane exposure), the authors assumed without verification that 100% of the CYP2E1 activity was eliminated by the inhibitor when using those data. In contrast, Mathews et al. (1997) found that pretreatment of F344 rats by tDCE (100 mg/kg intraperitoneally) only yielded 65% inhibition of CYP2E1. If a significant fraction of the CYP2E1 activity was not eliminated in the dichloromethane experiments, then that activity is erroneously assigned to the GST pathway in the parameter estimation of Marino et al. (2006).

Table 3-11. Parameters in the mouse, rat, and human PBPK model for dichloromethane used by EPA

Parameter	Mouse[a] mean	Rat[b] value	Human[c] Mean	Human[c] CV/*GSD* (shape, bounds)	Human[c] Sources
Fractional flow rates (fraction of cardiac output)[b]					David et al. (2006); *then normalized:* $Qi = \frac{QC \cdot QiC}{\sum QjC}$
QFC Fat	0.05	0.09	0.05	0.3 (N, 0.1–1.9)	
QLC Liver	0.24	0.20	0.26	0.35 (N, 0.0385–2.05)	
QRC Rapidly perfused tissues	0.52	0.56	0.50	0.2 (N, 0.4–1.6)	
QSC Slowly perfused tissues	0.19	0.15	0.19	0.15 (N, 0.553–1.453)	
Fractional tissue volumes (fraction of BW)[b]					Fat mean: §2.2.3.6; Liver mean: §2.2.3.7; otherwise David et al. (2006); *then normalized:* $Vi = \frac{0.9215 \cdot BW \cdot ViC}{\sum VjC}$
VFC Fat	0.04	0.07	*f (age, gender)*	0.3 (N, 0.1–1.9)	
VLC Liver	0.04	0.04	*f (age)*	0.05 (N, 0.85–1.15)	
VluC Lung	0.0115	0.0115	0.0115	0.14 (N, 0.58–1.42)	
VRC Rapidly perfused tissues	0.05	0.05	0.064	0.1 (N, 0.7–1.3)	
VSC Slowly perfused tissues	0.78	0.75	0.63	0.3 (N, 0.684–1.32)	
Partition coefficients[c]					GM and GSD/GM values converted from arithmetic mean & SDs of David et al. (2006)
PB Blood/air	23.0	19.4	9.7	*1.1* (LN, 0.738–1.34)	
PF Fat/blood	5.1	6.19	11.9	*1.34* (LN, 0.413–2.41)	
PL Liver/blood	1.6	0.73	1.43	*1.22* (LN, 0.552–1.81)	
PLu Lung/blood	0.46	0.46	1.43	"	
PR Rapidly perfused/blood	0.52	0.73	1.43	"	
PS Slowly perfused/blood	0.44	0.41	0.80	*1.22* (LN, 0.555–1.83)	
Flow rates					QCC: §2.2.3.5; vprv = VPR/VPR_{mean}: David et al. (2006); QAlvC: §2.2.3.4;
QCC Cardiac output (L/hr/$kg^{0.74}$)	24.2	14.99	$QCC_{mean} = f$(QAlvC)	QCC = QCC_{mean}/vprv	
VPR Ventilation/perfusion ratio	1.45	0.94	(variable)	*(varies)* (LN, 0.69–1.42)	
QAlvC	QCC/VPR	QCC/VPR	*f (age, gender)*	*f (age)* (N, 5^{th}–95^{th}%)	

(Table 3-11; page 1 of 2)

Table 3-11. Parameters in the mouse, rat, and human PBPK model for dichloromethane used by EPA

Parameter	Mouse[a] mean	Rat[b] value	Human[c]		
			Mean	CV/*GSD* (shape, bounds)	Sources
Metabolism parameters					
V_{maxC} Maximum CYP metabolic rate (mg/hr/kg^{Xvmax})	9.27	3.97	*LN(m = 9.34, s = 1.14 | lb = 6.96, ub = 11.88)*	*1.73* (LN, [unbounded])	V_{maxC}: §2.2.2; others: David et al. (2006)
Xvmax CYP allometric scaling power	0.7	0.7	0.88 for age <18; 0.7 for age ≥18)	*1.39* (LN, 0.376–2.68)	(GM & GSD/GM values converted from arithmetic mean & SDs)
K_m CYP affinity (mg/L)	0.574	0.510	0.41		
$k_{fC(mean)}$ First-order GST metabolic rate constant (kg$^{0.3}$/hr)	1.41	2.47	*LN(m = 0.6944, s = 1.896 | lb = 0.1932, ub = 2.496)*		$k_{fC(mean)}$: Combined data set posterior, Table 4 of David et al. (2006)
$k_{fC}/k_{fC(mean)}$	1	1	0 (-/-)[d] 0.8929 (+/-)[d] 1.7896 (+/+)[d]	-/-: NA +/-: 0.182 (N, 0–1.91) +/+: 0.127 (N, 0–1.64)	k_{fC} / $k_{fC(mean)}$: rescaled from Table 2 of David et al. (2006)
A1 Ratio of lung V_{maxC} to liver V_{maxC}	0.207	0.002	0.00092	*1.47* (LN, 0.316–3.17)	
A2 Ratio of lung k_{fC} to liver k_{fC}	0.196	0.149	0.0083	*1.92* (LN, 0.140–6.99)	

[a]Based on Marino et al. (2006) (source for all mouse parameters).
[b]Based on Andersen et al. (1991), with the addition of lung metabolism of dichloromethane via the CYP (4% of liver metabolite production) and GST (14% of liver metabolite production) pathways. Physiological parameters and partition coefficients are from Andersen et al. (1991). The values for dichloromethane metabolism in the lung (as a fractional yield of liver metabolism for each pathway) were estimated from the in vitro ratios of enzyme activity (nmol/min/mg protein) in lung and liver cytosolic (GST) and microsomal (CYP) tissue fractions (Reitz et al., 1989a). Metabolic parameters were re-optimized against the inhalation data of Andersen et al. (1991) using a heteroscedasticity parameter value of 2, which uses relative error for the model fitting algorithm. See Appendix C for further details.
[c]Based on David et al. (2006), with changes as noted. Additional sources include Clewell et al. (2004), Arcus-Arth and Blaisdell (2007), and Lipscomb et al. (2003). See identified sections for details. Distribution values (mean and a measure of dispersion) are provided with the CV (mean/SD) presented for normal (N) distributions and the GSD (italicized) presented for log-normal (LN) distributions. Distributions were truncated, bounds are (upper-lower bound)/mean.
[d]Values for the homozygous (-/-), heterozygous (+/-), and homozygous (+/+) GST-T1 genotypes, respectively.

In addition to the possibility that incomplete inhibition of CYP2E1 affects the data interpretation, the Michaelis-Menten rate equation used in the dichloromethane PBPK models, including that of Marino et al. (2006), has not been shown to accurately describe the CYP2E1-mediated metabolism of dichloromethane in the relevant concentration range. While the Michaelis-Menten equation usually describes CYP-mediated oxidation data quite well, if there is some departure of the actual kinetics from this equation and incomplete inhibition from the tDCE treatment, the model parameters obtained under those assumptions would be compromised. If pathway-specific metabolite data were used to define or bound the ratio of GST to CYP metabolism, the resulting estimates would be less sensitive to errors in the CYP rate equation.

EPA compared model predictions of total CYP metabolism in mice, which should match with CO elimination at 24 hours after bolus exposures to dichloromethane since only the CYP pathway produces CO. At 50 mg/kg (in water), the model predicts that 10% of the dichloromethane is metabolized by the CYP pathway, agreeing with the observed values of 11–12% in Angelo et al. (1986a). However, at 500 and 1,000 mg/kg, the model predicts that 1.8 and 1.0% will be metabolized by the CYP pathway, while Angelo et al. (1986a) observed 9–10 and 3.6–7%, respectively. Thus, the extent of CYP saturation predicted by the model does not match these pathway-specific metabolite data. As shown in Appendix C, the rat model predicts total exhaled CO quite well, indicating an error in the fraction of metabolism via the GST pathway of <13% in that species. That the *mouse* model does not describe well the dose-dependent shift in metabolism shown by those CO data suggests that the dose-dependence of the CYP Michaelis-Menten rate-equation may not be adequate. As will be shown, an alternative equation for CYP kinetics may fit the existing dichloromethane data better than Michaelis-Menten kinetics, with the result that a higher portion of total dichloromethane metabolism would be interpreted as being CYP-mediated. Thus, there is some uncertainty in the choice of equation for the CYP pathway, which leads to some uncertainty in the estimated GST:CYP metabolic ratio, upon which current risk predictions are based. However the extent of the error appears quite limited in the rat and more predominant at high exposures versus low exposures in the mouse.

The potential error in assuming Michaelis-Menten kinetics for CYP-mediated oxidation of dichloromethane is reinforced by examining the in vitro oxidative (i.e., CYP-specific) kinetics of dichloromethane reported by Reitz et al. (1989a). When extrapolated from in vitro to in vivo, the apparent values of the oxidative saturation constant, K_m, identified by Reitz et al. (1989a) for mice, rats, and humans are over 2 orders of magnitude greater than those obtained in vivo with the PBPK model. This apparent discrepancy is partly explained by the disparate concentration ranges investigated: Reitz et al. (1989a) used much higher dichloromethane concentrations in vitro than those observed in or predicted for the various in vivo pharmacokinetic studies. In particular, the oxidation of dichloromethane could involve two oxidative processes, one with a high affinity (low K_m) corresponding to the nonlinearity observed in vivo and one with a low affinity (high K_m) corresponding to the nonlinearity observed in vitro. Such a low-affinity

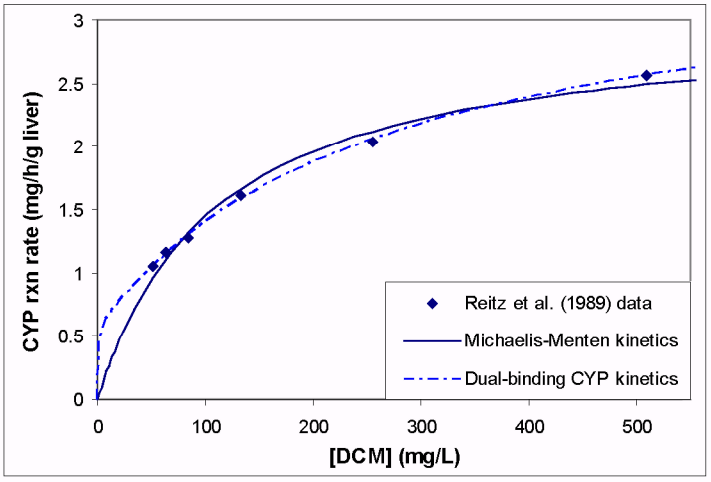
process might account for the higher CO production observed in vivo (see above) than predicted by the current model. Further, a low-affinity process would have nearly linear kinetics in the exposure range used for the in vivo dosimetry studies and, hence, would be difficult to distinguish from GST-mediated metabolism unless pathway-specific metabolite data are used. If this second oxidative process is not inhibited by tDCE, then it may correspond to the 35% of oxidative metabolism observed to remain in rats after tDCE treatment by Mathews et al. (1997).

The data of Reitz et al. (1989a) could indicate a second CYP with low-affinity dichloromethane activity, but that possibility is contradicted by the results of Kim and Kim (1996) who observed that another CYP2E1-specific inhibitor, disulfiram, completely abolished dichloromethane-induced increases on COHb in rats. Another possible explanation which supports the findings observed in Kim and Kim (1996), as well as Reitz et al. (1989a) and the various in vivo data, is that a number of CYPs exhibit "atypical" kinetics, not described by the classic Michaelis-Menten equation, consistent with the enzymes having dual binding sites as proposed by Korzekwa et al. (1998). (Korzekwa et al. [(1998) demonstrated atypical kinetics for several CYP-isozyme/substrate pairs, but not specifically for CYP2E1.) The application of this alternate kinetic model to dichloromethane dosimetry in mice was explored by Evans and Caldwell (2010), who demonstrate that the dichloromethane gas-uptake data in mice can be explained with this model in the hypothetical case where the GST pathway is not included. The Evans and Caldwell (2010) alternate PBPK model is not considered further here because GST-mediated metabolism of dichloromethane clearly occurs in mice, rats, and humans based on the in vitro observations of Reitz et al. (1989a) and a model which excludes GST-mediated metabolism is not consistent with the overall database concerning dichloromethane metabolism and carcinogenesis research (see Section 4.5)

Figure 3-6 shows kinetic model fits to the in vitro mouse dichloromethane oxidation kinetic data of Reitz et al. (1989a), expressing the data on a per gram of liver basis. The standard Michaelis-Menten kinetic equation (solid line) and the dual-binding equation (dashed line) given by Korzekwa et al. (1998) are shown. The high-affinity (low) K_m for the dual-binding equation was set equal to that obtained from the PBPK modeling by Marino et al. (2006). The dual binding model is not only consistent with the apparent high-affinity saturation obtained from in vivo PBPK modeling [K_m of Marino et al. (2006)], but also with the apparent low-affinity (high K_m) data of Reitz et al. (1989a). The figure also describes those in vitro data better than the standard Michaelis-Menten equation. Reitz et al. (1989a) used classic Lineweaver-Burk plots to display their kinetic data (i.e., 1/reaction rate versus 1/concentration). The systematic discrepancy between the data of this study and Michaelis-Menten kinetics evident in Figure 3-6 is much less obvious with that scaling, which may be why the study authors made no note of it.

In summary, the current PBPK model used the standard Michaelis-Menten equation to describe CYP2E1-catalyzed oxidation of small volatile organic compounds. Analysis of the dichloromethane pharmacokinetic data and evaluation of the inconsistencies described above

suggest that an alternate equation, which would impact risk predictions, may better represent CYP2E1-induced oxidation of dichloromethane. The analysis provided here demonstrates shortcomings in the existing model which the alternate model may address, indicating that this is a substantial model uncertainty. However, the hypothesis that CYP2E1 kinetics for dichloromethane should be described by this alternate rate equation requires further laboratory testing. For example, dichloromethane oxidation in a bacterial expression system where only CYP2E1 is expressed could be measured over a concentration range sufficient to firmly distinguish between the two kinetic forms Figure 3-6. Such experiments would indicate if the metabolic kinetics are due to atypical kinetics occurring with a single enzyme (CYP2E1), versus involvement of a second, low-affinity enzyme. Also, the alternate equation would need to be incorporated into a PBPK model which also included the GST pathway, and the resulting model calibrated not only for the mouse, but also for the rat and the human. Until such additional experiments and modeling are available, the existing PBPK model remains the best available science for dose- and risk-extrapolation from rodents to humans despite this uncertainty. Analysis of the GST-mediated metabolism of dichloromethane measured by Reitz et al. (1989a) shows that those results are within a factor of three of the GST kinetic parameters used in the current PBPK model, indicating that any error in the GST:CYP balance is in that range.



Dichloromethane oxidation data obtained with mouse liver microsomes by Reitz et al. (1989a) (points), expressed on a per gram of liver basis, with a fitted Michaelis-Menten equation (solid line) or a fitted dual-binding-site equation as described by Korzekwa et al. (1998) (dashed line), where the high affinity saturation constant of the dual-binding-site equation is set equal to the mean K_m determined for mice via PBPK modeling by Marino et al. (2006). The K_m for the Michaelis-Menten equation (108 mg/L) is inconsistent with the in vivo dichloromethane dosimetry data, while the in vitro data shown here are inconsistent with the K_m estimated in vivo (0.42 mg/L) if that equation is used.

Figure 3-6. Comparison of dichloromethane oxidation rate data with alternate kinetic models.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS

4.1.1. Introduction—Case Reports, Epidemiologic, and Clinical Studies

There has been considerable interest in the influence of occupational exposure to dichloromethane in relation to a variety of conditions. The recognition that dichloromethane can be metabolized and bound to hemoglobin to form COHb, resulting in a reduction in the oxygen carrying capacity of the blood (Stewart et al., 1972a), prompted investigations into risk of ischemic heart disease and other cardiovascular effects. Reports of neurological effects from acute, high-exposure situations contributed to concern about neurological effects of chronic exposure to lower levels of dichloromethane. A general interest in potential cancer risk became more focused on lung and liver cancer because of the observation of these specific tumors in the NTP (1986) experiments in mice. Studies pertaining to the experimental and epidemiologic studies of effects other than cancer (e.g., cardiac, neurologic) are summarized in Section 4.1.2; studies of cancer risk are summarized in Section 4.1.3, with additional details of cohort and case-control studies presented in Appendix D, Sections D.1 and D.2, respectively.

4.1.2. Studies of Health Effects Other Than Cancer

4.1.2.1. *Case Reports of Acute, High-dose Exposures*

Numerous case reports describe health effects resulting from acute exposure to dichloromethane (Bakinson and Jones, (1985); Rioux and Myers, (1988); Chang et al., (1999). Most describe health effects resulting from inhalation of dichloromethane or dermal contact. The COHb levels in some of these cases were relatively low (7.5–13%), so the initial toxic effects of acute dichloromethane exposure appear to be due to its anesthetic properties as opposed to metabolic conversion of dichloromethane to CO. Many of the incidents described in recent reports involve inadequately ventilated occupational settings (Jacubovich et al., 2005; Raphael et al., 2002; Fechner et al., 2001; Zarrabeitia et al., 2001; Goullé et al., 1999; Mahmud and Kales, 1999; Kim et al., 1996; Tay et al., 1995; Manno et al., 1992; Leikin et al., 1990; Shusterman et al., 1990). CNS depression and resulting narcosis, respiratory failure, and heart failure are common features of these cases. In a survey of workers in furniture stripping shops, 10 of the 21 workers stated that they sometimes experienced dizziness, nausea, or headache during furniture stripping operations (Hall and Rumack, 1990).

4.1.2.2. *Controlled Experiments Examining Acute Effects*

Several controlled experiments were conducted in the 1970s examining neurophysiological effects and levels of COHb resulting from short-term (1–4 hours) exposures to dichloromethane at levels up to 1,000 ppm, or longer-term exposures at levels up to 500 ppm.

The 8-hour threshold limit value before 1975 was 500 ppm (NIOSH, 1986). These studies are described in Appendix D (Section D-3). Stewart et al. (1972a, b) reported that acute (1-hour) exposures to dichloromethane concentrations above 500 ppm resulted in COHb saturation levels that exceeded and were more prolonged than those seen with the threshold limit value exposures to CO. The exposures also resulted in symptoms of CNS depression indicated by visual evoked response changes and reports of light-headedness. Exposure to 800 ppm dichloromethane resulted in a statistically significant decrease in the performance of 10 of 14 psychomotor tasks in a study of 38 women exposed to dichloromethane at levels of 300–800 ppm for 4 hours (Winneke, (1974). In a double-blind experiment by Putz et al. (1979), 12 healthy volunteers were each exposed for 4-hours to 70 ppm CO and on a separate occasion to 200 ppm dichloromethane; COHb levels were estimated to reach 5% from each of these exposures. The tests of eye-hand coordination, tracking tasks, and auditory vigilance revealed significant impairment with both exposures under the more difficult task conditions, and effects were similar or stronger in magnitude for dichloromethane compared with CO.

4.1.2.3. *Observational Studies Focusing on Neurological Effects and Suicide Risk*

Studies in currently exposed workers. Cherry et al. (1983; 1981) conducted two health evaluation studies of triacetate film production workers. Cherry et al. (1981) recruited 46 of the 76 male workers at a triacetate film factory, where workers were exposed to dichloromethane and methanol in a ratio of 9:1 at air levels of dichloromethane ranging from 75 to 100 ppm. A small comparison group (n = 12) of workers at this factory who worked a similar shift pattern (rapidly rotating shifts) but who were not exposed to dichloromethane was also included. The men were asked whether they had ever experienced cardiac symptoms (pain in the arms, chest pain sitting or lying, or chest pain when walking or hurrying) and were asked about the presence in the past 12 months of neurological disorders (frequent headaches, dizziness, loss of balance, difficulty remembering things, numbness and tingling in the hands or feet), affective symptoms (irritability, depression, tiredness), and stomachache (as an indicator of symptom over reporting). No difference in response was found in history of stomachache (reported by 15% of exposed workers compared with 17% nonexposed workers). Six of the exposed and none of the unexposed men responded positively to the cardiac symptoms. The exposed group reported an excess of neurological symptoms; the number (and proportion) reporting zero, one, two, and three or more symptoms were 26 (0.56), 8 (0.17), 9 (0.20), and 3 (0.07), respectively, in exposed workers compared with 11 (0.92), 1 (0.12), 0 (0.00), and 0 (0.00), respectively, in controls ($p <$ 0.02 for χ^2 test of linear trend). With respect to affective symptoms, the number (and proportion) reporting zero, one, two, and three symptoms were 28 (0.61), 6 (0.13), 7 (0.15), and 5 (0.11), respectively, among the exposed workers, and 9 (0.75), 2 (0.17), 1 (0.08), and 0 (0.0), respectively, among the unexposed workers. The authors concluded that there was no difference between exposed and nonexposed in reporting of affective symptoms based on a χ^2 test of linear

trend. There was no discussion of the statistical power of this test or of tests of the proportion reporting a specified number of symptoms, but the statistical power of this study was very low. Taking the simple case of the comparison of the proportion reporting two or more symptoms and using the estimates from this study (25 and 10% in the exposed and unexposed, respectively, the actual power with the sample size of 46 and 12 is <0.10.

Based on these results, a follow-up study was conducted with a larger referent group. This study included the symptom list described previously, a standardized clinical exam (including an electrocardiograph), and neurological and psychological tests of nerve conduction, motor speed and accuracy, intelligence, reading, and memory (Cherry et al., 1981). Twenty-nine of the original 46 exposed workers participated in the follow-up. The nonparticipants in the follow-up were similar in age and symptoms to the participants. The new referent group was recruited from another plant with a very similar process but without dichloromethane exposure. One control, age-matched within 3 years, was selected for each exposed worker. No differences between the groups were found in the clinical exam, electrocardiogram, or nerve conduction tests. A statistically significant ($p < 0.05$) deficit among exposed workers was found for coarse motor speed. On two tests of overall intelligence, the exposed group did significantly better than the referent, but on a reading ability test designed to assess premorbid educational level, scores for the exposed group were slightly lower than for the referent group. (Only one of these three differences, the trail making intelligence test, was statistically significant.) With respect to the report of neurological symptoms in the past year, the number (and proportion) reporting zero, one, two, and three symptoms were 17 (0.59), 4 (0.14), 6 (0.21), and 2 (0.07), respectively, among the exposed workers, and 21 (0.72), 6 (0.21), 0 (0.0), and 2 (0.07), respectively, among the unexposed workers, with a test of linear trend that was not statistically significant. The authors suggest that the differences in neurological symptoms seen in the initial study were due to chance and that, taken as a whole, the exposed workers had no detrimental effect attributable to dichloromethane exposure. The limitations of the statistical power of the analysis and approaches to addressing the resulting imprecision were not discussed.

Cherry et al. (1983) compared dichloromethane-exposed workers at an acetate film factory to nonexposed workers (from the same plant but from areas without solvent contact or from another film production factory in which solvents were not used). The 56 exposed and 36 unexposed workers were matched to within 3 years of age. Both factories were on rapid rotating shifts. Exposure to dichloromethane ranged from 28 to 173 ppm, using individual air sampling pumps. Blood samples were taken to monitor dichloromethane levels at the beginning and end of the shift. Study participants were asked to rate sleepiness, physical and mental tiredness, and general health on visual analog scales with the extreme responses at either end. Participants were also given a digit symbol substitution test and a test of simple reaction time. No differences were seen between exposed and unexposed groups at the beginning of the shift on the four visual analog scales, but the exposed deteriorated more on each of the scales than did the

controls. This difference in deterioration was statistically significant ($p < 0.05$) during the morning shift but was not statistically significant during the afternoon or night shifts. A significant correlation was shown between change in mood over the course of the shift and level of dichloromethane in the blood (correlation coefficients –0.37, –0.31, –0.43, and –0.50 for sleepiness, general health, mental exhaustion, and physical exhaustion, respectively; each $p < 0.05$). No difference was seen between the exposed and referents on the tests of reaction time or digit substitution conducted at the beginning of a workshift. However, among the exposed, deterioration in the digit substitution tests at the end of the shift was significantly related to blood dichloromethane levels (correlation coefficients = –0.37, $p < 0.01$).

Studies in retired workers. Lash et al. (1991) examined the hypothesis that long-term exposure to dichloromethane produces lasting CNS effects as measured by long-term impairment on memory and attention centers. Retired aircraft maintenance workers employed in at least 1 of 14 targeted jobs with dichloromethane exposure for ≥6 years between 1970 and 1984 were compared to unexposed workers (retired aircraft mechanics at the same base who held 1 of 10 jobs in the jet shop where little solvent was used). The exposed group, made up of painters and mechanics in the overhaul department, was chosen to maximize the exposure contrast yet minimize differences in potential confounders between exposed and nonexposed groups. Exposures were typically within state and federal guidelines for dichloromethane exposure. From 1974 to 1986, when 155 measurements for dichloromethane exposure were made, mean breathing zone TWAs ranged from 82 to 236 ppm and averaged 225 ppm for painters and 100 ppm for mechanics. The mean length of retirement among the study participants was 5.3 years in the exposed group and 5.1 years in the unexposed group.

Data collection occurred in three phases: (1) an initial questionnaire was given to all retired members of the airline mechanics union to identify eligible workers; (2) a telephone survey was conducted to collect medical, demographic, and general employment criteria; and (3) subjects who qualified were then recruited to participate in the medical evaluation. Sixty percent of the 1,758 retirees responded to the questionnaire; 259 met the eligibility criteria. Ninety-one men qualified for the medical evaluation based on the telephone survey; 25 retirees exposed to solvents and 21 unexposed retirees participated in the evaluation. All were men between the ages of 55 and 75 without a history of alcoholism or any neurological disorder. The 25 exposed participants worked an average of 11.6 years in dichloromethane-exposed jobs during the target period and 23.8 years in the industry.

The medical evaluation asked about the occurrence of 33 different symptoms in the past year, physiological measurement of odor and color vision senses, auditory response potential, hand grip strength, and measures of reaction time (simple, choice, and complex), short-term visual memory and visual retention, attention, and spatial ability. The only large differences (i.e., effect size, or mean difference between groups divided by the SD of the outcome measure,

of ≥ 0.4) between the two groups were a higher score on verbal memory tasks (effect size approximately 0.45, $p = 0.11$) and lower score on attention tasks (effect size approximately –0.55, $p = 0.08$) and complex reaction time (effect size approximately –0.40, $p = 0.18$) in the exposed compared with the control group. (Although not noted by the authors, the power to detect a statistically significant difference between the groups given this sample size was low [i.e., approximately 0.30 for an effect size of 0.40, using a two-tailed alpha of 0.05]). In an analysis of potential response bias, attempts were made to contact 30% of the questionnaire nonrespondents, with 46% contacted and 31% completing the telephone interview. The only difference found between those who responded to the mailed questionnaire and those who did not was a higher percentage of diagnosed heart disease among the nonrespondents who were 2.5 years older and had been retired 1.7 more years than the respondents. Those who were eligible but did not participate in the medical evaluation were similar to the exam participants on all characteristics included in the interview. The only difference was a higher prevalence of gout among the unexposed who did not participate compared to the unexposed who did participate.

Studies of Suicide Risk. Several cohort studies (see Section D.2) examined the relation between dichloromethane exposure and various causes of mortality, including suicide. Suicide risk is not an outcome that was a primary hypothesis of the cohort studies, but it may be relevant given the potential neuropsychological effects of dichloromethane. In a triacetate film production cohort in Rochester, New York, Hearne and Pifer (1999) an SMR of 1.8 (95% confidence interval [CI] 0.98–3.0) (Table 4-1). A similar relative risk estimate was seen in the highest exposure group in the study of triacetate fiber production workers in Maryland (Gibbs, 1992), but this increased risk was not seen in the updated study by Tomenson . There are no case-control studies of suicide risk and dichloromethane exposure.

Table 4-1. Suicide risk in two cohorts of dichloromethane-exposed workers

		Obs	Exp	SMR	95% CI
Triacetate film production					
Hearne and Pifer (1999)	Cohort 1	14	7.8	1.8	0.98–3.0
	Cohort 2	9	5.1	1.8	0.81–3.4
Tomenson	Men	6	4.4	0.83	0.30–1.80
Triacetate fiber production[a]					
Gibbs (1992)	50–100 ppm	8	6.4	1.3	0.54–2.5
	350–700 ppm	8	4.4	1.8	0.78–3.6

[a]In Lanes et al. (1993), 4 observed and 5.21 expected cases were reported which would be an SMR of 0.77, but the SMR reported with these data was 1.19 (95% CI 0.39–2.8). Information on suicide was not included in the analysis of civilian Air Force base workers (Radican et al., 2008; Blair et al., 1998).

Exp = number of expected deaths; Obs = number of observed deaths

4.1.2.4. *Observational Studies of COH and Cardiac Function*

Ott et al. (1983c) presented results from an investigation of changes in COHb, alveolar CO, and oxygen half-saturation pressure in relation to dichloromethane exposure. Blood samples were collected before and after shifts from 136 Rock Hill and 132 Narrows workers. For the Rock Hill workers, personal monitoring for dichloromethane exposure was done during the shift. The TWA for dichloromethane ranged from 0 to 900 ppm, with a bimodal distribution (peaks around 150 and 500 ppm) resulting from the layout of the plant. The blood samples were used to determine blood COHb, alveolar CO levels, and the partial oxygen pressure (P_{50}; that is, the pressure required to keep 50% of the blood oxygen-carrying capacity saturated with oxygen at pH 7.4 and 37°C). Separate analyses were conducted for smokers and nonsmokers to account for the smoking-related effects on COHb. Linear relationships were seen between dichloromethane exposure and the before-shift COHb and alveolar CO levels, reflecting residual metabolism from the previous day's exposure. There were significant quadratic relationships between dichloromethane exposure and postshift COHb and alveolar CO levels, indicating a partial saturation of the enzyme system metabolizing dichloromethane. The P_{50} group means were lower among exposed compared with referents, among smokers compared with nonsmokers, and among men compared with women. Given the relationship between COHb and P_{50}, an expected decrease in P_{50} during the shift was observed among the exposed.

Soden et al. (1996) studied male workers exposed to dichloromethane at a Hoechst Celanese triacetate film production plant in Belgium. The production process was the same as the process at the Hoechst Celanese Rock Hill plant, except the Belgium plant was newer with better engineering controls to significantly reduce overall levels of the dichloromethane, acetone, and methanol used in the process. The objectives of the study were to determine the impact of varying levels of dichloromethane exposure on COHb levels, whether successive days of dichloromethane exposure affected the COHb levels, and what impact smoking had on COHb levels in conjunction with dichloromethane exposure. Workers were monitored semiannually for COHb at the end of the work shift and were personally monitored for exposure to the three solvents. Smoking status was defined based on a health assessment questionnaire, with smokers smoking at least one cigarette per day. Among nonsmokers, a dose response was found among COHb levels and average dichloromethane exposure levels in the range of 7–90 ppm. The maximum COHb was 4.00% at an average exposure of 90 ppm (correlation coefficient = 0.58, $p < 0.05$). Smokers' COHb levels were elevated when compared with those of nonsmokers with similar dichloromethane air levels, but the dose-response correlation between dichloromethane air levels and COHb levels was weaker and not statistically significant (correlation coefficient 0.20). The maximum COHb level for smokers was 6.35% at an average dichloromethane air level of 99 ppm. The authors concluded that dichloromethane exposures up to the levels observed did not produce COHb levels that are likely to cause cardiac symptoms.

Continuous 24-hour cardiac monitoring was evaluated in 24 dichloromethane-exposed workers from a triacetate fiber production plant in Rock Hill, South Carolina and 26 workers from a comparison plant in Narrows, Virginia. This study (Ott et al., 1983e) was limited to white men ages ≥35 years. Special efforts were made to recruit men with a history of heart disease because this group was postulated to be most likely to demonstrate positive findings. The estimated TWA dichloromethane exposure ranged from 60 to 475 ppm in the exposed group. The evaluation examined ventricular and supraventricular ectopic activity and S-T segment depression in the exposed and nonexposed groups. Comparisons were also made between cardiac performance during work hours and nonwork hours to discern possible short-term effects of recent exposure. Comparing the findings for the 24 exposed and 26 referent volunteers indicated no difference in ventricular or supraventricular ectopic activity or S-T-segment depression. There was no difference comparing work and nonwork hours among exposed volunteers.

4.1.2.5. *Summary of Studies of Health Effects Other Than Cancer*

The clinical and workplace studies of the influence of dichloromethane exposure on health effects other than cancer are summarized below:

Neurological effects. The acute effects of dichloromethane exposure on neurological function seen in numerous case reports were also seen in experimental studies in humans (Putz et al., 1979; Winneke, 1974; Stewart et al., 1972a, b). Relatively less is known about the potential long-term effects of chronic exposures in humans. Some data from studies of workers suggest that the effects of dichloromethane are relatively short-lived. For example, in the study by Cherry et al. (1983) of 56 exposed and 36 unexposed workers, alterations in mood or in digit substitution test results were seen during the course of a work shift but were not seen at the beginning of a shift. No difference in four neurological symptoms was seen in an analysis of exposed workers (average exposure 475 ppm, ≥10-year duration) and an unexposed comparison group by Soden (1993). Other data suggest an increase in prevalence of neurological symptoms among workers (Cherry et al., 1981) and possible detriments in attention and reaction time in complex tasks among retired workers (Lash et al., 1991). These latter two studies are limited by the small sample size. Thus, Cherry et al. (1981) and Lash et al. (1991) have low power for detecting statistically significant results and consequently should not be interpreted as definitive analyses showing no effects. Rather, these analyses provide evidence of an increased prevalence of neurological symptoms among workers with average exposures of 75–100 ppm (Cherry et al., 1981) and long-term effects on specific neurological measures (i.e., attention and reaction time) in workers whose past exposures, at least for part of their work history, were in the 100–200 ppm range (Lash et al., 1991). The increased risk of suicide (approximately a twofold increased risk) seen in two of the worker cohort studies (Hearne and Pifer, 1999; Gibbs, 1992) is an additional

indication of potential neurological consequences of dichloromethane exposure. Adequate studies addressing these specific issues are not available. Thus, given the suggestions from the currently available studies, the statement that there are no long-term neurological effects of chronic exposures to dichloromethane cannot be made with confidence.

Cardiac effects. The effect of dichloromethane on the formation of COHb (Stewart et al., 1972a) raised concerns about potential risk of cardiovascular damage. To date, there is little evidence of cardiac damage related to dichloromethane exposure in the cohort studies of dichloromethane-exposed workers that examined ischemic heart disease mortality risk (Table 4-2) or in two small cardiac monitoring studies (Ott et al., 1983e; Cherry et al., 1981). However, limitations in these cohort mortality studies should be noted, including the healthy worker effect and the absence of data pertaining to workers who died before the establishment of the analytic cohort (Gibbs et al., 1996; Gibbs, 1992).

Table 4-2. Ischemic heart disease mortality risk in four cohorts of dichloromethane-exposed workers

		Obs	Exp	SMR	95% CI
Triacetate film production					
Hearne and Pifer (1999)	Cohort 1 (men)	117	136.7	0.86	0.71–1.03
	Cohort 2 (men)	122	143.3	0.85	0.71–1.02
Tomenson	Men	156	not reported	0.88	0.75–1.03
Triacetate fiber production					
Lanes et al. (1993)	Men and women	43	47.8	0.90	65–121
Gibbs et al. (1996)	Men				
	50–100 ppm	96	100.1	0.96	0.78–1.2
	350–700 ppm	98	106.8	0.92	0.75–1.1
	Women				
	50–100 ppm	32	45.8	0.70	0.48–0.99
	350–700 ppm	0	3.4	–	0.0–1.1

CI = confidence interval; Exp = number of expected deaths; Obs = number of observed deaths

Infectious disease and immune-related effects. Only limited and somewhat indirect evidence pertaining to immune-related effects of dichloromethane in humans is available. No risk was seen in the broad category of infectious and parasite-related mortality reported by Hearne and Pifer (1999), but there was some evidence of an increased risk for influenza and pneumonia-related mortality at two cellulose triacetate fiber production work sites in Maryland and South Carolina (Gibbs, 1992). Slightly elevated risks of mortality due to influenza and pneumonia were seen among the male workers in the high exposure group in Maryland (7 observed, 5.62 expected, SMR 1.25) and in South Carolina (3 observed, 1.33 expected, SMR

2.26). Among females, there were few observed or expected cases (in Maryland, 1 observed, 0.23 expected, SMR 4.36; in South Carolina, 0 observed, 0.74 expected). In the Maryland facility, an increased risk of cervical cancer was seen among the 938 female workers, with an SMR of 3.0 (95% CI 0.96–6.9) in the 50–100 ppm group and 5.4 (95% CI 0.13–30.1) in the 350–700 ppm group (Gibbs et al., 1996). Cervical cancer is viral mediated (human papilloma virus), and immunosuppression is a risk factor for development of this disease, as seen by the increased risk in immunocompromised patients and people taking immunosuppressant medications (Leitao et al., 2008; Ognenovski et al., 2004). In a cohort study of civilian Air Force base workers, an increased risk of bronchitis-related mortality, based on four exposed cases, was seen among the men who had been exposed to dichloromethane, with a hazard ratio of 9.21 (95% CI 1.03–82.69) (Radican et al., 2008). This collection of studies indicates that immune suppression, and a potentially related susceptibility to specific types of infectious diseases, may be a relevant health outcome for consideration with respect to dichloromethane exposure.

Hepatic effects. Three studies, described in Appendix D (Section D.4), provide data pertaining to markers of hepatic damage (i.e., serum enzymes and bilirubin levels) (Soden, 1993; Kolodner et al., 1990; Ott et al., 1983c). Two of these studies were based in the Rock Hill, South Carolina, cellulose triacetate fiber plant (Soden, 1993; Ott et al., 1983a), with the most recent focusing on workers with >10 years duration in a high exposure area (average exposure estimated as 475 ppm). There is some evidence of increasing levels of serum bilirubin with increasing dichloromethane exposure in Ott et al. (1983a) and Kolodner et al. (1990), but there are no consistent patterns with respect to the hepatic enzymes examined (serum γ-glutamyl transferase, serum AST, serum ALT). These studies do not provide clear evidence of hepatic damage in dichloromethane-exposed workers, to the extent that this damage could be detected by these serologic measures; however, these data are limited and, thus, the absence, presence, or extent of hepatic damage is not known with certainty.

Reproductive effects. Studies pertaining to various reproductive effects and dichloromethane exposure from workplace settings or environmental settings have examined possible associations with spontaneous abortion (Taskinen et al., 1986), low birth weight (Bell et al., 1991), and oligospermia (Wells et al., 1989; Kelly, 1988) (Appendix D, Section D.5). Of these, the data pertaining to spontaneous abortion provide the strongest evidence of an adverse effect of dichloromethane exposure, particularly with respect to the case-control study in which the strongest association was seen specifically with the higher frequency category of dichloromethane exposure (Taskinen et al., 1986). However, it is a small study (44 cases, 130 controls) with limited quantitative exposure assessment and multiple exposures (although the association seen with dichloromethane was among the highest seen among the solvents) and so cannot be considered to firmly establish the role of dichloromethane in induction of miscarriage.

The high exposure scenario, including the potential for substantial dermal exposure in the study of Kelly (1988), also suggests the potential for adverse male reproductive effects.

4.1.3. Cancer Studies

4.1.3.1. *Identification and Selection of Studies for Evaluation of Cancer Risk*

Seventeen epidemiologic studies of cancer risk were identified and included in this evaluation: four cohorts for which the primary solvent exposure was to dichloromethane (two in film production settings and two in cellulose triacetate fiber production), one large cohort of civilian employees at a military base with exposures to a variety of solvents but that included an assessment specifically of dichloromethane exposure, and twelve case-control studies of specific cancers with data on dichloromethane exposure. One additional study (Ott et al., 1985), a cohort of 1,919 men employed at Dow Chemical facilities, was identified but was not included in the summary. The analysis was based on exposure to a combined group of chlorinated methanes (e.g., carbon tetrachloride, chloroform, methyl chloride, and dichloromethane), and it was not possible from the data presented to assess the individual effects of dichloromethane.

The study setting, methods (including exposure assessment techniques), results pertaining to incidence or mortality from specific cancers, and primary strengths and limitations are summarized in Appendix D (Sections D.1 and D.2) for each of the identified studies. When two papers of the same cohort were available, the results from the longer period of follow-up are emphasized in the summary. Information from earlier reports is used when these reports contain more details regarding working conditions, study design, and exposure assessment.

4.1.3.2. *Summary of Cancer Studies by Type of Cancer*

The cohort and case-control studies with data relevant to the issue of dichloromethane exposure and cancer risk are summarized in Tables 4-3 and 4-4, respectively. This summary is adapted from the review by Cooper et al. (2011)), but includes the updated cohort study by Tomenson et al. that was not found in the previous search. The strongest of the cohort studies in terms of design are two of the triacetate film base production cohorts (Cohort 1 in New York and the United Kingdom cohort, reported in Hearne and Pifer (1999) and Tomenson et al. , respectively). These are the cohorts with the most extensive exposure assessment information. The start of eligibility for cohort entrance corresponds with the beginning of the time when the exposure potential at the work site began, and the follow-up period is relatively long (mean $\leq$25 years). Although Cohort 2 of the New York film base production study has similar exposure data and follow-up, this cohort was limited to workers employed between 1964 and 1970 and, therefore, would have missed anyone leaving (possibly because of illness or death) before this time. In addition, because of the overlap between Cohort 1 and Cohort 2, including both cohorts in an evaluation would be double-counting experiences of some individuals. Several limitations of the triacetate film base production cohorts should be noted, however. One of these limitations

concerns the generalizability of the results given the relatively low exposure level (mean 8-hour TWA <40 ppm). Exposures in small, poorly ventilated work areas are also often much higher than those seen in these film base production cohorts (Estill and Spencer, 1996; Anundi et al., 1993). Other limitations include the limited power to detect a risk of low-incidence cancers (including brain, liver, leukemia, and other forms of hematopoietic cancers) and the lack of women and, thus, lack of data pertaining to breast cancer. In addition, these cohorts used mortality rather than incidence data, which is of particular concern for cancers with a relatively high survival rate, such as non-Hodgkin lymphoma. Although the exposure levels in the cohorts involved in cellulose triacetate fiber production were much higher than those of the film production cohorts, the duration of exposure was relatively short in the South Carolina cohort (Lanes et al., 1993), and the majority of workers were missing job history data. In the Maryland triacetate fiber production plant, duration of exposure was not reported and the length of follow-up was relatively short (mean 17 years) (Gibbs et al., 1996). Also, the cohort began in 1970, even though production began in 1955, and the missing personnel records made it impossible to recreate an inception cohort. The exposure assessment in the study of civilian Air Force base workers (Radican et al., 2008; Blair et al., 1998) allowed for only a dichotomized classification of exposure, and there was considerable exposure to other solvents among these workers. This Air Force base study was the largest of the cohort studies that included women and presented data pertaining to breast cancer.

Table 4-3. Summary of cohort studies of cancer risk and dichloromethane exposure

Reference and Cohort	Total n, exposure level[a] and duration, follow-up period	Inclusion criteria[b]	Exposure assessment; outcome assessment	Results[c]
Hearne and Pifer (1999) Cellulose triacetate film base production; New York Cohort 1	n = 1,311 men; mean 39 ppm; mean duration, 17 yrs; follow-up through 1994; mean follow-up, 35 yrs	Began working after 1945; worked at least 1 yr	Work history (job records) and personal/air monitoring; death certificate (underlying cause)	See Table D-1. Brain cancer SMR 2.16 (95% CI 0.79–4.69); leukemia SMR 2.04 (95% CI 0.88–4.03). SMRs <1.0 for lung cancer and pancreatic cancers
Hearne and Pifer (1999) Cellulose triacetate film base production; New York Cohort 2	n = 1,013 men; mean 26 ppm; mean duration, 24 yrs; follow-up through 1994; mean follow-up, 26 yrs	Employed at least 1 yr between 1964 and 1970 (potential exposure began 1946)	Work history (job records) and personal/air monitoring; death certificate (underlying cause)	See Table D-1. Results similar to Cohort 1 except for pancreatic cancer, SMR 1.55 (95% CI 0.67–3.06)
Tomenson et al. Cellulose triacetate film base production; United Kingdom	n = 1,473 men; mean 19 ppm; median duration, 5 yrs; follow-up through 2006; median follow-up, 37 yrs	Employed anytime between 1946 and 1988	Work history (job records) and personal/air monitoring; death certificate (underlying cause)	See Table D-3. Brain cancer SMR 1.83 (95% CI 0.79–3.60). Lung cancer SMR 0.48 (95% CI 0.31–0.69)
Lanes et al. (1993) Cellulose triacetate fiber production; South Carolina	n = 551 men and 720 women (total n = 1,271); median 140, 280, and 475 ppm in low, moderate, and high, respectively; 56% <5-yr work duration; follow-up through 1990; mean follow-up, ~28 yrs	Worked at least 3 mo in the preparation or extrusion areas from 1954 to 1977	Job history data and personal/air monitoring of specific areas (but job history data available for 37%); death certificate (underlying and contributing causes)	See Table D-4. Liver cancer SMR 2.98 (95% CI 0.81–7.63), estimate from earlier follow-up SMR 5.75 (95% CI 1.82–13.8); lung cancer SMR 0.80 (95% CI 0.43–1.37)
Gibbs et al. (1996) Cellulose triacetate fiber production; Maryland	n = 1,931 men and 978 women (total n = 2,909); 50–100 ppm in low and 350–700 ppm in high exposure; duration not reported; follow-up through 1989; mean follow-up 17 yrs	Employed on or after January 1, 1970, for at least 3 mo (potential exposure began 1955)	Work history (job records) and personal/air monitoring; death certificate (fields used not stated)	See Table D-5. Increasing risk across exposure groups seen for prostate cancer and cervical cancer. In men, SMRs ~1.0 or <1.0 for lung cancer and, combining exposure groups, leukemia. In women, SMRs ~1.0 or <1.0 for breast cancer and, combining exposure groups, lung cancer

(Table 4-3; page 1 of 2)

Table 4-3. Summary of cohort studies of cancer risk and dichloromethane exposure

Reference and Cohort	Total n, exposure level[a] and duration, follow-up period	Inclusion criteria[b]	Exposure assessment; outcome assessment	Results[c]
Radican et al. (2008), Air Force Base, Utah	n = 10,461 men and 3,605 women (total n = 14,066)[d]; exposure dichotomized (yes, no); exposure duration not reported; follow-up through 2000; mean follow-up ~29 yrs	Employed at least 1 yr from 1952 to 1956 (potential exposure began 1939)	Work history (job records) and industrial hygiene assessment based on work site review (dichotomized exposure); death certificate (underlying and contributing causes)	In men, non-Hodgkin lymphoma RR 2.02 (95% CI 0.76–5.42) and multiple myeloma RR 2.58 (95% CI 0.86–7.76). In women, breast cancer RR 2.35 (95% CI 0.98–5.65)

[a]8-hour TWA.
[b]If dichloromethane was used at the plant before the first date of entrance into the cohort, the yr that potential exposure began is noted.
[c]Results for all studies are described as SMR and 95% CI except for Blair et al. (1998), in which results are presented as RR (relative risks) and 95% CI. Results are presented cancers based on a minimum of four observed cases. More comprehensive information, when available for other cancers, is shown in the summary tables for each study (see Appendix D).
[d]Includes whites and unknown race.

Adapted from Cooper et al. (2011)
(Table 4-3; page 2 of 2)

Table 4-4. Summary of case-control studies of cancer risk and dichloromethane exposure

Cancer type, reference	Location n cases, n controls (source), time period, demographic group	Exposure assessment	Results[a]
Brain Heineman et al. (1994)	Louisiana, New Jersey, Philadelphia; 300 cases, 320 controls (death certificates); 1978–1981; cancer confirmed by hospital records; white men	Job exposure matrix applied to detailed information on all jobs held (at least 1 yr) since age 15, as obtained from next-of-kin interviews; probability, duration, intensity, and cumulative exposure scores; six solvents evaluated	See Section D.2.1. OR 1.3 (0.9–1.8) for any exposure; increased risk with increased probability (trend p-value < 0.05, OR 2.4 [1.0, 5.9] for high probability), increased duration, increased intensity; strongest effects seen in high probability plus high duration, OR 6.1 (1.1–43.8) or high intensity and high duration, OR 6.1 (1.5–28.3) combinations; no association with cumulative exposure score
Brain Cocco et al. (1999)	24 states (United States); 12,980 cases, 51,920 controls (death certificates); 1984–1992; women	Job exposure matrix applied to death certificate occupation; probability, and intensity scores; 11 exposures evaluated	See Section D.2.1. Weak association overall, OR 1.2 (1.1, 1.3), no trend with probability or intensity scores
Breast Cantor et al. (1995)	24 states (United States); 33,509 cases, 117,794 controls (death certificates); 1984–1989; black and white women	Job exposure matrix applied to death certificate job data, probability, and exposure level; 31 substances evaluated	See Section D.2.2. Little evidence of association with exposure probability; weak association with highest exposure in whites, OR 1.17 (1.1–1.3) or in blacks, OR 1.46 (1.2–1.7)
Pancreas Kernan et al. (1999)	24 states (United States); 63,037 cases, 252,386 controls (death certificates); 1984–1993; black and white men and women	Job exposure matrix applied to death certificate occupation, probability, and intensity scores; 11 chlorinated solvents and formaldehyde evaluated	See Section D.2.3. Little evidence of associations with intensity or probability
Kidney (renal cell) Dosemeci et al. (1999)	Minnesota; 438 incident cases (state cancer registry), 687 controls (random digit dialing and Medicare records); 1988–1990; cancer confirmed by histology; men and women	Job exposure matrices applied to most recent and usual job, as ascertained from interviews; nine solvents evaluated	See Section D.2.4. No evidence of increased risk associated with dichloromethane in men, OR 0.85 (0.6–1.2) or women, OR 0.95 (0.4–2.2)
Rectum Dumas et al. (2000)	Montreal, Canada; 257 incident cases, 1,295 other cancer controls from 19 hospitals; 533 population-based controls (electoral rolls and random digit dialing), 1979–1985; cancer confirmed by histology; men	Job exposure matrix applied to detailed information on all jobs held, as ascertained from interviews; 294 substances evaluated	See Section D.2.5. Little evidence of association with any exposure, OR 1.2 (0.5–2.8). Increased risk in "substantial exposure" group, OR 3.8 (1.1–12.2) using cancer controls; analysis using population controls not given)

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Table 4-4. Summary of case-control studies of cancer risk and dichloromethane exposure

Cancer type, reference	Location n cases, n controls (source), time period, demographic group	Exposure assessment	Results[a]
Non-Hodgkin lymphoma Seidler et al. (2007)	Germany (6 areas); 710 incident lymphoma cases (589 non-Hodgkin lymphoma), 710 population-based controls (area population files), 1999–2003; ages 18–80 yrs, men and women	Job exposure matrix applied to work history (all jobs held at least 1 yr) ascertained through interviews, job-specific and industry-specific questionnaires (for solvent- and other chemical-related jobs). Probability and intensity ratings; 8 specific solvents	See Section D.2.6. Cumulative exposure (ppm-yrs): lymphoma / non-Hodgkin lymphoma 0: 1.0 (referent) / 1.0 (referent) >0 to ≤26.3: 0.4 (0.7–5.2) / 0.4 (0.2–1.1) >26.3 to ≤175: 0.8 (0.3–1.9) / 0.9 (0.3–2.3) >175: 2.2 (0.4–11.6) / 2.7 (0.5–14.5)
Non-Hodgkin lymphoma Wang et al. (2009); Barry et al. (2011)	Connecticut, United States; 601 incident cases, 717 population-based controls (random digit dialing and Medicare files), 1996–2000; ages 21–84 yrs, women. Barry et al. (2011) is limited to 518 cases and 597 controls with blood or buccal cell sample for genotyping	Job exposure matrix applied to work history (all jobs held at least 1 yr) ascertained through interviews (job and industry titles, duties). Probability and intensity ratings; 8 specific solvents	See Section D.2.6. Association seen with dichloromethane exposure: Ever OR 1.5 (1.0–2.3); Little difference in risk by probability or intensity score. Stronger risk seen in diffuse large B-cell lymphoma: OR 2.10 (1.15–3.85), and in TT genotype of CYP2E1 rs20760673: OR 4.42 (2.03–9.62)
Non-Hodgkin lymphoma Miligi et al. (2006)	Italy (8 areas) 1,428 incident cases, 1,530 population-based controls (area population files), 1991–1993; cancer classification based on NCI protocol; ages 20–74 yrs, men and women	Job exposure matrix applied to work history (all jobs held at least 5 yrs) ascertained through interviews, job-specific and industry-specific questionnaires (for solvent- and other chemical-related jobs). Probability and intensity ratings; 10 specific solvents	See Section D.2.6. Association with intensity measure: very low/low OR 0.9 (0.5–1.6); medium/high OR 1.7 (0.7–4.3); In small lymphocytic subtype, any exposure OR 3.2 (1.0–10.1)
Chronic lymphoid leukemia Costantini et al. (2008)	Italy (7 areas) 586 incident cases, 1,278 population-based controls (area population files), 1991–1993; cancer classification based on NCI protocol; ages 20–74 yrs, men and women	Job exposure matrix applied to work history (all jobs held at least 5 yrs) ascertained through interviews, job-specific and industry-specific questionnaires (for solvent- and other chemical-related jobs). Probability and intensity ratings; 10 specific solvents	See Section D.2.6. Association with intensity measure: very low/low OR 0.7 (0.3–1.7) medium/high OR 0.5 (0.1–2.3)
Multiple myeloma Costantini et al. (2008)	Italy (6 areas) 263 incident cases, 1,100 population-based controls (area population files), 1991–1993; cancer classification based on NCI protocol; ages 20–74 yrs, men and women	Job exposure matrix applied to work history (all jobs held at least 5 yrs) ascertained through interviews, job-specific and industry-specific questionnaires (for solvent- and other chemical-related jobs). Probability and intensity ratings; 10 specific solvents	See Section D.2.6. Only four exposed cases; association not estimated

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Table 4-4. Summary of case-control studies of cancer risk and dichloromethane exposure

Cancer type, reference	Location n cases, n controls (source), time period, demographic group	Exposure assessment	Results[a]
Multiple myeloma Gold et al. (2010)	Seattle, Washington and Detroit, Michigan (2 SEER sites), United States 180 incident cases; 481 population-based controls (random digit dialing and Medicare files), 2000–2002; ages 35–74 yrs, men and women	Job exposure matrix applied to work history (all jobs held since age 15) ascertained through interviews, job-specific questionnaires (for solvent-related jobs held at least 2 yrs). Probability, frequency, intensity and confidence ratings; 6 specific solvents	See Section D.2.6. In analyses classifying low-confidence jobs as unexposed: Ever exposed OR 2.0 (1.2–3.2) Trends with duration (p = 0.01), cumulative exposure (p = 0.08) and 10-yr lagged cumulative exposure (p = 0.06)
Childhood leukemia (acute lymphoblastic leukemia) Infante-Rivard et al. (2005)	Quebec, Canada 790 incident cases (hospitals—all provinces), 790 population-based controls (government population registries), 1980–2000; cancer based on oncologist or hematologist diagnosis ages 0–14 yrs,[b] boys and girls	Systematic review of detailed information on all jobs held by the mother from 2 yrs before pregnancy through birth of the child; 21 individual substances and six mixtures evaluated (mostly solvents); confidence, frequency, and concentration of exposure ratings	See Section D.2.6. Little evidence of association with any exposure, OR 1.34 (0.54–3.34), but stronger associations with probable or definite, OR 3.22 (0.88–11.7) (referent group = possible/no exposure) and with combinations of frequency and concentration

[a]Results given as OR and (95% CI).
[b]From 1980 to 1993, study was limited to diagnoses of ages 0–9, but this was expanded between 1994 and 2000 to ages 0–14.

Adapted from Cooper et al. (2011)
(Table 4-4; page 3 of 3)

Case-control studies offer the potential for increased statistical power for assessing associations with relatively rare cancers such as brain cancer and leukemia. Case-control studies are often designed to examine incidence rather than mortality, which is of particular importance in etiologic research for diseases with relatively high survival rates and diseases in which survival may be strongly related to factors that are difficult to adjust for without detailed data collection (e.g., access to health care). There is a considerable range, however, in the detail and quality of the exposure assessment used in case-control studies. Case-control studies rarely include specific measurements taken at specific work sites of individual study participants. Although it is more difficult to determine absolute exposure levels without these individual measurements, the exposure assessment methodology used in case-control studies can result in useful between-group comparisons of risk if the intra-group variability is less than the intergroup variability in potential exposure levels. The use of death certificate data to classify disease and occupational exposures in the three studies using the large 24-state death certificate database [brain cancer, Cocco et al. (1999); breast cancer, Cantor et al. (1995); pancreatic cancer, Kernan et al. (1999)] is likely to have resulted in nondifferential misclassification of both outcome and exposure (and thus attenuated associations). Several other case-control studies included diagnoses based on medical records, detailed job-specific and industry-specific questionnaire modules focusing on potential exposure to specific solvents, and included assessment of intensity and probability of exposure (Gold et al., 2010; Costantini et al., 2008; Seidler et al., 2007; Miligi et al., 2006). The assessment methodology used in Wang et al. (2009) and Heineman et al. (1994) also included the assessment of exposure intensity and probability, but were somewhat more limited in that specific follow-up modules designed to obtain more detailed information for jobs with potentially relevant exposures were not included. Each of these seven studies did, however, obtain detailed information about all jobs held (or, in the case of the study of childhood leukemia, jobs held in the two years before and during the pregnancy), rather than just the usual or most recent job, and focused on a relatively small number of exposures. In addition, Heineman et al. (1994) included more detailed coding of specific jobs within the 4-digit industry and occupation code categories to distinguish those of particular relevance to a specific exposure (e.g., production of paint removers within the broader category of production of paints, varnishes, lacquers, enamels, and allied products) (Dosemeci et al., 1994). Heineman et al. (1994) obtained the work history from interviews with next-of-kin, however, which is also likely to have resulted in nondifferential misclassification of exposure, and thus attenuation in the observed associations.

Considering the issues described above with respect to the strengths and limitations of the available epidemiologic studies, a summary of the epidemiologic evidence relating to dichloromethane exposure and specific types of cancer can be made, as described below. The available epidemiologic data suggest an association between dichloromethane and brain cancer, liver cancer, and specific hematopoietic cancers, but not lung cancer.

4.1.3.2.1. ***Brain and CNS cancer.*** An increased risk of brain and CNS cancers was seen in the strongest cohort studies; SMRs were 2.16 in Cohort 1 in New York (Hearne and Pifer, 1999) and 1.83 in the United Kingdom cohort (Tomenson, In Press). These estimates are based on a small number of observations (six cases in New York and four in the United Kingdom) and so are relatively imprecise. In both of these studies, an increasing risk was seen with cumulative exposure in the middle exposure groups (e.g., 400 to 800 ppm-years), with a decrease in risk above 800 ppm-years; the small number of observations and resulting imprecision in relative risk estimates makes it difficult to interpret these patterns. Two case-control studies of dichloromethane exposure and brain cancer have been conducted (Cocco et al., 1999; Heineman et al., 1994). The Heineman et al. (1994) study, which is the stronger study in terms of exposure assessment strategy and confirmation of diagnosis, reported relatively strong trends ($p < 0.05$) with increasing probability, duration, and intensity measures of exposure, but a nonlinear trend was seen with the cumulative exposure metric. This difference could reflect a more valid measure of relevant exposures in the brain from the intensity measure, as suggested by the study in rats reported by Savolainen et al. (1981) in which dichloromethane levels in the brain were much higher with a higher intensity exposure scenario compared with a constant exposure period with an equivalent TWA (see Section 3.2). The combination of high intensity of exposure and long (>20 years) duration of employment in exposed jobs was strongly associated with brain cancer risk (OR 6.1, 95% CI 1.5–28.3) in the Heineman et al. (1994) study; similar associations were seen with the high probability in combination with long duration measures. The available epidemiologic studies provide evidence of an association between dichloromethane and brain cancer, and this area of research represents a data gap in the understanding of the carcinogenic potential of dichloromethane.

4.1.3.2.2. ***Liver and biliary duct cancer.*** Liver and biliary duct cancer are relatively uncommon (age-adjusted incidence 7.3 per 100,000 person-years) (SEER website, seer.cancer.gov, accessed May 2, 2011), and therefore are difficult cancers to study in most occupational cohorts of limited size. The cohort study with the higher exposures, the Rock Hill triacetate fiber production plant, suggested an increased risk of liver cancer (Lanes et al., 1993; Lanes et al., 1990). The SMR for liver and biliary duct cancer was 2.98 (95% CI 0.81–7.63) in the latest update of this cohort. This observation was based on four cases; three of these cases were biliary duct cancers. The authors estimated a total of 0.15 expected cases of biliary tract cancer in the first of the follow-up studies (Lanes et al., 1990); this subset of cancers may represent a particularly relevant form of cancer with respect to dichloromethane exposure. As the follow-up period has increased, the strength of this association has decreased, although it is relatively strong (albeit with wide CIs). The decrease in the SMR with increasing follow-up reflects the increase in number of expected cases because the four observed cases were seen earlier in the follow-up period. No other cohort

study has reported an increased risk of liver cancer mortality, although it should be noted that there is no other inception cohort study of a population with exposure levels similar to those of the Rock Hill plant, and no data from a case-control study of liver cancer are available pertaining to dichloromethane exposure. The available epidemiologic studies, with biological plausibility inferred from the localization of GST-T1 in the nuclei of bile duct epithelial cells in human samples (Sherratt et al., 2002), provide some evidence of an association between dichloromethane and liver and biliary duct cancer, although it should be noted that this evidence is based on limited epidemiologic data in that these observations were based on one study. The SMRs of <1.0 seen in other cohort studies may reflect the healthy worker effect, which is seen not just for cardiovascular disease, but for many specific types of cancer, including liver cancer (Leonard et al., 2007).

4.1.3.2.3. ***Lung cancer.*** In the stronger cohort studies [Cohort 1 in the New York Eastman Kodak Company triacetate film production study reported by Hearne and Pifer (1999) and the United Kingdom triacetate film production study reported by Tomenson et al.], the SMRs for lung cancer were well below 1.0. The New York study had also obtained data on smoking history that indicated that it was unlikely that differences in smoking could be masking an effect of dichloromethane (Hearne et al., 1987). Lung cancer is a common cancer (age-adjusted incidence 62 per 100,000 person-years) (SEER website, seer.cancer.gov, accessed May 2, 2011) so the expected rates, even in small cohorts, are based on relatively robust estimates. The only group in any study that had an increased risk for lung cancer was the high-exposure women in the triacetate fiber production cohort in Maryland (Gibbs et al., 1996). However, this was based on only two cases and was a highly imprecise estimate (SMR 2.3 [95% CI 0.28–8.3]). No case-control study of dichloromethane exposure and lung cancer risk is available. The available epidemiologic studies do not provide evidence for an association between dichloromethane and lung cancer, although it should be noted that this conclusion is based on a relatively limited database.

4.1.3.2.4. ***Pancreatic cancer.*** An early study (Hearne et al., 1990) of Cohort 2 of the New York triacetate film production cohort had reported 8 observed and 4.2 expected pancreatic cancer deaths, for a twofold increased SMR ($p = 0.13$). This association was reduced in the subsequent follow-up (SMR 1.5 [95% CI 0.7–3.0]) (Hearne and Pifer, 1999) but was not seen in the more methodologically sound Cohort 1 (SMR 0.92) or in any of the other cohorts. A meta-analysis of the cohort studies [using the data of Hearne et al. (1990)] reported a summary association of 1.42 (95% CI 0.80–2.53) (Ojajärvi et al., 2001). This summary measure would be further reduced with the updated data for Cohort 2 and the addition of Cohort 1 from Hearne and Pifer (1999). The only case-control study of pancreatic cancer mortality risk and dichloromethane exposure (based on death certificate data) did not report consistent patterns with respect to

intensity or exposure among the race-sex groups studied (Kernan et al., 1999). The available epidemiologic studies do not provide evidence for an association between dichloromethane and pancreatic cancer.

4.1.3.2.5. ***Leukemia and lymphoma.*** Each of the individual hematopoietic cancers is relatively uncommon, with age-adjusted incidence rates ranging from 1.6 to 4.2 per 100,000 person-years for specific types of leukemias (SEER website, seer.cancer.gov, accessed May 2, 2011); the incidence rate for non-Hodgkin lymphoma is 19.8 per 100,000 person-years. The relatively inconsistent (point estimates ranging from approximately 0.50 to 2.0) and imprecise measures of association in the cohort studies between dichloromethane exposure and leukemia are thus expected, given the relatively small size of the available cohorts. Three large population-based case-control studies of incident non-Hodgkin lymphoma in adults in Germany (Seidler et al., 2007), Italy (Miligi et al., 2006), and Connecticut (Barry et al., 2011; Wang et al., 2009) observed ORs between 1.5 and 2.7 with dichloromethane exposure (ever exposed, or highest category of exposure). There was also some evidence of higher risk among specific subsets of disease, such as small lymphocytic non-Hodgkin lymphoma (Miligi et al., 2006) or diffuse large B-cell lymphoma (Barry et al., 2011). An extensive exposure assessment protocol was used in the studies from Italy and Germany, including job-specific and industry-specific questionnaire modules focusing on potential exposure to specific solvents. No association was seen in a related study of chronic lymphoid leukemia, conducted in Italy using the same study protocol (Costantini et al., 2008). Only four cases of multiple myeloma within this study were exposed to dichloromethane, and risk estimates were not presented. In a population-based case-control study of multiple myeloma conducted in two SEER sites in the United States (also using an extensive exposure assessment protocol focusing on specific solvents), a twofold increased risk was seen with ever exposure to dichloromethane (OR 2.0, 95% CI 1.2–3.2), with trends seen with duration, cumulative exposure, and cumulative exposure lagged by 10 years (Gold et al., 2010). One case-control study of childhood leukemia (acute lymphoblastic leukemia) examining maternal occupational history and dichloromethane exposure is available (Infante-Rivard et al., 2005). This is a large, population-based study of incident cases of leukemia, with a detailed exposure assessment pertaining to the period before and during pregnancy. A threefold increased risk was seen with probable or definite exposure (OR 3.22 [95% CI 0.88–11.7]) compared with possible or no exposure. The available epidemiologic studies show consistent observations of associations between dichloromethane and non-Hodgkin lymphoma, but do not definitively establish that dichloromethane exposure is related to an increased cancer risk. These studies are limited by a relatively small number of exposed cases, resulting in imprecise effect estimates.

4.1.3.2.6. ***Breast cancer.*** Only one large cohort study included women and reported data pertaining to breast cancer risk (Radican et al., 2008; Blair et al., 1998), and this is a cohort with a limited exposure assessment (dichotomized) and multiple exposures. A twofold increased risk was seen between dichloromethane exposure and breast cancer mortality in this study (rate ratio 2.35 [95% CI 0.98–5.65]). Similar associations were seen with several other chemicals, and the potential effect of confounding and misclassification of these exposures may have biased the estimate in either direction. The only case-control study of breast cancer risk and dichloromethane exposure used the 24-state death certificate data to classify exposure and disease (Cantor et al., 1995),. The available epidemiologic studies do not provide an adequate basis for the evaluation of the role of dichloromethane in breast cancer because there are currently no cohort studies with adequate statistical power and no case-control studies with adequate exposure methodology to examine this relationship.

4.2. CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

4.2.1.1. *Overview of Noncancer and Cancer Effects*

Results from studies of animals exposed by the oral route for short-term, subchronic, and chronic durations identify the liver and the nervous system as the most sensitive targets for noncancer toxicity from repeated oral exposure to dichloromethane. In a 90-day exposure study, nonneoplastic histopathologic changes in the liver were observed in F344 rats exposed to drinking water doses of ≥166 mg/kg-day (males) or ≥209 mg/kg-day (females) (Kirschman et al., 1986). This subchronic oral toxicity study is summarized in more detail in Appendix E (Section E.1). Similar changes were seen in F344 rats in a 2-year exposure of ≥50 mg/kg-day (Serota et al., 1986a).

The 2-year oral exposure study in F344 rats did not produce evidence of increasing incidence of liver tumors across all of the dose groups in males or females (Serota et al., 1986a). In a parallel study in $B6C3F_1$ mice (Serota et al., 1986b; Hazleton Laboratories, 1983), a clearer trend with respect to hepatic cancer was seen in males but not females.

None of the chronic oral exposure studies included a systematic measurement of potential neurological effects. One 14-day study focusing on neurobehavioral changes is available, however. Changes in autonomic, neuromuscular, and sensorimotor functions were observed in F344 rats exposed for 14 days to gavage doses ≥337 mg/kg-day (Moser et al., 1995) (see Section 4.4.2 for more details).

No effects on reproductive parameters were observed in Charles River CD rats exposed for 90 days to gavage doses as high as 225 mg/kg-day (General Electric Company, 1976) or in pregnant F344 rats exposed to gavage doses of up to 450 mg/kg-day on GDs 6–19 (Narotsky and

Kavlock, 1995). However, no oral two-generation and no oral exposure studies examining developmental neurobehavioral effects have been conducted (see Section 4.3 for more details).

4.2.1.2. *Toxicity Studies of Chronic Oral Exposures: Hepatic Effects and Carcinogenicity*

Chronic (up to 2-year) oral exposure studies in mice and rats are summarized in Table 4-5 and described in more detail below. These studies provide additional information pertaining to hepatotoxicity and carcinogenicity.

Table 4-5. Studies of chronic oral dichloromethane exposures (up to 2 years)

Reference, strain/species	Number per group	Exposure information	Comments
Serota et al. (1986a) F344 rats	85/sex/dose + 135 controls	Drinking water, 2 yrs, target dose 0, 5, 50, 125, 250 mg/kg-d, beginning at 7 wks of age Mean intake: males: 0, 6, 52, 125, 235 mg/kg-d females: 0, 6, 58, 136, 263 mg/kg-d	Nonneoplastic liver effects (foci/areas of alteration) in males and females; jagged stepped pattern of increasing incidence of neoplastic nodules or hepatocellular carcinoma in females (i.e., increased in the 50 and 250 mg/kg-d but not 125 mg/kg-d groups) (see Table 4-6)
Serota et al. (1986b); Hazleton Laboratories (1983) B6C3F$_1$ mice	Males: 125, 200, 100, 100, 125 Females: 100, 100, 50, 50, 50	Drinking water, 2 yrs, target dose 0, 60, 125, 185, 250 mg/kg-d, beginning at 7 wks of age Mean intake: males: 0, 61, 124, 177, 234 mg/kg-d females: 0, 59, 118, 172, 238 mg/kg-d	Increasing trend of liver cancer (hepatocellular adenoma or carcinoma) in males (see Table 4-7)
Maltoni et al. (1988) Sprague-Dawley Rats	50/sex/dose	Gavage, up to 64 wks 0, 100, 500 mg/kg-d, 4–5 d/wk, beginning at 12 wks of age	High mortality in high dose group led to termination of study at 64 wks; statistically nonsignificant increase in malignant mammary tumors in female rats
Maltoni et al. (1988) Swiss mice	50/sex/dose + 60 controls	Gavage, up to 64 wks 0, 100, 500 mg/kg-d, 4–5 d/wk, beginning at 9 wks of age	High mortality in high dose group led to termination of study at 64 wks

4.2.1.2.1. *Chronic oral exposure in F344 rats (Serota et al., 1986a).* Treatment with dichloromethane in drinking water did not induce adverse clinical signs or affect survival in the F344 rats (Serota et al., 1986a). BWs of rats in the 125 and 250 mg/kg-day groups were generally lower than in controls throughout the study. The authors stated that the differences, although small, were statistically significant, but the data were not shown in the published report. Water consumption was lower throughout the study in both sexes of rats from the 125 and 250 mg/kg-day groups relative to controls; food consumption was also lower in these groups during the first 13 weeks of treatment. Mean hematocrit, hemoglobin, and red blood cell count

were increased in both sexes at dichloromethane levels of 50, 125, and 250 mg/kg-day for 52 and 78 weeks. Half of these increases were reported to be statistically significant, but the report did not provide the numerical values or specify which parameters were significant. Clinical chemistry results showed decreases in alkaline phosphatase (AP), creatinine, blood urea nitrogen, total protein, and cholesterol in both sexes at 250 mg/kg-day, and most of these changes were statistically significant at one or both of the intervals evaluated. (Significant parameters not specified and the mean group values were not presented in the published report.) No significant deviations in urinary parameters were observed. Organ weights were not significantly affected by treatment with dichloromethane.

No treatment-related histopathological effects were noted in the tissues examined except for the liver (Serota et al., 1986a). Examination of liver sections showed a dose-related positive trend (positive Cochran-Armitage trend test) in the incidences of foci/areas of cellular alteration in treated F344 rats (Table 4-6). Comparisons of incidences with control incidences indicated statistically significant elevations at all dose levels except 5 mg/kg-day. These liver changes were first noted after treatment for 78 weeks and progressed until week 104. Livers of animals treated with dichloromethane also showed an increased incidence of fatty change, but incidence data for this lesion were not presented in the published report. The recovery group also showed an increased incidence of areas of cellular alterations, but the fatty changes were less pronounced than in the 250 mg/kg-day group dosed for 104 weeks. The authors indicate that 5 mg/kg-day was a NOAEL and 50 mg/kg-day was a LOAEL for liver changes (foci/areas of cellular alteration) in male and female F344 rats exposed to dichloromethane in drinking water for 2 years.

Dichloromethane-exposed male rats showed no statistically significant increased incidence of liver tumors. In females, there was a positive trend for increasing incidence of hepatocellular carcinoma or neoplastic nodules with increasing dose (Table 4-6) (Serota et al., 1986a). Statistically significant increases in tumor incidences were observed in the 50 and 250 mg/kg-day groups (incidence rates of 10 and 14%, respectively) but not in the 125 mg/kg-day group (incidence rate of 3%). Incidence of neoplastic nodules was also increased (10%) in a group exposed for 78 weeks followed by a 26-week period of no exposure; however, the characterization of malignant potential of the nodules was not described. The incidence of hepatocellular carcinoma or neoplastic nodules in this control group (0%) was lower than that seen in historical controls from the same laboratory (324 female F344 rats; 4 with carcinoma, 21 with neoplastic nodules; 25/324 = 7.7%), further suggesting the variation seen across exposure groups is unlikely to be exposure-related.

Table 4-6. Incidences of nonneoplastic liver changes and liver tumors in male and female F344 rats exposed to dichloromethane in drinking water for 2 years

	Target dose (mg/kg-d)						
	0[a] (Controls)	5	50	125	250	Trend *p*-value[b]	250 with recovery[c]
Males							
Estimated mean intake (mg/kg-d)	0	6	52	125	235		232
total n	135	85	85	85	85		25
n at terminal kill[d]	76	34	38	35	41		17
Number (%) with: Liver foci/areas of alteration	52 (68)	22 (65)	35 (92)[e]	34 (97)[e]	40 (98)[e]	< 0.0001	15 (100)[e]
Neoplastic nodules	9 (12)	1 (3)	0 (0)	2 (6)	1 (2)	Not reported	2 (13)
Hepatocellular carcinoma	3 (4)	0 (0)	0 (0)	0 (0)	1 (2)	Not reported	0 (0)
Neoplastic nodules and hepatocellular carcinoma	12 (16)	1 (3)	0 (0)	2 (6)	2 (5)	Not reported	2 (13)
Females							
Estimated mean intake (mg/kg-d)	0	6	58	136	263		269
total n	135	85	85	85	85		25
n at terminal kill[d]	67	29	41	38	34		20
Number (%) with: Liver foci/areas of alteration	34 (51)	12 (41)	30 (73)[e]	34 (89)[e]	31 (91)[e]	< 0.0001	17 (85)[e]
Neoplastic nodules	0 (0)	1 (3)	2 (5)	1 (3)	3 (9)	Not reported	2 (10)
Hepatocellular carcinoma	0 (0)	0 (0)	2 (5)	0 (0)	2 (6)	Not reported	0 (0)
Neoplastic nodules and hepatocellular carcinoma	0 (0)	1 (3)	4 (10)[f]	1 (3)	5 (14)[f]	$p < 0.01$	2 (10)[f]

[a]Two control groups combined. Sample size (incidence of liver foci) in group 1 and 2, respectively, was 36 (75%) and 40 (63%) in males and 31 (55%) and 36 (47%) in females.
[b]Cochran-Armitage trend test was used for trend test of liver foci/areas of alteration. For tumor mortality-unadjusted analysis, a Cochran-Armitage trend test was used, and for tumor mortality-adjusted analyses, tumor prevalence analytic method by Dinse and Lagakos (1982) was used. Similar results were seen in these two analyses.
[c]Recovery group was exposed for 78 wks and then had a 26-wk period without dichloromethane exposure; n = 15 for nonneoplastic lesions and n = 17 for neoplastic lesions.
[d]Excludes 5, 10, and 20 per group sacrificed at 25, 52, and 78 wks, respectively, and unscheduled deaths, which ranged from 5 to 19 per group.
[e]Significantly ($p < 0.05$) different from controls with Fisher's exact test.
[f]Significantly ($p < 0.05$) different from controls with Fisher's exact test, mortality-unadjusted and mortality-adjusted analyses; incidence in historical controls = 7.7%.

Source: Serota et al. (1986a).

4.2.1.2.2. Chronic oral exposure in B6C3F1 mice *(Serota et al., 1986b; Hazleton Laboratories, 1983)*. A 2-year drinking water study similar to the previously described study in F344 rats was also conducted in B6C3F$_1$ mice (Serota et al., 1986b; Hazleton Laboratories, 1983). The mice received target doses of 0, 60, 125, 185, or 250 mg/kg-day of dichloromethane in deionized drinking water for 24 months. Treatment groups consisted of 100 female mice in the low-dose (60 mg/kg-day) group and 50 in the remaining treatment groups; larger sample sizes were used in the male bioassay, with 200, 100, 100, and 125 male mice in the 60, 125, 185, and 250 mg/kg-day groups, respectively. One hundred females (in two groups of 50) and 125 males (in two groups of 60 and 65 mice) served as controls. The authors indicate that this study design involving two groups of control mice was used because of the high and erratic incidence of liver tumors in historical control B6C3F$_1$ mice; when the results were similar in the two control groups, the groups could be combined to provide a more statistically precise estimate for comparisons with the exposed groups. Based on water consumption and BW measurements, mean intakes were reported to be 61, 124, 177, and 234 mg/kg-day for males and 59, 118, 172, and 238 mg/kg-day for females. Endpoints examined included clinical signs, BW and water consumption, hematology at weeks 52 and 104, and gross and microscopic examinations of tissues and organs at termination. All tissues from the control and 250 mg/kg-day groups were examined microscopically, as well as the livers and neoplasms from all groups and the eyes of all males from all groups.

Throughout the 2-year study, mice from both control and treatment groups exhibited a high incidence of convulsions (Serota et al., 1986b; Hazleton Laboratories, 1983). The convulsions were noted only during handling for BW determinations, and efforts to establish a basis for this response were unsuccessful. The incidence of convulsions did not correlate with an increased mortality rate. Survival to 104 weeks was high (82% in males and 78% in females), and no evidence for exposure-related negative effects on survival were found. Exposure had no significant effect on BW or water consumption. Mean leukocyte count was significantly elevated in males and females dosed with 250 mg/kg-day dichloromethane for 52 weeks, but the authors indicated that the mean values were within the normal historical range for the laboratory. Treatment-related nonproliferative histopathologic effects were restricted to the liver and consisted of a marginal increase in the amount of Oil Red O-positive material in the liver of males and females dosed with 250 mg/kg-day (group incidences for this lesion, however, were not presented in the published report). The results indicate that 185 mg/kg-day was a NOAEL and 250 mg/kg-day was a LOAEL for marginally increased amounts of fat in livers of male and female B6C3F$_1$ mice.

Incidences of liver tumors in female mice were not presented in the published paper (Serota et al., 1986b; Hazleton Laboratories, 1983) or in the full report of the study (Serota et al., 1986b; Hazleton Laboratories, 1983), but it was reported that exposed female mice did not show increased incidences of proliferative hepatocellular lesions. In the male B6C3F$_1$ mice,

incidences for hepatic focal hyperplasia showed no evidence of an exposure-related effect (Table 4-7). The incidence of hepatocellular adenomas or carcinomas was 18 and 20% in each of the two control groups. Because of the similarity in the results for these groups, the combined group is presented in this table and used as the comparison group for the analysis. The incidence of hepatocellular adenomas or carcinomas across exposure groups was 26, 30, 31, and 28% in the 60, 125, 185 and 250 mg/kg-day groups, respectively. Similar patterns are seen with the mortality-adjusted incidences (Table 4-7). The trend tests and the tests of the comparisons between individual exposure groups and the controls were not reported by Serota et al. (1986b) but were reported in the full report of the study prepared by Hazleton Laboratories (1983). Exposed male mice showed a marginally increased combined incidence of hepatocellular adenomas and carcinomas, with a linear trend p-value = 0.058; the individual p-values for the 60, 125, 185, and 250 mg/kg-day dose groups were 0.071, 0.023, 0.019, and 0.036, respectively.

Table 4-7. Incidences for focal hyperplasia and tumors in the liver of male B6C3F$_1$ mice exposed to dichloromethane in drinking water for 2 years

	Target dose (mg/kg-d)					
	0[a] (Controls)	60	125	185	250	Trend p-value[b]
n per group[c]	125	200	100	99	125	
Estimated mean intake (mg/kg-d)	0	61	124	177	234	
Number (%) with: Focal hyperplasia[d]	10 (8)	14 (7)	4(4)	10 (10)	13 (10)	Not reported
Hepatocellular adenoma (mortality-adjusted percent) p-value[e]	10 (8) (9)	20 (10) (12) $p = 0.24$	14 (14) (17) $p = 0.064$	14 (14) (16) $p = 0.076$	15 (12) (12) $p = 0.13$	0.172
Hepatocellular carcinoma (mortality-adjusted percent) p-value[e]	14 (11) (13)	33 (17) (19) $p = 0.082$	18 (18) (21) $p = 0.073$	17 (17) (19) $p = 0.11$	23 (18) (21) $p = 0.044$	0.147
Hepatocellular adenoma or carcinoma (mortality-adjusted percent) p-value[e]	24 (19) (21)	51 (26) (29) $p = 0.071$	30 (30) (34) $p = 0.023$	31 (31) (34) $p = 0.019$	35 (28) (32) 0.036	0.058

[a]Two control groups combined. Sample size (incidence of hepatocellular adenoma or carcinoma) in group 1 and 2, respectively, was 60 (18%) and 65 (20%). Two additional sets of analyses using the individual control groups were also presented in Hazleton Laboratories (1983).
[b]Cochran-Armitage trend test [source: Hazleton Laboratories (1983)].
[c]Number at start of treatment.
[d]Some mice with hyperplasia also had hepatocellular neoplasms, but the exact number was unspecified by Serota et al. (1986b).
[e]Percent calculated based on number at risk, using Kaplan-Meier estimation, taking into account mortality losses; p-value for comparison with control group, using asymptotic normal test [source: Hazleton Laboratories (1983)].

Sources: Serota et al. (1986b); Hazleton Laboratories (1983).

Serota et al. (1986b) summarized these results as showing slight increases in proliferative hepatocellular lesions in exposed male B6C3F$_1$ mice that were not dose-related and were within

the range of historical controls, with no effect seen in female $B6C3F_1$ mice. Serota et al. (1986b) concluded that dichloromethane "did not induce a treatment-related carcinogenic response in $B6C3F_1$ mice" under the conditions of this study. An alternative conclusion, as determined by EPA based on the results of the analysis shown in Hazleton Laboratories (1983), is that dichloromethane induced a carcinogenic response in male $B6C3F_1$ mice as evidenced by small but statistically significant ($p < 0.05$) increases in hepatocellular adenomas and carcinomas at dose levels of 125, 185, and 250 mg/kg-day, and by a marginally increased trend test ($p = 0.058$) for this endpoint. The incidence in the control groups was almost identical to the mean seen in the historical controls (17.8%, based on 354 male $B6C3F_1$ mice), so there is no indication that the observed trend is being driven by an artificially low incidence in controls. There is also no indication that the experimental conditions resulted in a systematic increase in the incidence of hepatocellular adenomas and carcinomas. Given the information provided regarding the incidence in historical controls (mean 17.8%, range 5 to 40%), the pattern of results (increased incidence in all four dose groups, with three of these increases significant at a p-value < 0.05) is consistent with a treatment-related increase.

One reason for the difference between Serota et al. (1986b) and EPA in the interpretation of these data is the difference in the significance level used to evaluate the between-group comparisons. EPA used the standard two-tailed significance level of $p = 0.05$. Serota et al. (1986b) state that a two-tailed significance level of $p = 0.05$ was used for all tests, but this does not appear to correctly represent the statistic used by Serota et al. (1986b). As can be seen by the p-values in Table 4-7, each of the p-values for the comparison of the 125, 185, and 250 mg/kg-day dose groups with the controls was $p < 0.05$. As noted previously, these p-values were found in the full report of this study, see Hazleton Laboratories (1983), but were not included in the Serota et al. (1986b) publication. Interpretation of the study findings in Serota et al. (1986b) appears to be based on a statistical significance level of 0.0125 rather than 0.05; none of the group comparisons shown in Table 4-7 are statistically significant when a p-value of 0.0125 is used. Hazleton Laboratories (1983) indicated that a correction factor for multiple comparisons was used specifically for the liver cancer data, reducing the nominal p-value from 0.05 to 0.0125; the rationale for using a multiple comparisons correction for this endpoint was not provided in this report, and Serota et al. (1986b) did not mention that the correction factor had been used (stating, erroneously, that a significance value of 0.05 was used). A multiple comparisons correction is sometimes advocated in studies examining many different types of effects (e.g., ≥20 individual causes of death) or many different types of exposures (≥20 different chemicals or several hundred genes) with no a priori focus, to protect against inappropriately focusing on spurious findings. EPA concluded that the use of this multiple comparisons correction factor specifically for a primary hypothesis under investigation in the 2-year mouse oral carcinogenicity assay conducted by Hazleton Laboratories is not warranted.

4.2.1.2.3. ***Chronic oral exposure in Sprague-Dawley rats and Swiss mice (*****Maltoni et al., 1988*****).*** Maltoni et al. (1988) conducted gavage carcinogenicity studies in Sprague-Dawley rats and in Swiss mice. Groups of rats (50/sex/dose level) were gavaged with dichloromethane (99.9% pure) in olive oil at dose levels of 0 (olive oil), 100, or 500 mg/kg-day 4–5 days/week for 64 weeks. This dosing regime was also used for groups of Swiss mice (50/sex/dose level plus 60/sex as controls). Endpoints monitored included clinical signs, BW, and full necropsy at sacrifice (when spontaneous death occurred). For each animal sacrificed, histopathologic examinations were performed on the following organs: brain and cerebellum, zymbal glands, interscapular brown fat, salivary glands, tongue, thymus and mediastinal lymph nodes, lungs, liver, kidneys, adrenals, spleen, pancreas, esophagus, stomach, intestine, bladder, uterus, gonads, and any other organs with gross lesions. High mortality was observed in male and female high-dose rats (data not shown) and achieved statistical significance ($p < 0.01$) in males. The increased mortality became evident after 36 weeks of treatment and led to the termination of treatment at week 64. Explanation of the mortality was not provided by the study authors. As with the rats, high mortality occurred in male and female mice from the high-dose group ($p < 0.01$), and the exposure was terminated after 64 weeks.

Little information is provided regarding nonneoplastic effects (Maltoni et al., 1988). Treatment with dichloromethane did not affect BW in the Sprague-Dawley rats. A reduction in BW was apparent in treated mice after 36–40 weeks of treatment, but no data were shown to determine the magnitude of the effect. The lack of reporting of nonneoplastic findings from the histopathologic examinations precludes assigning NOAELs and LOAELs for possible nonneoplastic effects in these studies.

The Maltoni et al. (1988) studies of Sprague-Dawley rats and Swiss mice did not find distinct exposure-related carcinogenic responses following gavage exposure to dichloromethane at dose levels up to 500 mg/kg-day, although the early termination of the study (at 64 weeks) limits the interpretation of this finding. Dichloromethane exposure was not related to the percentage of either study animal bearing benign and/or malignant tumors or to the number of total malignant tumors per 100 animals. High-dose female rats showed an increased incidence in malignant mammary tumors, mainly due to adenocarcinomas (8, 6, and 18% in the control, 100, and 500 mg/kg dose groups, respectively; the number of animals examined was not provided), but the increase was not statistically significant. A dose-related increase, although not statistically significant, in pulmonary adenomas was observed in male mice (5, 12, and 18% in control, 100, and 500 mg/kg-day groups, respectively). When mortality was taken into account, high-dose male mice that died in the period ranging from 52 to 78 weeks were reported to show a statistically significantly ($p < 0.05$) elevated incidence for pulmonary tumors (1/14, 4/21, and 7/24 in control, 100, and 500 mg/kg-day groups, respectively). Details of this analysis were not provided. EPA applied a Fisher's exact test to these incidences and determined a p-value of 0.11 for the comparison of the 500 mg/kg-day group (7/24) to the controls (1/14).

4.2.2. Inhalation Exposure: Overview of Noncancer and Cancer Effects

4.2.2.1. *Overview of Noncancer and Cancer Effects*

Inhalation dichloromethane exposure studies in rats and mice using subchronic and chronic durations identify the CNS, liver, and lungs as potential toxicity targets. Data from other studies indicate that hamsters are less susceptible to the nonneoplastic and neoplastic effects of dichloromethane than are rats and mice. Subchronic inhalation toxicity studies are summarized in detail in Appendix E; the results of chronic toxicity studies of inhaled dichloromethane are summarized in Section 4.2.2.2 below.

Increased incidences of nonneoplastic liver lesions were observed in Sprague-Dawley rats exposed to ≥500 ppm for 2 years (Nitschke et al., 1988a; Burek et al., 1984), F344 rats exposed to ≥1,000 ppm for 2 years (Mennear et al., 1988; NTP, 1986), and B6C3F$_1$ mice exposed to ≥2,000 ppm for 2 years (Mennear et al., 1988; NTP, 1986).

Two-year inhalation exposure studies at concentrations of 2,000 or 4,000 ppm dichloromethane produced increased incidences of lung and liver tumors in B6C3F$_1$ mice (Mennear et al., 1988; NTP, 1986). Additional studies examining mechanistic issues regarding these effects are summarized in Sections 4.5.2 and 4.5.3 (Foley et al., 1993; Kari et al., 1993). Significantly increased incidences of benign mammary tumors (primarily fibroadenomas) were also observed in male and female F344/N rats exposed by inhalation to 2,000 or 4,000 ppm for 2 years (Mennear et al., 1988; NTP, 1986). In the male rats, the incidence of fibromas or sarcomas originating from the subcutaneous tissue around the mammary gland was also increased, but the difference was not statistically significant. In other studies in Sprague-Dawley rats with exposures of 50–500 ppm (Nitschke et al., 1988a) and 500–3,500 ppm (Burek et al., 1984), the incidence of benign mammary tumors was not increased, but in females, the number of tumors per tumor-bearing rat increased at the higher dose levels.

No obvious clinical signs of neurological impairment were observed in the 2-year bioassays involving exposure concentrations up to 2,000 ppm in F344 rats (Mennear et al., 1988; NTP, 1986) or 3,500 ppm in Sprague-Dawley rats (Nitschke et al., 1988a; Burek et al., 1984). In B6C3F$_1$ mice exposed to 4,000 ppm, there was some evidence of hyperactivity during the first year of the study and lethargy during the second year, with female mice appearing to be more sensitive (Mennear et al., 1988; NTP, 1986). Evaluation of batteries of neurobehavioral endpoints following subchronic or chronic inhalation exposure is limited to one study in F344 rats exposed to concentrations up to 2,000 ppm for 13 weeks (Mattsson et al., 1990). No effects were observed >64 hours postexposure in an observational battery, a test of hind-limb grip strength, a battery of evoked potentials, or histologic examinations of brain, spinal cord, or peripheral nerves (see Section 4.4.2).

No effects on reproductive performance were found in a two-generation reproductive toxicity study with F344 rats exposed to concentrations up to 1,500 ppm for 14 and 17 weeks before mating of the F0 and F1 generations, respectively (Nitschke et al., 1988b) (see Section

4.3). Developmental effects following exposure of Long-Evans rats to 4,500 ppm for 14 days prior to mating and during gestation (or during gestation alone) included decreased offspring weight at birth and changed behavioral habituation of the offspring to novel environments (Bornschein et al., 1980; Hardin and Manson, 1980) (see Section 4.3). In standard developmental toxicity studies involving exposure to 1,250 ppm on GDs 6–15, no adverse effects on fetal development were found in Swiss-Webster mice or Sprague-Dawley rats (Schwetz et al., 1975) (see Section 4.3).

4.2.2.2. *Toxicity Studies from Chronic Inhalation Exposures*

Chronic inhalation exposure studies are summarized in Table 4-8, and details of each study are summarized in the following sections.

Table 4-8. Studies of chronic inhalation dichloromethane exposures

Reference, strain/species	Number per group	Exposure information	Comments
Mennear et al. (1988); NTP (1986) F344 rats	50/sex/dose	2 yrs, 6 hrs/d, 5 d/wk 0, 1,000, 2,000, 4,000 ppm, beginning at 7-8 wks of age	Nonneoplastic liver effects and hemosiderosis in males and females (see Table 4-9) Weak trend for neoplastic nodule or hepatocellular carcinoma in females, benign mammary tumors in males and females (see Table 4-10)
Mennear et al. (1988); NTP (1986) B6C3F$_1$ mice	50/sex/dose	2 yrs, 6 hrs/d, 5 d/wk 0, 2,000, 4,000 ppm, beginning at 8-9 wks	Varied nonneoplastic effects (see Table 4-11) Liver and lung tumors (adenomas or carcinomas) in males and females (see Table 4-12)
Burek et al. (1984) Syrian hamsters	95/sex/dose	2 yrs, 6 hrs/d, 5 d/wk 0, 500, 1,500, 3,500 ppm, beginning at 8 wks	Decreased mortality Increased COHb at 500 ppm, but no dose-response seen beyond this level (see Section 4.2.2.2.3)
Burek et al. (1984) Sprague-Dawley rats	92–97/sex/dose	2 yrs, 6 hrs/d, 5 d/wk 0, 500, 1,500, 3,500 ppm, beginning at 8 wks	Nonneoplastic liver effects in males and females (see Table 4-13) Increased COHb at 500 ppm, but no dose-response seen beyond this level Increased number of benign mammary tumors per tumor bearing rat (females) (see Table 4-13)
Nitschke et al. (1988a) Sprague-Dawley rats	90/dose/sex	2 yrs, 6 hrs/d, 5 d/wk 0, 50, 200, 500 ppm, beginning at 8-10 wks	Nonneoplastic liver effects in males and females (statistically significant in females) (see Table 4-14) Increased COHb beginning at 50 ppm, but no duration-dependence in this effect Increased number of benign mammary tumors per animal in females (see Table 4-15)
Maltoni et al. (1988) Sprague-Dawley rats, female	54–60/dose	2 yrs, 4 hrs/d, 5 d/wk for 7 wks; 7 hrs/d, 5 d/wk for 97 wks 0, 100 ppm, beginning at 12 wks	No effects seen on total number of benign or malignant cancers

4.2.2.2.1. *Chronic inhalation exposure in F344/N rats (Mennear et al., 1988; NTP, 1986)*.

NTP conducted a 2-year inhalation exposure study in F344/N rats (Mennear et al., 1988; NTP,

1986). The rats (50/sex/exposure level) were exposed to dichloromethane (>99% pure) by inhalation (0, 1,000, 2,000, or 4,000 ppm) in exposure chambers 6 hours/day, 5 days/week for 2 years. Mean daily concentrations never exceeded 110% of target and were <90% of target in only 23 of 1,476 analyses. Endpoints monitored included clinical signs, mortality, and gross and microscopic examinations of 32 tissues at study termination. Clinical examinations were conducted weekly for 3.5 months and biweekly until month 8. After 8 months, the animals were clinically examined and palpated for tumors and masses monthly until the end of the study.

Dichloromethane exposure did not significantly alter BW gain or terminal BWs (Mennear et al., 1988; NTP, 1986). Survival of male rats was low in all groups (including controls), and no significant exposure-related differences were apparent. Most deaths occurred during the last 16 weeks of the study. Survival at week 86 was 36/50, 39/50, 37/50, and 33/50 for the control, 1,000, 2,000, and 4,000 ppm groups, respectively. In female rats, there was a trend towards decreased survival, and the survival of high-dose female rats was significantly reduced, possibly due to leukemia. Survival in the females at 86 weeks was 30/50, 22/50, 22/50, and 15/50 for the control, 1,000, 2,000, and 4,000 ppm groups, respectively. Nonneoplastic lesions with statistically significantly elevated incidences compared with controls included hepatocyte cytoplasmic vacuolation and necrosis and liver hemosiderosis in males and females (Table 4-9). The results indicate that 1,000 ppm (6 hours/day, 5 days/week), the lowest dose in the study, was a LOAEL for liver changes (hepatocyte cytoplasmic vacuolation and necrosis, hepatic hemosiderosis) in male and female F344/N rats.

Incidences of mammary fibroadenomas were significantly increased in 4,000 ppm males and 2,000 and 4,000 ppm females compared with controls (Table 4-10). Similar patterns were seen with the combination of fibroadenomas and adenomas (not shown in Table 4-10). In males, subcutaneous tissue fibroma or sarcoma was seen in 1/50, 1/50, 2/50, and 5/50 rats in the 0, 1,000, 2,000, and 4,000 ppm groups, respectively, but these lesions were not seen in females. Incidences of female rats with liver neoplastic nodules or carcinomas (combined) showed a significant trend test after survival adjustment only, but the incidences at the two highest dose levels were not significantly increased relative to the controls (Table 4-10). NTP (1986) considered the relationship between exposure to dichloromethane and mononuclear cell leukemia (Table 4-10) to be equivocal, noting the relatively high incidence seen in all exposure groups in the males. The pattern seen in females was not considered to be treatment-related (Mennear et al., 1988), although the rationale for this interpretation was not discussed in detail. Other neoplasms that had increased incidences included mesotheliomas (predominantly in the tunica vaginalis) in males (0/50, 2/50, 5/50, and 4/50 in controls, 1,000, 2,000, and 4,000 ppm rats, respectively). This lesion was not considered to be related to dichloromethane exposure because the concurrent control incidence (0/50) for this neoplasm was low relative to earlier inhalation studies conducted at this laboratory (4/100, 4%) and in other NTP studies with male F344/N rats (44/1,727) (mean historical percentage across NTP studies = 3 ± 2%).

Table 4-9. Incidences of nonneoplastic histologic changes in male and female F344/N rats exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Lesion, by sex	Exposure (ppm)[a]			
	Controls			
	0	1,000	2,000	4,000
Males				
n per group[b]	50	50	50	50
Number (%)[c] with:				
Liver changes				
Hepatocyte cytoplasmic vacuolation	8 (16)	26 (53)[d]	22 (44)[d]	25 (50)[d]
Hepatocyte focal necrosis	7 (14)	23 (47)[d]	6 (12)	16 (32)[d]
Hepatocytomegaly	2 (4)	10 (20)	6 (12)	5 (10)
Hemosiderosis	8 (16)	29 (59)[d]	37 (74)[d]	42 (84)[d]
Bile duct fibrosis	8 (16)	10 (20)	17 (34)	23 (46)[d]
Renal tubular cell degeneration	11 (22)	13 (26)	23 (46)[d]	10 (20)[d]
Splenic fibrosis	2 (4)	6 (12)	11 (22)[d]	8 (16)[d]
Females				
n per group[e]	50	50	50	50
Number (%)[c] with:				
Liver changes				
Hepatocyte cytoplasmic vacuolation	10 (20)	43 (86)[d]	44 (88)[d]	43 (86)[d]
Hepatocyte focal necrosis	2 (4)	32 (64)[d]	19 (38)[d]	9 (18)[d]
Hepatocytomegaly	3 (6)	10 (20)[d]	18 (36)[d]	5 (10)
Hemosiderosis	19 (38)	29 (58)[d]	38 (76)[d]	45 (90)[d]
Bile duct fibrosis	4 (8)	3 (6)	10 (20)[d]	3 (6)
Renal tubular cell degeneration	14 (28)	20 (40)	22 (44)	25 (51)[d]
Splenic fibrosis	0 (0)	2 (4)	4 (8)	4 (8)
Nasal cavity squamous metaplasia	1 (2)	2 (4)	3 (6)	9 (18)[d]

[a]1,000 ppm = 3,474 mg/m^3, 2,000 ppm = 6,947 mg/m^3, 4,000 ppm = 13,894 mg/m^3.
[b]Number of male rats necropsied per group; only 49 1,000 ppm livers were examined microscopically.
[c]Percentages were based on the number of tissues examined microscopically per group.
[d]Statistical significance not reported in publications but significantly ($p \leq 0.05$) different from controls as calculated by Fisher's exact test.
[e]Number of females necropsied per group; only 49 4,000 ppm kidneys and spleens were examined microscopically.

Sources: Mennear et al. (1988); NTP (1986); Appendix C, Tables C1 and C2 of the NTP (1986) report.

NTP (1986) concluded that there was "some evidence of carcinogenicity of dichloromethane" in male F344/N rats as shown by increased incidence of benign mammary gland tumors and "clear evidence of carcinogenicity" of dichloromethane in female F344/N rats as shown by increased incidence of benign mammary gland tumors. The summary of hepatic effects in rats also notes the positive trend in the incidence of hepatocellular neoplastic nodules or carcinomas in females (Table 4-10) which "may have been due to dichloromethane exposure."

Table 4-10. Incidences of selected neoplastic lesions in male and female F344/N rats exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Neoplastic lesion, by sex	Exposure (ppm)[a]												Trend *p*-value[d]
	0 (Controls)			1,000			2,000			4,000			
	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	
Males: n per group	50	–	–	50	–	–	50	–	–	50	–	–	–
Liver—neoplastic nodule or hepatocellular carcinoma	2	(4)	(6)	2	(4)	(9)	4	(8)	(19)	1	(2)	(2)	0.43
Lung—bronchoalveolar adenoma or carcinoma	1			1	(2)		2	(4)		1	(2)		Not reported
Mammary gland:													
Adenoma, adenocarcinoma, or carcinoma	0	(0)		0	(0)		0	(0)		1	(2)		
Subcutaneous tissue fibroma or sarcoma	1	(2)	(6)	1	(2)	(6)	2	(4)	(9)	5	(10)	(23)	0.029
Fibroadenoma	0	(0)	(0)	0	(0)	(0)	2	(4)	(12)	4	(8)	(34)	0.009
Mammary gland or subcutaneous tissue adenoma, fibroadenoma or fibroma	1	(2)	(6)	1	(2)	(6)	4	(8)	(21)	9[e]	(18)	(49)	< 0.001
Brain (carcinoma, not otherwise specified, invasive)	0	(0)		1	(2)		0	(0)		0	(0)		Not reported
Mononuclear cell leukemia	34	(68)	(80)	26	(52)	(77)	32	(64)	(80)	35	(70)	(89)	Not reported
Females: n per group	50	–	–	50	–	–	50	–	–	50	–	–	–
Liver—neoplastic nodule or hepatocellular carcinoma	2	(4)	(7)	1	(2)	(2)	4	(8)	(14)	5	(10)	(20)	0.08
Lung—bronchoalveolar adenoma or carcinoma	1	(2)		1	(2)		0	(0)		0	(0)		Not reported
Mammary gland:													
Adenocarcinoma or carcinoma	1	(2)		2	(4)		2	(4)		0	(0)		
Adenoma, adenocarcinoma, or carcinoma	1	(2)		2	(4)		2	(4)		1	(2)		
Fibroadenoma	5	(10)	(16)	11[e]	(22)	(41)	13[e]	(26)	(44)	22[e]	(44)	(79)	< 0.001
Adenoma, fibroadenoma, or adenocarcinoma	6	(12)	(18)	13	(26)	(44)	14[e]	(28)	(45)	23[e]	(46)	(84)	< 0.001
Brain (carcinoma, not otherwise specified, invasive[f]	1	(2)		0	(0)		2	(4)		0	(0)		Not reported
Mononuclear cell leukemia	17	(34)	(41)	17	(34)	(44)	23	(46)	(64)[e]	23	(46)	(58)[e]	0.086

(Table 4-10, page 1 of 2)

Table 4-10. Incidences of selected neoplastic lesions in male and female F344/N rats exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Neoplastic lesion, by sex	Exposure (ppm)[a]												Trend *p*-value[d]
	0 (Controls)			1,000			2,000			4,000			
	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	

[a]1,000 ppm = 3,474 mg/m^3, 2,000 ppm = 6,947 mg/m^3, 4,000 ppm = 13,894 mg/m^3.
[b]Percentages based on the number of tissues examined microscopically per group. Incidence in historical controls reported in NTP (1986) were 3% for male mammary gland fibroadenomas, 27% male for mononuclear cell leukemia, 1% for female liver tumors, 16% for female mammary fibroadenomas, and 17% for female mononuclear cell leukemia.
[c]Mortality-adjusted percentage.
[d]Life-table trend test, as reported by NTP (1986).
[e]Life-table test comparison dose group with control < 0.05, as reported by NTP (1986).
[f]Also includes one oligodendroglioma in the 2,000 ppm group.

Sources: Mennear et al. (1988); NTP (1986); Appendix A and Appendix E, Tables A1, A2, E1 and E2 of the NTP (1986) report.

(Table 4-10, page 2 of 2)

4.2.2.2.2. ***Chronic inhalation exposure in B6C3F$_1$ mice (Mennear et al., 1988; NTP, 1986).*** A 2-year inhalation exposure study in B6C3F$_1$ mice, similar to that in F344/N rats, was also conducted by NTP (Mennear et al., 1988; NTP, 1986). The mice (50/sex/exposure level) were exposed to dichloromethane (>99% pure) by inhalation at concentrations of 0, 2,000, or 4,000 ppm in exposure chambers 6 hours/day, 5 days/week for 2 years. As with the study in rats, mean daily concentrations in the mice never exceeded 110% of target and were <90% of target in only 23 of 1,476 analyses. Endpoints monitored included clinical signs, mortality, and gross and microscopic examinations of 32 tissues at study termination. Clinical examinations were conducted weekly for 3.5 months and biweekly until month 8. After 8 months, the animals were clinically examined and palpated monthly for tumors and masses until the end of the study.

The BW of 4,000 ppm males was comparable to controls until week 90 and 8–11% below controls thereafter. The BW of 4,000 ppm females was 0–8% lower than that of controls from week 51 to 95 and 17% lower at study termination. No information was provided regarding food consumption during the study. Male and female mice from the high-dose groups (4,000 ppm) were hyperactive during the first year of the study; during the second year, high-dose females appeared lethargic. Exposure was associated with decreased survivability of both male and female mice (males: 39/50, 24/50, and 11/50 and females: 25/50, 25/50, and 8/50 in controls, 2,000, and 4,000 ppm, respectively, at 104 weeks). In 4,000 ppm mice, statistically significant incidences of nonneoplastic lesions were found in the liver (cytologic degeneration), testes (atrophy), ovary and uterus (atrophy), kidneys (tubule casts in males only), stomach (dilatation), and spleen (splenic follicles in males only) (Table 4-11). In 2,000 ppm mice, the only nonneoplastic lesions showing statistically significantly elevated incidences were ovarian atrophy, renal tubule casts, and hepatocyte degeneration in female mice (Table 4-11). The results indicate that 2,000 ppm, the lowest exposure level, was a LOAEL for nonneoplastic changes in the ovaries, kidneys, and livers of female B6C3F$_1$ mice. A NOAEL was not established because effects occurred at the lowest exposure level.

At both exposure levels, statistically significantly elevated incidences were found for hepatocellular adenomas (males only), hepatocellular carcinomas, hepatocellular adenomas and carcinomas combined, bronchoalveolar adenomas, bronchoalveolar carcinomas, and bronchoalveolar adenomas and carcinomas combined (Table 4-12). Statistically significant positive trend tests were found for each of these tumor types in female mice. The trend tests were significant for the liver tumors in male mice after life-table adjustment for reduced survival. The only other statistically significant carcinogenic response was for increased incidence of hemangiosarcomas or combined hemangiomas and hemangiosarcomas in male mice exposed to 4,000 ppm. NTP (1986) concluded that the elevated incidences of liver and lung tumors provided clear evidence of carcinogenicity in male and female B6C3F$_1$ mice.

Table 4-11. Incidences of nonneoplastic histologic changes in B6C3F$_1$ mice exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Lesion, by sex	Exposure (ppm)[a]		
	Controls		
	0	2,000	4,000
Males—n per group[b]	50	50	50
Number (%)[c] with:			
Liver changes			
Hepatocyte cytoplasmic vacuolation	Not reported	Not reported	Not reported
Hepatocyte focal necrosis	0 (0)	0 (0)	2 (4)
Cytologic degeneration	0 (0)	0 (0)	22 (45)[d]
Testicular atrophy	0 (0)	4 (8)	31 (62)[d]
Renal tubule casts	6 (12)	11 (22)	20 (40)[d]
Stomach dilatation	3 (6)	7 (15)	9 (18)[d]
Splenic follicular atrophy	0 (0)	3 (6)	7 (15)[d]
Females—n per group[e]	50	49	49
Number (%)[c] with:			
Liver changes			
Hepatocyte cytoplasmic vacuolation	Not reported	Not reported	Not reported
Hepatocyte focal necrosis	3 (6)	1 (2)	2 (4)
Cytologic degeneration	0 (0)	23 (48)[d]	21 (44)[d]
Ovarian atrophy	6 (12)	28 (60)[d]	32 (74)[d]
Uterus atrophy	0 (0)	1 (2)	8 (17)[d]
Renal tubule casts	8 (16)	23 (48)[d]	23 (49)[d]
Glandular stomach dilatation	1 (2)	2 (4)	10 (20)[d]
Splenic follicular atrophy	0 (0)	0 (0)	1 (2)

[a]2,000 ppm = 6,947 mg/m^3, 4,000 ppm = 13,894 mg/m^3.
[b]Number of male mice necropsied per group. The number biopsied in the 0, 2,000, and 4,000 ppm dose groups was 50, 49, and 49 for liver; 50, 49, and 50 for renal tubules; 49, 47, and 49 for stomach; and 49, 49, and 48 for spleen.
[c]Percentages were based on the number of tissues examined microscopically per group.
[d]Statistical significance not reported in publications but significantly different ($p \leq 0.05$) from control as calculated by EPA using Fisher's exact test.
[e]Number of females necropsied per group. The number biopsied in the 0, 2,000, and 4,000 ppm dose groups was 50, 48, and 48 for liver; 50, 47, and 43 for ovaries; 50, 48, and 47 for uterus; 49, 48, and 47 for renal tubule; 49, 47, and 48 for stomach; and 49, 48, and 47 for spleen.

Sources: Mennear et al. (1988); NTP (1986); Appendix D, Tables D1 and D2 of the NTP (1986) report.

Table 4-12. Incidences of neoplastic lesions in male and female B6C3F$_1$ mice exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Neoplastic lesion, by sex	Exposure (ppm)[a]									Trend *p*-value[d]
	0 (Controls)			2,000			4,000			
	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	
Males										
Liver										
Hepatocellular adenoma	10	(20)	(23)	14	(29)	(47)	14	(29)	(68)	0.19
Hepatocellular carcinoma	13	(26)	(30)	15	(31)	(44)	26[e]	(53)	(76)	0.004
Hepatocellular adenoma or carcinoma	22	(44)	(48)	24	(49)	(67)	33[e]	(67)	(93)	0.013
Lung										
Bronchoalveolar adenoma	3	(6)	(8)	19[e]	(38)	(56)	24[e]	(48)	(79)	< 0.001
Bronchoalveolar carcinoma	2	(4)	(5)	10[e]	(20)	(34)	28[e]	(56)	(93)	< 0.001
Bronchoalveolar adenoma or carcinoma	5	(10)	(12)	27[e]	(54)	(74)	40[e]	(80)	(100)	< 0.001
Mammary adenocarcinoma[f]	–			–			–			
Hemangioma or hemangiosarcoma, combined	2	(4)	(5)	2	(4)	(8)	6	(12)	(26)	0.08
Females										
Liver										
Hepatocellular adenoma	2	(4)	(7)	6	(13)	(21)	22[e]	(46)	(83)	< 0.001
Hepatocellular carcinoma	1	(2)	(4)	11	(23)	(34)	32[e]	(67)	(97)	< 0.001
Hepatocellular adenoma or carcinoma	3	(6)	(10)	16[e]	(33)	(48)	40[e]	(83)	(100)	< 0.001
Lung										
Bronchoalveolar adenoma	2	(4)	(7)	23[e]	(48)	(67)	28[e]	(58)	(91)	< 0.001
Bronchoalveolar carcinoma	1	(2)	(4)	13[e]	(27)	(46)	29[e]	(60)	(92)	< 0.001
Bronchoalveolar adenoma or carcinoma	3	(6)	(11)	30[e]	(63)	(83)	41[e]	(85)	(100)	< 0.001
Mammary adenocarcinoma	2	(4)	(8)	3	(6)	(10)	0	(0)	(0)	0.21
Hemangioma or hemangiosarcoma, combined[f]	–			–			–			

[a]2,000 ppm = 6,947 mg/m^3, 4,000 ppm = 13,894 mg/m^3.
[b]Percentages based on the number of tissues examined microscopically per group; for males, 49 livers were examined in the 2,000 and 4,000 ppm groups; for females, 48 livers and lungs and 49 mammary glands were microscopically examined in the 2,000 and 4,000 ppm groups. For comparison, incidence in historical controls reported in NTP (1986) were 28% for male liver tumors, 31% for male lung tumors, 5% for female liver tumors, and 10% for female lung tumors.
[c]Mortality-adjusted percentage.
[d]Life-table trend test (Cochran-Armitage), as reported by NTP (1986).
[e]Life-table test comparison dose group with control < 0.05, as reported by NTP (1986).
[f]Data not reported.

Sources: Mennear et al. (1988); NTP (1986); Appendix E, Tables E3 and E4 of the NTP (1986) report.

4.2.2.2.3. ***Chronic inhalation exposure in Syrian hamsters (Burek et al., 1984).*** Burek et al. (1984) conducted a chronic toxicity and carcinogenicity study in rats and hamsters. In the hamster study, groups of 95 Syrian golden hamsters of each sex were exposed to 0 (filtered air), 500, 1,500, or 3,500 ppm dichloromethane (>99% pure) under dynamic airflow conditions in whole-body exposure chambers 6 hours/day, 5 days/week for 2 years. Exposure started when the animals were approximately 8 weeks of age. Interim sacrifices were conducted at 6, 12, and 18 months. The hamsters were observed daily during exposure days and were palpated monthly for palpable masses starting the third month of the study. BWs were monitored weekly for the first 8 weeks of the study and monthly thereafter. Hematologic determinations included packed cell volume, total erythrocyte counts, total red blood cells, differential leukocyte counts, and hemoglobin concentration. The mean corpuscular volume, mean corpuscular hemoglobin, and MCHC were also determined. A reticulocyte count was also performed on all animals at the 18-month kill and on 10 animals/sex/dose at 24 months. Clinical chemistry determinations included serum AP and ALT activities, blood urea nitrogen levels, and total protein and albumin. Urinary parameters measured were specific gravity, pH, glucose, ketones, bilirubin, occult blood, protein, and urobilinogen. Hematology, clinical chemistries, and urinalysis were performed at interim sacrifices and at termination. COHb was measured after a single 6-hour exposure and following 22 months of exposure. Gross and microscopic examinations were conducted on all tissues. In addition, the weights of the brain, heart, liver, kidneys, and testes were recorded.

In the study using Syrian hamsters (Burek et al., 1984), hamsters were exposed to analytical concentrations of dichloromethane of 510 ± 27, 1,510 ± 62, and 3,472 ± 144 ppm for the target concentrations of 500, 1,500, and 3,500 ppm, respectively. No exposure-related clinical signs were observed in the hamsters throughout the study. Significantly decreased mortality was observed in females exposed to 3,500 ppm from the 13th through the 24th month and from the 20th to the 24th month in females exposed to 1,500 ppm. Exposure to dichloromethane had no significant effect on BW or on mean organ weights. Regarding hematology parameters (actual data were not shown), Burek et al. (1984) stated that a few statistically significant changes occurred, but no obvious pattern could be discerned, and most values were within the expected range for the animals. There were no exposure-related alterations in clinical chemistry or urinalysis values. Male and female hamsters in all dose groups had significantly elevated COHb values after a single 6-hour exposure and after 22 months of exposure, but at both time points, there was no dose-response relationship above the first dose level and no apparent significant differences in the magnitude of the changes between the two time points. For example, mean values (± SD) for percentage COHb in male hamsters after 22 months of exposure were 3.3 (± 3.5), 28.4 (± 5.9), 27.8 (± 2.9), and 30.2 (± 4.9), for the control through 3,500 ppm groups, respectively. Similar values were obtained for females at 22 months and for males and females after the first day of exposure. Pathological evaluation of hamsters showed a lack of evidence of definite target organ toxicity.

The results indicate that 3,500 ppm was a NOAEL for adverse changes in clinical chemistry and hematological variables, as well as for histologic changes in tissues in male and female Syrian golden hamsters. A LOAEL was not established based on the lack of adverse changes in clinical chemistry and hematological variables as well as the absence of histologic changes in tissues in male and female Syrian golden hamsters.

Evaluation of the total number of hamsters with a tumor, the number with a benign tumor, or the number with a malignant tumor revealed no exposure-related differences in male hamsters. In the high-dose female group, there was a statistically significant increase in the total number of benign tumors at any tissue site (the report did not specify which sites), but this was considered to be secondary to the increased survival of this group. Incidences of male or female hamsters with tumors in specific tissues were not statistically significantly elevated in exposed groups compared with control incidences. The results indicate that no statistically significant, exposure-related carcinogenic responses occurred in male or female Syrian golden hamsters exposed (6 hours/day, 5 days/week) to up to 3,500 ppm dichloromethane for 2 years.

4.2.2.2.4. ***Chronic inhalation exposure in Sprague-Dawley rats (Burek et al., 1984).*** In the rat study, groups of 92–97 Sprague-Dawley rats of each sex were exposed (similar to the hamster study described in the previous section) to 0, 500, 1,500, or 3,500 ppm dichloromethane 6 hours/day, 5 days/week for 2 years (Burek et al., 1984). Rats were approximately 8 weeks old when exposure started. Interim sacrifices were conducted at 6, 12, 15, and 18 months. Endpoints monitored in rats were the same as in hamsters except that total protein and albumin in blood were not determined in rats. In addition to measurement at scheduled sacrifices, serum ALT activity was also measured after 30 days of exposure. COHb was measured after 6, 11, 18, and 21 months of exposure. Bone marrow cells were collected for cytogenetic studies from 5 rats/sex/dose after 6 months of exposure. The scope of the pathological examinations of the rats was the same as in the hamster study.

No significant exposure-related signs of toxicity were observed in the rats during the study. A significant increase in mortality was seen in high-dose female rats from the 18th to the 24th month of exposure, and this appeared to be exposure-related. Exposure to dichloromethane had no significant effect on BW gain in either males or females. The only exposure-related alterations in organ weights was a significant increase in both absolute and relative liver weight in high-dose males at the 18-month interim kill and a significant increase in relative liver weight in high-dose females also at 18 months. Statistically significant changes in hematologic parameters were restricted to increased mean corpuscular volume and mean corpuscular hemoglobin values at 15 months in males. The clinical chemistry tests revealed no significant exposure-related effects. Male and female rats in all exposed groups had significantly elevated COHb values at all time points, but no dose-response relationship was apparent. For example, mean (± SD) values for percentage COHb after 21 months of exposure were 0.4 (± 0.7),

12.8 (± 2.6), 14.8 (± 4.4), and 12.2 (± 5.7) for the control through 3,500 ppm female rat groups, respectively. Exposure-related, statistically significant increases in incidences of nonneoplastic lesions were restricted to the liver (Table 4-13). The incidences of males or females with hepatocellular vacuolation consistent with fatty change increased as the exposure concentration increased. Hepatocellular necrosis occurred at elevated incidences in male rats exposed to 1,500 or 3,500 ppm compared with controls, but this endpoint was not reported in the female data. Liver lesions were initially observed after 12 months of treatment. There was some evidence that exposure at the two highest levels provided some inhibition of the age-related glomerulonephropathy observed in the control rats at termination. The results indicate that the lowest exposure level, 500 ppm, was a LOAEL for fatty changes in the liver of male and female Sprague-Dawley rats and that exposure to ≥1,500 ppm induced hepatocellular necrosis in males.

In females, an increasing trend was seen in the incidence of foci or areas of altered hepatocytes. Female rats in all exposed groups showed increased incidence of multinucleated hepatocytes in the centrilobular region compared with controls, but there was no evidence of increasing incidence or severity with increasing exposure level (Table 4-13). The foci and areas were apparent after 12 months, and their number and size increased thereafter, but incidences for neoplastic nodules in the liver or hepatocellular carcinomas were not increased in any exposure group. A statistically significant increased incidence of salivary gland sarcomas was reported for male rats exposed to 3,500 ppm. Burek et al. (1984) considered this finding unusual and inconsistent with other existing data because the primary target organ for dichloromethane seems to be the liver. Incidences of rats with benign mammary gland tumors were not statistically significantly higher in exposed male or female groups compared with controls, and exposed male and female groups showed no significantly increased incidences for malignant mammary gland tumors. The average number of benign mammary tumors per tumor-bearing rat increased with increasing exposure level. In females, the values were 2.1, 2.7, 3.1, and 3.5 in the control through 3,500 ppm groups, respectively; males showed a similar response with increasing exposure level, albeit to a lesser extent (Table 4-13). Burek et al. (1984) concluded that the significance of this benign mammary tumor response (i.e., increase in number of tumors per tumor-bearing rat) was unknown but speculated that the predisposition of this strain of rats (historical control incidences of females with benign mammary tumors normally exceeded 80%) plus the high exposure to dichloromethane may have resulted in the response.

Table 4-13. Incidences of selected nonneoplastic and neoplastic histologic changes in male and female Sprague-Dawley rats exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Lesion, by sex	Exposure (ppm)[a]			
	0 (Controls)	500	1,500	3,500
Males—n per group	92	95	95	97
Number (%) with:				
Liver changes				
Hepatocellular necrosis	2[b] (2)	8 (8)	10 (11)[c]	11 (11)[c]
Coagulation necrosis	–[d]	–	–	–
Hepatic vacuolation (fatty change)	16[b] (17)	36 (38)[c]	43 (45)[c]	52 (54)[c]
Foci of altered hepatocytes	–	–	–	–
Foci of altered hepatocytes, basophilic	–	–	–	–
Area of altered hepatocytes	–	–	–	–
Multinucleated hepatocytes	–	–	–	–
Glomerulonephropathy				
Severe	70[b] (76)	62 (65)	53 (56)[c]	39 (40)[c]
Any degree	92[b,e] (100)	91 (96)	93 (98)	90 (93)
Mammary changes				
Rats with benign mammary tumors	7[b] (8)	3 (3)	7 (7)	14 (14)
Total number of benign mammary tumors	8	6	11	17
Number of tumors per tumor-bearing rat[f]	1.1	2.0	1.6	1.2
Females—n per group	96	95	96	97
Number (%) with:				
Liver changes				
Hepatocellular necrosis	–	–	–	–
Coagulation necrosis	1[b] (1)	0 (0)	2 (2)	7 (7)
Hepatic vacuolation (fatty change)	33[b] (34)	49 (52)[c]	56 (58)[c]	63 (65)[c]
Foci of altered hepatocytes	35[b] (36)	36 (38)	27 (28)	50 (52)[c]
Foci of altered hepatocytes, basophilic	3[b] (3)	0 (0)	4 (4)	10 (10)
Area of altered hepatocytes	19[b] (20)	24 (25)	28 (29)	35 (36)[c]
Multinucleated hepatocytes	7[b] (7)	36 (38)[c]	34 (35)[c]	29 (30)[c]
Glomerulonephropathy				
Severe	5 (5)	3 (3)	4 (4)	5 (5)
Any degree	62[b] (65)	64 (67)	59 (61)	48 (49)[c]
Mammary changes				
Rats with benign mammary tumors	79 (82)	81 (85)	80 (83)	83 (86)
Total number of benign mammary tumors	165	218	245	287
Number of tumors per tumor-bearing rat[f]	2.1	2.7	3.1	3.5

[a]500 ppm = 1,737 mg/m^3, 1,500 ppm = 5,210 mg/m^3, 3,500 ppm = 12,158 mg/m^3.
[b]Significant dose-related trend—Cochran-Armitage trend test $p < 0.05$.
[c]Significantly higher than control incidence by Fisher's exact test.
[d]Reported as "no exposure effect" by Burek et al. (1984); data not given.
[e]Reported as 93/92 male mice in the control group had glomerulonephropathy; EPA corrected this to 92/92.
[f]Calculated by EPA.

Source: Burek et al. (1984).

4.2.2.2.5. ***Chronic inhalation exposure in Sprague-Dawley rats (Nitschke et al., 1988a).***
Nitschke et al. (1988a) examined the toxicity and carcinogenicity of lower concentrations of dichloromethane in Sprague-Dawley rats. Groups of 90 male and 90 female rats were exposed to 0, 50, 200, or 500 ppm dichloromethane (>99.5% pure) 6 hours/day, 5 days/week for 2 years. Interim sacrifices were conducted at 6, 12, 15, and 18 months (five rats/sex/interval). An additional group of 30 female rats was exposed to 500 ppm for 12 months and then exposed to room air for up to an additional 12 months, and another group of 30 female rats was exposed to room air for the first 12 months, followed by exposure to 500 ppm for the last 12 months of the study. These latter groups were included to examine temporal relationships between exposure and potential carcinogenic response. All groups of rats were examined daily for signs of toxicity and all rats were examined for palpable masses prior to the initial exposure and at monthly intervals after the first 12 months. BW was checked twice a month for the first 3 months and monthly thereafter. Blood samples were collected at interim sacrifices and analyzed for total bilirubin, cholesterol, triglycerides, potassium, estradiol, follicle-stimulating hormone, and luteinizing hormone levels. In addition, COHb was determined at multiple times in blood collected from the tail vein. DNA synthesis (incorporation of ^{3}H-thymidine as a measure of cellular proliferation) was measured in the liver of separate groups of female rats after exposure to the various concentrations for 6 and 12 months (four females/exposure group/interval). All rats were subjected to a complete necropsy, and sections from most tissues were processed for microscopic examination.

Exposure to dichloromethane at any of the exposure levels did not significantly alter mortality rates, BWs, organ weights, clinical chemistry values, or plasma hormone levels (Nitschke et al., 1988a). Blood COHb was elevated in a dose-related manner but not in an exposure duration-related fashion, suggesting lack of accumulation with repeated exposures. For example, mean (± SD) values for percentage COHb were 2.2 (± 1.3), 6.5 (± 1.1), 12.5 (± 0.8), and 13.7 (± 0.6) for male rats in the control through 500 ppm groups, respectively, at the terminal sacrifice. A similar pattern was seen at the 6-month and 12-month intervals (e.g., respective values for males were 4.8 [± 2.6], 8.8 [± 2.0], 14.3 [± 1.3], and 16.7 [± 2.4] at the 6-month sacrifice).

The results of the thymidine incorporation experiment revealed no detectable alteration in the rate of liver DNA synthesis in the exposed groups compared with controls. Statistically significantly increased incidences of nonneoplastic liver lesions (hepatic lipid vacuolation consistent with fatty change and multinucleated hepatocytes) occurred only in females in the 500 ppm group (Table 4-14). Male rat incidence for hepatocyte vacuolation was elevated at 500 ppm but not to a statistically significant degree. In the group of female rats exposed for only 12 months to 500 ppm, significantly increased incidences of nonneoplastic lesions compared with controls were restricted to liver cytoplasmic vacuolization (16/25 = 64%) and multinucleated hepatocytes (9/25 = 36%) in rats exposed during the first 12 months of the study;

rats exposed only during the last 12 months of the study showed no elevated incidences of the liver lesions. The observation of the increased incidence of hepatic (lipid) vacuolation indicates 500 ppm is a LOAEL and 200 ppm is a NOAEL in this study.

Table 4-14. Incidences of selected nonneoplastic histologic changes in male and female Sprague-Dawley rats exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Lesion, by sex	Exposure (ppm)[a]						
	0 (Controls)	50	200	500	Trend *p*-value[b]	Late 500[c]	Early 500[c]
Males—n per group	70	70	70	70		NA[d]	NA
Number (%) with:							
Hepatic vacuolation (fatty change)	22 (31)	–[e]	–	28 (40)			
Multinucleated hepatocytes	–	–	–	–			
Females—n per group	70	70	70	70		25	25
Number (%) with:							
Hepatic vacuolation (fatty change)	41 (59)	42 (60)	41 (59)	53 (76)[f]	0.01	15 (60)	16 (64)[f]
Multinucleated hepatocytes	8 (11)	6 (9)	12 (17)	27 (39)[f]	< 0.0001	3 (12)	9 (36)[f]

[a]50 ppm = 174 mg/m^3, 200 ppm = 695 mg/m^3, 500 ppm = 1,737 mg/m^3.
[b]Cochran-Armitage trend test.
[c]Late 500 = no exposure for first 12 mo followed by 500 ppm for last 12 mo; early 500 = 500 ppm for first 12 month followed by no exposure for last 12 month.
[d]NA = there were no male rats in these exposure groups.
[e]– = Incidences not reported.
[f]Significantly ($p \leq 0.05$) higher than control incidence by Fisher's exact test (Nitschke et al., 1988a).

Source: Nitschke et al. (1988a).

A few fibrosarcomas or undifferentiated sarcomas in the mammary gland were seen in the exposed rats, but these incidences were not statistically significant (Table 4-15). Significantly increased incidences of rats with neoplastic lesions were restricted to benign mammary tumors in female rats exposed for 2 years to 200 ppm compared with controls (61/69 = 87%) (Table 4-15). However, significantly elevated incidences of this tumor type were not observed in 500 ppm females, and the 200 ppm incidence was within the range of historical control values for benign mammary tumors in female Sprague-Dawley rats (79–82%) from two other chronic toxicity/carcinogenicity studies from the same laboratory. A slight but statistically significant increase in the number of palpable masses in subcutaneous or mammary regions (at

Table 4-15. Incidences of selected neoplastic histologic changes in male and female Sprague-Dawley rats exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Lesion, by sex	Exposure (ppm)[a]					
	0 (Controls)	50	200	500	Late 500[b]	Early 500[b]
Males—n per group	70	70	70	70	0	0
Number (%) with:						
Liver tumors[c]	0 (0)	0 (0)	0 (0)	0 (0)		
Lung tumors[c]	0 (0)	0 (0)	0 (0)	0 (0)		
Mammary gland tumors[c]						
Adenocarcinoma or carcinoma	0 (0)	0 (0)	0 (0)	0 (0)		
Fibroadenoma	2 (4)	0 (0)	2 (3)	2 (3)		
Fibroma	6 (11)	1 (6)	6 (11)	10 (16)		
Fibrosarcoma	0 (0)	1 (6)	1 (6)	0 (0)		
Undifferentiated sarcoma	0 (0)	2 (4)	0 (0)	0 (0)		
Fibroma, fibrosarcoma, or undifferentiated sarcoma[d]	6 (11)	4 (6)	7 (12)	10 (16)		
Brain tumors[c]						
Astrocytoma or glial cell	0 (0)	1 (1)	2 (3)	1 (1)		
Granular cell	0 (0)	0 (0)	0 (0)	1 (1)		
Females—n per group	70	70	70	70	25	25
Number (%)with:						
Liver tumors[c]						
Neoplastic nodule(s)	4 (6)	4 (6)	3 (4)	4 (6)	0 (0)	1 (4)
Hepatocellular carcinoma	1 (1)	0 (0)	2 (3)	1 (1)	0 (0)	0 (0)
Lung tumors[c]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mammary gland tumors[c]						
Adenocarcinoma or carcinoma	4 (6)	5 (7)	4 (6)	3 (4)	3 (12)	2 (8)
Adenoma	1 (1)	1 (1)	2 (3)	1 (1)	2 (8)	0 (0)
Fibroadenoma	51 (74)	57 (83)	60 (87)	55 (80)	22 (88)	23 (92)
Fibroma	0 (0)	1 (1)	0 (0)	1 (1)	1 (4)	0 (0)
Fibrosarcoma	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Number with palpable masses in subcutaneous or mammary region	55 (79)	56 (80)	60 (86)	59 (84)	22 (88)	23 (92)
Number of palpable masses in subcutaneous or mammary region per tumor-bearing rat	1.8	2.1	2.0	2.2[e]	2.3[e]	2.7[e]
Number with benign tumors	52 (74)	58 (83)	61[f] (87)	55 (79)	23 (92)	23 (92)
Number of benign tumors per tumor-bearing rat	2.0	2.3	2.2	2.7	2.2	2.6
Brain tumors[c]						
Astrocytoma or glial cell	0 (0)	0 (0)	0 (0)	2 (3)	0 (0)	0 (0)
Granular cell	1 (1)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)

[a]50 ppm = 174 mg/m^3, 200 ppm = 695 mg/m^3, 500 ppm = 1,737 mg/m^3.
[b]Late 500 = no exposure for first 12 mo followed by 500 ppm for last 12 mo; early 500 = 500 ppm for first 12 month followed by no exposure for last 12 month (only females included in these exposure groups).
[c]Percentages were based on number of tissues examined microscopically per group (varying between 64 to 70).
[d]EPA summed across these three tumors, assuming no overlap.
[e]Significantly ($p \leq 0.05$) higher than control by Haseman's test (Nitschke et al., 1988a).
[f]Significantly ($p \leq 0.05$) higher than control incidence by Fisher's exact test (Nitschke et al., 1988a).

Source: Nitschke et al. (1988a).

23 months) per tumor-bearing rat was observed only in the 500 ppm female group. The numbers of benign mammary tumors per tumor-bearing rat were slightly elevated in the exposed groups compared with control groups, but no statistical analysis of this variable was performed. In female rats exposed to 500 ppm (during the first or second 12 months of the study), slight but statistically significant elevations were found in the number of palpable masses in subcutaneous or mammary regions per tumor-bearing rat; the numbers of benign mammary tumors per tumor-bearing rat were elevated compared with those of the study controls, (and with historical controls), but statistical analysis of this variable was not performed.

A statistically significant increased incidence of brain or CNS tumors was not observed, but six astrocytoma or glioma (mixed glial cell) tumors were seen in the exposed groups (four in males, two in females); no tumors of this type were seen in either male or female control groups. The authors concluded that there was no distinct exposure-related malignant carcinogenic response in male or female Sprague-Dawley rats exposed (6 hours/day, 5 days/week) to up to 500 ppm dichloromethane for 2 years (Nitschke et al., 1988a).

4.2.2.2.6. ***Chronic inhalation exposure in Sprague-Dawley rats (*****Maltoni et al., 1988*****).***
Maltoni et al. (1988) conducted an inhalation exposure study in Sprague-Dawley rats. Two groups of female rats (54–60/dose) were exposed to 0 or 100 ppm dichloromethane for 104 weeks. The exposure period was 4 hours/day, 4 days/week for 7 weeks and then 7 hours/day, 5 days/week for 97 weeks. Endpoints monitored included clinical signs, BW, and full necropsy at sacrifice (when spontaneous death occurred). For each animal sacrificed, histopathologic examinations were performed on the following organs: brain and cerebellum, zymbal glands, interscapular brown fat, salivary glands, tongue, thymus and mediastinal lymph nodes, lungs, liver, kidneys, adrenals, spleen, pancreas, esophagus, stomach, intestine, bladder, uterus, gonads, and any other organs with gross lesions.

There was no evidence of increased mortality in the exposed group, and there was no effect on BW (Maltoni et al., 1988). Little information was provided regarding nonneoplastic effects, precluding identification of NOAELs and LOAELs for nonneoplastic effects in this study. Dichloromethane exposure was not related to the percentage of rats with benign and malignant tumors, malignant tumors, or the number of total malignant tumors per 100 animals. The percentage of rats with benign mammary tumors was 40.0% in controls and 64.8% in the exposed group, and the percentage of malignant mammary tumors was 3.3 and 5.5% in controls and exposed rats, respectively. Neither of these differences was statistically significant.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Reproductive and development studies of dichloromethane exposure are summarized in Table 4-16 and described in detail in Appendix E, Sections E.2 and E.3. No effects on reproductive performance were observed in a 90-day gavage study in Charles River CD rats with

doses up to 225 mg/kg-day (General Electric Company, 1976) or in a two-generation reproductive toxicity study with F344 rats exposed to concentrations up to 1,500 ppm for 14 weeks before mating of the F0 generation, during the F1 gestational period (GDs 0–21), and 17 weeks prior to mating in the F1 generation beginning PND 4 (Nitschke et al., 1988b). Reproductive parameters (e.g., number of litters, implants/litter, live fetuses/litter, percent dead/litter, percent resorbed/litter, or fertility index[3]) were also examined in a study of male Swiss-Webster mice administered dichloromethane (250 or 500 mg/kg) by subcutaneous injection 3 times/week for 4 weeks and in a similar study involving inhalation exposure to 0, 100, 150, or 200 ppm dichloromethane. No statistically significant effects were seen in either protocol, although some evidence of a decrease in fertility index was seen in the 150 and 200 ppm groups (Raje et al., 1988).

Following exposure of pregnant F344 rats to gavage doses of up to 450 mg/kg-day on GDs 6–19, maternal weight gain was decreased, but no effects were found on the number of resorption sites, pup survivability, or pup weights at postnatal days (PNDs) 1 or 6 (Narotsky and Kavlock, 1995). The developmental effects following exposure of Long-Evans rats to 4,500 ppm for 14 days prior to mating and during gestation (or during gestation alone) were decreased offspring weight at birth and changed behavioral habituation of the offspring to novel environments (Bornschein et al., 1980; Hardin and Manson, 1980). In standard developmental toxicity studies involving exposure to 1,250 ppm on GDs 6–15, no adverse effects on fetal development were found in Swiss-Webster mice or Sprague-Dawley rats, but the incidence of minor skeletal variants (e.g., delayed ossification of sternebrae) was increased (Schwetz et al., 1975).

The potential for gestational exposure to CO and to dichloromethane (through its transfer across the placenta) resulting from maternal dichloromethane exposure via oral and inhalation routes raises concerns regarding neurodevelopmental effects. Although few developmental effects were observed at high exposures to dichloromethane (Bornschein et al., 1980; Schwetz et al., 1975), there are no studies that have adequately evaluated neurobehavioral and neurochemical changes resulting from gestational dichloromethane exposure. The available data identify changes in behavior habituation at 4,500 ppm (Bornschein et al., 1980) and increases in COHb at 1,250 ppm (Schwetz et al., 1975). The behavioral changes observed at 4,500 ppm indicate developmental neurotoxic effects; this is the only dose group used in the Bornschein et al. (1980) study. No other neurological endpoints have been evaluated in the available developmental studies of dichloromethane. The potential for developmental neurotoxicity occurring at lower exposures to dichloromethane represents a data gap.

[3]Fertility index defined as number of females impregnated divided by total number of females mated times 100.

Table 4-16. Summary of studies of reproductive and developmental effects of dichloromethane exposure in animals

Species and n	Exposure dose	Exposure period	Results	Reference
Gavage or subcutaneous				
Charles River rats (males and females), 10/sex/dose group	0, 25, 75, 225 mg/kg (gavage)	90 d before mating (10 d between last exposure and mating period)	No effects on fertility index, number of pups per litter, pup survival, or F1 BW, hematology, and clinical chemistry tests (up to 90 d of age)	General Electric Company (1976)
Swiss-Webster mice (males), 20/group	0, 250, 500 mg/kg (subcutaneous injection), 3 × per wk	4 wks prior to mating (1 wk between last exposure and mating period)	No effects on fertility index, number of litters, implants per litter, live fetuses per litter, resorption rate; no testicular effects	Raje et al. (1988)
F344 rats (females), 17–21/dose group	0, 337.5, 450 mg/kg-d (gavage)	GDs 6–19	Decreased maternal weight gain; no effect on resorption rate, number of live litters, implants, live pups, or pup weight	Narotsky and Kavlock (1995)
Inhalation				
F344 rats (males and females, two generation), 30/sex/dose group (F0 and F1)	0, 100, 500, 1,500 ppm, 6 hrs/d	14 wks prior to mating (F0), GDs 0–21, and 17 wks prior to mating, beginning PND 4, (F1)	No effect on fertility index, litter size, neonatal survival, growth rates, or histopathologic lesions	Nitschke et al. (1988b)
Swiss-Webster mice (males), 20/group	0, 100, 150, 200 ppm, 2 hrs/d	6 wks, prior to mating (2 d between last exposure and mating period)	Fertility index decreased in 150 and 200 ppm group (statistical significance depends on test used); no effects on number of litters, implants per litter, live fetuses per litter, resorption rate; no testicular effects	Raje et al. (1988)
Long-Evans rats (female), 16–21/dose group	0, 4,500 ppm	12–14 d before mating and/or GDs 1–17	Gestational exposure resulted in increased absolute and relative maternal liver weight, decreased fetal BW	Hardin and Manson (1980)
Long-Evans rats (female), 16–21/dose group	0, 4,500 ppm	12–14 d before mating and/or GDs 1–17	Altered rate of behavioral habituation to novel environment (at 4 d of age). No effect on crawling (at 10 d), movement in photocell cage (15 d), use of running wheel (45–108 d), and shock avoidance (4 mo)	Bornschein et al. (1980)
Swiss-Webster mice (females), 30–40/group	0, 1,250 ppm, 7 hrs/d	GDs 6–15	Increased incidence of extra center of ossification in sternum, increased (~10%) maternal blood COHb, increased maternal weight, increased maternal absolute liver weight	Schwetz et al. (1975)
Sprague-Dawley rats (females), 20–35/group	0, 1,250 ppm, 7 hrs/d	GDs 6–15	Decreased incidence of lumbar ribs or spurs, increased incidence of delayed ossification of sternebrae, increased (~10%) maternal blood COHb, increased maternal absolute liver weight	Schwetz et al. (1975)

PND = postnatal day

4.4. OTHER ENDPOINT-SPECIFIC STUDIES

4.4.1. Immunotoxicity Studies in Animals

Aranyi et al. (1986) studied the effects of acute inhalation exposures to 50 or 100 ppm dichloromethane on two measures of immune response (susceptibility to respiratory infection and mortality due to *Streptococcus zooepidemicus* exposure and ability of pulmonary macrophages to clear infection with *Klebsiella pneumoniae*). Female CD1 mice that were 5–7 weeks of age at the start of the exposure portion of the experiment were used for both assays. Up to five replicate groups of about 30 mice were challenged with viable *S. zooepidemicus* during simultaneous exposure to dichloromethane or to filtered air. Deaths were recorded over a 14-day observation period. Clearance of ^{35}S-labeled *K. pneumoniae* by pulmonary macrophages was determined by measuring the ratio of the viable bacterial counts to the radioactive counts in each animal's lungs 3 hours after infection; 18 animals were used per dose group. A single 3-hour exposure to 100 ppm dichloromethane significantly increased the susceptibility to respiratory infection and greater mortality following exposure to *S. zooepidemicus* ($p \leq 0.01$). Twenty-six deaths occurred in 140 (18.6%) mice challenged during a 3-hour exposure to 100 ppm dichloromethane; in contrast, nine deaths occurred in 140 mice (6.4%) exposed to filtered air. The 3-hour exposure to 100 ppm dichloromethane was associated with a statistically significant ($p \leq 0.001$) 12% decrease in pulmonary bactericidal activity (91.6 and 79.6% of bacteria killed in controls and 100 ppm group, respectively). No difference was seen in either mortality rate or bactericidal activity in experiments using a single 3-hour exposure to 50 ppm or 3-hour exposures to 40 ppm dichloromethane repeated daily for 5 days compared with control animals exposed to filtered air. These results suggest that 3-hour exposure to 50 ppm dichloromethane was a NOAEL and 100 ppm was a LOAEL for decreased immunological competence (immunosuppression) in CD-1 mice.

Aranyi et al. (1986) also conducted a similar set of experiments with 13 other chemicals (acetaldehyde, acrolein, propylene oxide, chloroform, methyl chloroform, carbon tetrachloride, allyl chloride, benzene, phenol, monochlorobenzene, benzyl chloride, perchloroethylene, and ethylene trichloride). Perchloroethylene and ethylene trichloride were the only chemicals in this group for which an increased mortality risk from Streptococcal pneumonia was seen (mortality risk 15.0 and 31.4% in controls and 50 ppm exposure groups, respectively, for perchloroethylene and 13.4 and 58.1% in controls and 50 ppm exposure groups, respectively, for ethylene trichloride). Decreased bactericidal activity was also seen with acetaldehyde, acrolein, methyl chloroform, allyl chloride, benzene, benzyl chloride, perchloroethylene, and ethylene trichloride at one or more exposures. Results from several chemicals suggest that 5 days of exposure results in greater decrease in bactericidal activity (i.e., acetaldehyde, acrolein, and benzene), and others (e.g., perchloroethylene) suggest that 5 days of exposure does not result in greater suppression than a single exposure period.

There was considerable variation in both measures of immune response among the controls in the experiments (Aranyi et al., 1986). Among the controls in the experiments with the 13 chemicals other than dichloromethane, mortality in the Streptococcal infectivity model ranged from 5.7 to 22.1%, with a mean of 12.7%.[4] Bactericidal activity in the Klebsiella model among controls ranged from 67.9 to 94.7%, with a mean of 81.8%. The number of bacteria deposited in the lung in an inhalation bacterial infectivity model can show considerable variation (i.e., between 750 to 1,500 viable Streptococcus or Klebsiella organisms) (Ehrlich, 1980). Therefore, concurrent controls are particularly important due to the variation in preparation and aerosol administration of the bacteria in these assays.

Warbrick et al. (2003) evaluated immunocompetence in male and female Sprague-Dawley rats by measuring the immunoglobulin M (IgM) antibody responses following immunization with sheep red blood cells in addition to hematological parameters and histopathology of the spleen, thymus, lungs, and liver. Groups of rats (8/sex/dose level) were exposed to 0 or 5,000 ppm dichloromethane 6 hours/day, 5 days/week for 28 days. Rats injected with cyclophosphamide served as positive controls. Five days before sacrifice (day 23 of exposure) all rats were injected with sheep red blood cells. IgM levels in response to the sheep red blood cells were comparable between dichloromethane-exposed and air-exposed rats, indicating that dichloromethane did not produce immunosuppression in the animals under these exposure conditions. Cyclophosphamide-treated animals had significantly lower levels of IgM in the blood serum, indicating immunosuppression. Rats exposed to dichloromethane showed reduced response to sound, piloerection, and hunched posture during exposures. Neither BW gain nor the hematological parameters monitored were significantly affected by exposure to dichloromethane. Relative and absolute liver weights were significantly increased in females but not in males. Relative spleen weight was reduced in females, and no significant changes were seen in the weight of the thymus and lungs. Histopathology of the tissues examined was unremarkable. Exposure to 5,000 ppm dichloromethane did not affect antibody production to the challenge with sheep red blood cells.

In the 2-year drinking water study in F344 rats (Serota et al., 1986a, b) and 2-year inhalation study in Sprague-Dawley rats (Nitschke et al., 1988a), histopathologic analyses were conducted on the lymph nodes, thymus, and spleen among several other organs, and no significant changes were noted.

In summary, one study (Aranyi et al., 1986) demonstrated evidence of immuno-suppression, including increased risk of Streptococcal-pneumonia-related mortality and decreased clearance of Klebsiella bacteria following a single dichloromethane exposure at 100 ppm for 3 hours in CD-1 mice. The Streptococcal and Klebsiella bacterial inhalation assays are models of respiratory infection that test for local immune effects associated with inhalation

[4]EPA did not include the duplicate assay of perchloroethylene in calculating this summary statistic. If this additional assay is included, the mortality risk ranges from 5.7 to 45.7%, with a mean of 15.0%.

exposure rather than systemic immunosuppression. The NOAEL identified in this study was 50 ppm. In contrast, in a functional immune assay of systemic immunosuppression conducted in rats, Warbrick et al. (2003) did not observe changes in the antibody response to sheep red blood cells in a 28-day inhalation exposure to 5,000 ppm dichloromethane. Histopathologic analyses of immune system organs in chronic exposure studies for $B6C3F_1$ mice and F344 rats (Nitschke et al., 1988a; Serota et al., 1986a, b) revealed no changes from controls. However, no assays of functional immunity were included in these chronic studies. These two studies for dichloromethane do not suggest systemic immunosuppression, but the Aranyi et al. (1986) study provides evidence of route-specific local immunosuppression from acute inhalation exposure in CD1 mice. Due to the acute exposure duration used in Aranyi et al. (1986), the immune effects of short-term or chronic exposure to dichloromethane are unclear.

4.4.2. Neurotoxicology Studies in Animals

Neurological evaluations in animals during and after exposure to dichloromethane have resulted in CNS depressant effects similar to other chlorinated solvents (e.g., trichloroethylene, perchloroethylene) and ethanol. Overall, there are decreased motor activity, impaired memory, and changes in responses to sensory stimuli. Neurobehavioral, neurophysiological, and neurochemical/neuropathological studies have been used to characterize the effects of dichloromethane on the CNS. An overview of these types of studies and a summary of the results seen in these studies are provided below; a detailed description of individual studies is provided in Appendix E, Section E.4.

Neurobehavioral studies with dichloromethane used protocols to measure changes in spontaneous motor activity, a functional observational battery (FOB) test (to evaluate gross neurobehavioral deficits), and a task developed to assess learning and memory. The FOB protocol includes various autonomic parameters, neuromuscular parameters, sensorimotor parameters, excitability measures, and activity. Learning and memory changes with dichloromethane were studied by using a passive avoidance task. The oral and inhalation studies that examined neurobehavioral endpoints are summarized in Table 4-17.

Neurophysiological studies with dichloromethane exposure consisted of measuring evoked responses in response to sensory stimuli. In these studies, animals were implanted with electrodes over the brain region that responds to the particular stimuli. For example, an electrode would be implanted over the visual cortex in an animal presented with a visual stimulus. Once the stimulus is presented to the animal, an evoked response is elicited from the brain region and transmitted to the implanted electrode. During administration of a chemical, if there is a significant change in the magnitude, shape, and latency (among other measures) in the evoked response, then the chemical is considered to produce neurological effects. A summary of studies examining dichloromethane exposure and neurophysiological changes is shown in Table 4-18.

In neurochemical/neuropathological studies with dichloromethane, animals were first exposed to dichloromethane (orally or via inhalation or injection), and then the brains were removed. Changes in excitatory neurotransmitters, such as glutamate and acetylcholine and the inhibitory neurotransmitter, GABA, were measured. Additionally, dopamine and serotonin levels, which are associated with addiction and mood, were also measured. Other parameters that were measured included DNA/protein content and regional brain changes in the cerebellum and hippocampus. Measurement of neurochemical changes provides mechanistic information, and neurobehavioral and neurophysiological effects can be correlated to these results. Table 4-19 summarizes studies of neurochemical changes and dichloromethane.

Three studies evaluated the neurotoxic potential of dichloromethane by either administering the solvent orally or by injection; two of these studies (Herr and Boyes, 1997; Kanada et al., 1994) only evaluated acute effects (2–5 hours) from single-dose exposures. Observed neurological effects included decreased spontaneous activity (Moser et al., 1995), changes in flash-evoked potential (FEP) measurements (Herr and Boyes, 1997), and changes in catecholamine levels in the brain (Kanada et al., 1994).

The database pertaining to neurotoxic effects from inhalation exposure to dichloromethane is considerably larger than the oral exposure database. Acute (<1 day) and short-term (1–14 days) exposures resulted in an initial increase in spontaneous activity followed by a decrease for exposures between 500 and 2,500 ppm (Kjellstrand et al., 1985; Savolainen et al., 1977). Higher (5,000 ppm) acute and short-term exposures resulted in decreased spontaneous activity and lethargy (Weinstein et al., 1972; Heppel et al., 1944; Heppel and Neal, 1944). Longer-term exposures (up to 14 weeks) produced decreased motor activity and lethargy in several animals at 1,000 and 5,000 ppm (Haun et al., 1971), and exposures at 25 ppm for 14 weeks produced significant increases in activity in mice, starting at week 9. CNS depression was evidenced by decreased responses in the auditory, visual, and somatosensory regions of the brain in a study of sensory-evoked potential effects in 12 adult male F344 rats exposed to 0, 5,000, 10,000, and 15,000 ppm for 1-hour periods (Rebert et al., 1989). The significant changes noted in several somatosensory-evoked potential (SEP) measures during dichloromethane exposure were not observed after a subchronic exposure where animals were tested at least 65 hours after the last exposure (Mattsson et al., 1990). As a result, it is difficult to ascertain if tolerance is developed to dichloromethane-mediated changes in sensory potentials seen during an acute exposure, or if these effects are still maintained during repeated exposure, since measurements were not taken during the subchronic exposure. Altered learning and memory abilities were demonstrated in young (3-, 5-, and 8-week-old) male Swiss-Webster mice exposed to 168 mg/L (~47,000 ppm) dichloromethane for approximately 20 seconds (until there was a loss of the righting reflex) (Alexeeff and Kilgore, 1983).

Table 4-17. Studies of neurobehavioral changes from dichloromethane, by route of exposure and type of effect

Species	Exposure(s)	Duration	Neurobehavioral effect	Reference
Gavage exposure				
FOB				
F344 rat, female	101, 337, 1,012, 1,889 mg/kg, gavage	Acute—evaluated 4 and 24 hrs after dosing	FOB neuromuscular and sensorimotor parameters significantly different from controls at 1,012 and 1,889 mg/kg (337 mg/kg = NOAEL)	Moser et al. (1995)
F344 rat, female	34, 101, 337, 1,012 mg/kg-d, gavage	14 d—evaluated on d 4, 9, and 15	All FOB parameters (except activity) significantly affected from d 4 at doses of 337 and 1,012 mg/kg-d	Moser et al. (1995)
Inhalation exposure				
Spontaneous activity				
NMRI mouse, male	400–2,500 ppm	1 hr	Initial increase in activity followed by a pronounced decrease at exposures ≥600 ppm	Kjellstrand et al. (1985)
Rat, male	5,000 ppm	1 hr, every other d for 10 d	Decreased spontaneous locomotor activity	Heppel and Neal (1944)
Wistar rat, male	500 ppm	6 hrs/d, 6 d	Increased preening frequency	Savolainen et al. (1977)
ICR mouse, female	5,000 ppm	Continuous, 7 d	Increased spontaneous activity in first few hrs and then decreased activity	Weinstein et al. (1972)
Sprague-Dawley rat, male	1,000, 5,000 ppm	Continuous, 14 wks	No neurobehavioral changes	Haun et al. (1971)
ICR mouse, female	1,000, 5,000 ppm	Continuous, 14 wks	Incoordination, lethargy	Haun et al. (1971)
Beagle dog, female	1,000, 5,000 ppm	Continuous, 14 wks	Incoordination, lethargy	Haun et al. (1971)
Rhesus monkey, female	1,000, 5,000 ppm	Continuous, 14 wks	Incoordination, lethargy	Haun et al. (1971)
ICR mouse, female	25, 100 ppm	Continuous, 14 wks	Increased spontaneous activity at 25 ppm	Thomas et al. (1972)
FOB				
F344 rat, male and female	50, 200, 2,000 ppm	6 hrs/d, 5 d/wk, 13 wks + 65 hrs exposure free	No effects observed on FOB, grip strength	Mattsson et al. (1990)
Learning and memory				
Swiss-Webster mouse, male	47,000 ppm	Approximately 20 sec + 1 hr exposure free before training; retested at d 1, 2, and 4	Significant decrease in learning and recall ability	Alexeef and Kilgore (1983)

Table 4-18. Studies of neurophysiological changes as measured by evoked potentials resulting from dichloromethane, by route of exposure

Species	Exposure(s)	Duration	SEPs measured	Effect	Reference
Intraperitoneal					
Long-Evans rat, male	57.5, 115, 230, 460 mg/kg	Acute; tested at 15 min, 1 hr, and 5 hrs after dosing	FEP	Significant changes in FEPs were noted in animals dosed ≥115 mg/kg; FEP changes time- and dose- dependent	Herr and Boyes (1997)
Inhalation exposure					
F344 rat, male	5,000, 10,000, 15,000 ppm	Acute, 1 hr; tested during exposure	Electroencephalogram, BAER, CAEP, FEP, SEP	Significant changes in SEP, FEP, BAER, and CAEP responses at all exposures; slight recovery noted at 1 hr after exposure	Rebert et al. (1989)
F344 rat, male and female	50, 200, 2,000 ppm	Subchronic, 6 hr/d, 5 d/wk, 13 wks; tested 65 hrs after last exposure	FEP, CAEP, BAER, SEP	No significant changes noted in any evoked potential measurements	Mattsson et al. (1990)

BAER = brainstem-auditory-evoked response; CAEP = cortical-auditory-evoked potential; FEP = flash-evoked potential; SEP = somatosensory-evoked potential

Table 4-19. Studies of neurochemical changes from dichloromethane, by route of exposure

Species and sex	Exposure	Duration	Regions	Effect[a]	Reference
Oral exposure					
Sprague-Dawley rat, male	534 mg/kg	Acute, single dose; evaluated 2 hrs after dosed	Hippocampus, medulla, midbrain, hypothalamus	↑ acetylcholine in hippocampus ↑ dopamine and serotonin in medulla ↓ norepinephrine in midbrain ↓ norepinephrine and serotonin in hypothalamus	Kanada et al. (1994)
Inhalation exposure					
Wistar rat, male	1,000 ppm TWA (basal exposure of 100 ppm + 2,800 ppm, 1 hr peak exposures at hrs 1 and 4)	6 hrs/d, 5 d/wk, 2 wks	Cerebrum, cerebellum	↑ NADPH diaphorase, succinate dehydrogenase in cerebrum ↑ cerebral RNA ↓ succinate dehydrogenase in cerebellum	Savolainen et al. (1981)
Wistar rat, male	1,000 ppm TWA	6 hrs/d, 5 d/wk, 2 wks + 7 d exposure free	Cerebrum, cerebellum	↓ succinate dehydrogenase in both regions	Savolainen et al. (1981)
Wistar rat, male	1,000 ppm	6 hrs/d, 5 d/wk, 2 wks	Cerebrum, cerebellum	↑ acid proteinase ↓ succinate dehydrogenase in cerebellum	Savolainen et al. (1981)
Wistar rat, male	1,000 ppm	6 hrs/d, 5 d/wk, 2 wks + 7 d exposure free	Cerebrum	↓ cerebral RNA	Savolainen et al. (1981)
Sprague-Dawley rat, male	70, 300, 1,000 ppm	6 hrs/d, 3 d	Caudate nucleus—medial	↑ catecholamine levels (70 ppm) ↓ catecholamine levels (300 and 1,000 ppm) No effect on luteinizing hormone release	Fuxe et al. (1984)
Mongolian gerbil, male and female	210, 350 ppm	Continuous (24 hrs/d), 3 mo + 4 mo exposure free	Hippocampus, cerebellum cerebral cortex	↓ DNA concentration per wet weight in hippocampus (210, 350 ppm) and cerebellar hemispheres (350 ppm) ↑ astroglial proteins in frontal and sensory motor cerebral cortex	Rosengren et al. (1986)
Mongolian gerbil, male and female	210 ppm	Continuous (24 hrs/d), 3 mo	Frontal cortex, cerebellum	↓ glutamate, GABA, phosphoethanolamine in frontal cortex ↑ glutamate, GABA in posterior cerebellar vermis	Briving et al. (1986)

Table 4-19. Studies of neurochemical changes from dichloromethane, by route of exposure

Species and sex	Exposure	Duration	Regions	Effect[a]	Reference
Mongolian gerbil, male and female	210 ppm	Continuous (24 hrs/d), 3 mo + 4 mo exposure free	Hippocampus, olfactory bulbs, cerebral cortex	↓ DNA concentration per wet weight in hippocampus only	Karlsson et al. (1987)

[a]All effects shown in this table were statistically significant.

↑ = increase; ↓ = decrease; NADPH = nicotinamide adenine dinucleotide phosphate

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

4.5.1. Genotoxicity Studies

The application of genotoxicity data to predict potential carcinogenicity is based on the principle that genetic alterations are found in all cancers. Most tests for genotoxicity are designed to detect on a cellular or molecular level whether a substance has the capability of damaging genetic material, although some specific modifications of the epigenome, including proteins associated with DNA or RNA, can also cause transmissible changes. Genotoxicity assays that indicate mutagenicity are those that provide evidence of the ability of a chemical to alter the amount or structure of genetic material in a manner that permits changes to be transmitted during cell division. Certain genetic alterations, including gene mutations and chromosomal aberrations, are considered mutational events that can occur through a variety of mechanisms; 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 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 for Carcinogenic Risk Assessment* and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005a, b), the approach does not consider quantitative issues related to the probable production of specific metabolites in vivo. Instead, the analysis of genetic toxicity data presented here focuses on the identification of a genotoxic hazard of dichloromethane or its metabolites; a quantitative analysis of dichloromethane metabolism to reactive intermediates, via PBPK modeling, is considered in the derivation of the cancer toxicity values.

The following section summarizes available data on genotoxicity for dichloromethane by genotoxic endpoint and organism.

4.5.1.1. *In Vitro Genotoxicity Assays*

Genotoxicity assays in nonmammalian organisms. Numerous in vitro studies have demonstrated the mutagenic potential of dichloromethane in bacterial, yeast, and fungal mutagenesis assays. Several studies provide evidence that the mutagenicity of dichloromethane in bacterial systems is enhanced in the presence of GSH (Table 4-20). Considering that the response is primarily dependent on the presence of GSH, activation likely involves the GST-T1 metabolic pathway, which produces two proposed DNA-reactive metabolites, S-(chloromethyl)glutathione and formaldehyde.

Dichloromethane consistently induced mutations in *Salmonella typhimurium* strains TA100 and TA98. These effects were not markedly influenced by the addition of exogenous mammalian liver fractions, suggesting that endogenous metabolism in these strains was sufficient to activate dichloromethane (Green, 1983; Jongen et al., 1982; Gocke et al., 1981; Jongen et al., 1978). Dichloromethane exposure of NG-11, a glutathione-deficient variant of *S. typhimurium* strain TA100, produced twofold fewer base-pair substitution mutations compared with exposure of strain TA100, which produces normal levels of GSH. Furthermore, this difference was not apparent when the culture medium contained 1 mM GSH (Graves et al., 1994b). *S. typhimurium* strains TA1535, TA1537, and TA1538 that are deficient in GST activity did not develop base-pair mutations in response to dichloromethane exposure (Pegram et al., 1997; Simula et al., 1993; Thier et al., 1993; Osterman-Golkar et al., 1983; Gocke et al., 1981). However, when strain TA1535 was transfected with rat GST-T1, dichloromethane induced base-pair substitution mutations (Demarini et al., 1997; Pegram et al., 1997; Thier et al., 1993). A 60-fold higher concentration of dichloromethane was needed to induce a response (i.e., a sixfold increase over background levels in reverse mutations) in *S. typhimurium* strain TA100 than in TA1535 transfected with rat GST-T1 (Demarini et al., 1997). This study also included several trihalomethanes; dichloromethane was several-fold less genotoxic than dibromochloromethane or bromoform, but was similar in potency to bromodichloromethane (Demarini et al., 1997; Pegram et al., 1997).

The mutagenic effects of dichloromethane have also been examined in fungi and yeast with both systems reporting positive results. Fungal assays were positive for mitotic segregation in *Aspergillus nidulans* (Crebelli et al., 1988), but there was not a dose response relationship as only the 4,000 ppm dichloromethane exposure was positive (exposure up to 8,000 ppm). A yeast assay was positive for gene conversion and recombination in *Saccharomyces cerevisiae* for concentrations up to 209 mM (Callen et al., 1980).

Table 4-20. Results from in vitro genotoxicity assays of dichloromethane in nonmammalian systems

Endpoint	Test system	Dose/concentration and duration	Results[a]		Comments	Reference
			–S9	+S9		
Reverse mutation	*Salmonella typhimurium* TA98, TA100	48-hr exposure to 0, 5,700, 11,400, 17,100, 22,800, and 57,000 ppm	+ (DR)	++[b] (DR)	Vapor phase exposure in enclosed 37°C system. Toxic at highest dose only.	Jongen et al. (1978)
Reverse mutation	*S. typhimurium* TA98, TA100	8-hr exposure up to 750 µL/plate	+ (DR)	++[c] (DR)	Exposures in airtight desiccator.	Gocke et al. (1981)
	TA1535, TA1537, TA1538		–	–		
Reverse mutation	*S. typhimurium* TA100	6-hr exposure to 0, 3,500, 7,000, and 14,000 ppm	+ (DR)	++[d] (DR)	Vapor phase exposure in enclosed 37°C system.	Jongen et al. (1982)
Reverse mutation	*S. typhimurium* TA100	3-day exposure, up to 84,000 ppm	+	+[e]	Vapor phase exposure in sealed jars. Peak response at 12 h. Exogenous GST or GSH had no effect.	Green (1983)
Reverse mutation	*S. typhimurium* TA100, TA1950; *E. coli* WU361089	10 µL/plate	+	ND	Spot test.	Osterman-Golkar et al. (1983)
	S. typhimurium TA1535		–	ND		
	TA100	2-hr exposures; 0, 20, 40, and 80 mM	+ (DR)	ND	Standard plate incubation assay; no toxicity observed.	
Reverse mutation	*S. typhimurium* TA100, TA98	24-hr exposure to 0, 0.01, 0.05, 0.1, 0.25, 0.5, and 1.0 mL/chamber	+ (DR)	++[f] (DR)	Vapor phase exposure in sealed desiccator jars required for positive result. Toxicity at highest dose only.	Zeiger (1990)
Reverse mutation	*S. typhimurium* TA100	2- and 6-hr exposures to 0, 2,500, 5,000, 7,500, 10,000 ppm; 6- and 48-hr exposures up to 50,000 ppm	+ (DR)	+[g] (DR)	Vapor phase exposure in sealed jars. NG54=TA100 with 4-fold lower GSH levels. Exogenous GSH slightly increased mutation frequency. Peak response at 6 h.	Dillon et al. (1992)
	S. typhimurium TA100, NG54	6-hr exposure to 0, 2,500, 5,000, 7,500, 10,000, 20,000, 40,000 ppm	+ (DR)	+ (DR)		
	E. coli WP2 uvrA pKM101	6- and 48-hr exposures to 6,300, 12,500, 25,000, and 50,000 ppm	+ (DR)	+ (DR)		
Reverse mutation	*S. typhimurium* TA100 (+GSTA1-1 and GSTP1-1)	0, 50, 100, and 200 µL/plate	+ (DR)	ND	Mutagenicity in TA100 not enhanced by transfection with human GSTA1-1 or GSTP1-1.	Simula et al. (1993)

Table 4-20. Results from in vitro genotoxicity assays of dichloromethane in nonmammalian systems

Endpoint	Test system	Dose/concentration and duration	Results[a] –S9	Results[a] +S9	Comments	Reference
Reverse mutation	*S. typhimurium* TA1535 (+GST5-5)	0–2.0 mM/plate	+ (DR)	ND	5 min preincubation. Transfected with rat GST5-5. Negative with exogenous S-(1-acetoxymethyl)GSH or HCHO.	Thier et al. (1993)
	TA1535		–	ND	Parental strain negative with exogenous GSH or GST.	
Reverse mutation	*S. typhimurium* TA100	3-day exposure, up to 100,000 ppm	++ (DR)	ND	Vapor phase exposure in sealed jars. NG-11=TA100 without GSH; adding GSH increased mutagenicity of NG-11. Toxic at highest dose.	Graves et al. (1994b)
	NG-11		+ (DR)	ND		
Reverse mutation	*S. typhimurium* TA1535 (+GST5-5)	0, 200, 400, 800, and 1600 ppm (0, 0.03, 0.06, 0.13, and 0.26 mM in medium)	+ (DR)	ND	Plate incorporation assay; 24 h exposure in sealed Tedlar bags. Transfected with rat GST5-5. Toxic at highest dose.	Pegram et al. (1997)
	TA1535		– (T)	ND		
Reverse mutation	*S. typhimurium* TA100, RSJ100	Up to 24,000 ppm	+	ND	Plate incorporation assay; 24 h exposure in sealed Tedlar bags. RSJ100=TA1535+transfected rat GSTT1-1; TPT100= nonfunctional GSTT1-1 gene. Toxic at highest dose.	DeMarini et al. (1997)
	TA1535, TPT100		– (T)	ND		
Forward mutation	*S. typhimurium* BA13	0, 8, 20, 40, and 85 µmol/plate	+++	+[c]	Preincubation assay for L-arabinose resistance (Ara^R test). Toxic ≥85 µmol.	Roldán-Arjona and Pueyo (1993)
Forward mutation	*E. coli* K12 (wild type)	2-hr exposures to 0, 30, 60, and 130 mM/plate (aqueous concentrations)	–	+[h]	Vapor phase exposure in sealed jars. "+" with mouse liver S9 only, not rat. No cell death in these strains and doses.	Graves et al. (1994b)
	E. coli UvrA		–	–		
Forward mutation	*E. coli* Uvr^+	20,000 ppm	+	ND	Excision repair-proficient strain indicated by lacI gene expression.	Zielenska et al. (1993)
	E. coli $UvrB^-$		+	ND	Excision repair-absent strain.	
DNA repair	*S. typhimurium* TA1535/pSK1002	0, 2.5, 5.0, 10, and 20 mM	–	ND	SOS response indicated by umu gene expression.	Oda et al. (1996)
	S. typhimurium NM5004		+ (DR)	ND	TA1535/pSK1002 transfected with rat GST5-5. Toxic at highest dose.	
Prophage induction	*E. coli* K-39 (λ)	10 µL/plate	+++	ND	Spot test.	Osterman-Golkar et al. (1983)

Table 4-20. Results from in vitro genotoxicity assays of dichloromethane in nonmammalian systems

Endpoint	Test system	Dose/concentration and duration	Results[a] –S9	Results[a] +S9	Comments	Reference
Fungi and yeasts						
Mitotic segregation	*Aspergillus nidulans* -diploid strain P1	0, 800, 2,000, 4,000, 6,000, and 8,000 ppm	+ (T)	ND	Positive only at 4,000 ppm.	Crebelli et al. (1988)
Gene conversion	*Saccharomyces cerevisiae* -strain D7	0, 104, 157, and 209 mM	+ (T)	ND	Total cell death at 209 mM. Positive at 157 mM only with 58% cell death.	Callen et al. (1980)
Mitotic recombination			+ (T)	ND		
Reverse mutation			+ (T) (DR)	ND	Positive dose-response at 104 and 157 mM.	

[a] + = positive, – = negative, (T) = toxicity, ND = not determined, DR = dose-response observed.
[b] S9 liver fraction isolated from male Wistar rats induced with phenobarbital.
[c] S9 liver fraction isolated from rats induced with Aroclor 1254.
[d] S9 liver fraction isolated from male Wistar rats induced with Aroclor 1254 and phenobarbital and separated into microsomal and cytosolic fractions.
[e] S9 liver fraction isolated from male Sprague-Dawley rats induced with Aroclor 1254 and separated into microsomal and cytosolic fractions.
[f] S9 liver fraction isolated from male Sprague-Dawley rats induced with Aroclor 1254.
[g] S9 liver fraction isolated from male Fischer F344 rats induced with Aroclor and separated into microsomal and cytosolic fractions.
[h] S9 liver fractions isolated from male B6C3F$_1$ mice or male Alpk:APfSD (AP) rats.

Mammalian assays. In the in vitro mammalian system studies conducted with murine cell lines (Table 4-21), dichloromethane produced DNA single stranded breaks (SSBs) in mouse hepatocytes (Graves et al., 1994a) and mouse Clara cells (Graves et al., 1995). DNA SSBs were induced at lower concentrations in mouse hepatocytes (0.4 mM) than in rat hepatocytes (30 mM). The extent of DNA damage was reduced to the level seen in control (no exposure) conditions by pretreatment with buthionine sulfoximine to deplete cellular levels of GSH and thus inhibit dichloromethane metabolism via the GST pathway (Graves et al., 1995; Graves et al., 1994a). Similar results were seen in mouse lung Clara cells. Freshly isolated Clara cells from the lungs of $B6C3F_1$ mice also showed significantly increased, concentration-dependent amounts of DNA SSBs when incubated in vitro for 2 hours in the presence of 5–60 mM dichloromethane. Pretreatment with buthionine sulphoximine before Clara cell isolation or the presence of buthionine sulphoximine in the culture medium decreased the amount of induced in vitro DNA damage. These results are consistent with the evidence that exposure to dichloromethane results specifically in lung and liver tumors. Additionally, GST is localized in the nucleus of hepatocytes and lung cells in the mouse (Mainwaring et al., 1996), which would increase sensitivity of these particular cell fractions to genotoxic effects of dichloromethane.

In a series of experiments with freshly isolated hepatocytes from multiple species (Table 4-21), DNA-protein cross-links were detected in hepatocytes of $B6C3F_1$ mice but not in hepatocytes of F344 rats, Syrian golden hamsters, or three human subjects following 2-hour in vitro exposures to concentrations ranging from 0.5 to 5 mM dichloromethane (Casanova et al., 1997). Within the range of concentrations tested, DNA-protein cross-links in mouse hepatocytes appeared to increase with increasing concentrations of dichloromethane.

Dichloromethane-induced hypoxanthine-guanine phosphoribosyl transferase (*hprt*) gene mutations were observed in CHO cells incubated with GST-competent mouse liver cytosol preparations, and positive results were also seen for other genotoxicity indicator assays (DNA-protein cross-links and DNA SSBs) in this study (Graves and Green, 1996). Negative results for dichloromethane were generally seen in the in vitro test systems (DNA-protein cross-links, DNA SSBs, DNA and protein synthesis, sister chromatid exchanges, and unscheduled DNA synthesis) that used rat or hamster cell lines with low or no GST activity (Table 4-21) (Casanova et al., 1997; Graves et al., 1995; Thilagar et al., 1984; Andrae and Wolff, 1983; Garrett and Lewtas, 1983; Thilagar and Kumaroo, 1983; Jongen et al., 1981).

Table 4-21. Results from in vitro genotoxicity assays of dichloromethane with mammalian systems, by type of test

Assay	Test system	Concentrations	Results	Reference
Mouse				
DNA breaks by alkaline elution	Mouse hepatocytes ($B6C3F_1$)	0, 0.4, 3.0, 5.5 mM	Positive with dose-response. No toxicity at these doses as measured by trypan blue exclusion assay.	Graves et al. (1994a)
DNA SSBs by alkaline elution	Mouse Clara cells ($B6C3F_1$)	0, 5, 10, 30, 60 mM	Positive with dose-response; DNA damage reduced by addition of GSH depletor. No toxicity at these doses as measured by trypan blue exclusion assay.	Graves et al. (1995)
DNA-protein cross-links	Mouse hepatocytes ($B6C3F_1$)	0.5–5 mM	Positive	Casanova et al. (1997)
Rat				
DNA SSBs by alkaline elution	Rat hepatocytes (Alpk:APfSD [AP])	0, 30, 60, 90 mM	Positive with dose-response. Cytotoxicity at 90 mM as measured by trypan blue exclusion assay.	Graves et al. (1994a)
DNA-protein cross-links	Rat hepatocytes (Fischer-344)	0.5–5 mM	Negative	Casanova et al. (1997)
Unscheduled DNA synthesis	Rat hepatocytes	Up to 16 mM (measured)	Negative	Andrae and Wolff (1983)
Hamster with GST activity from mouse				
hprt mutation analysis	CHO cells	3,000 and 5,000 ppm	Positive with mouse liver cytosol	Graves and Green (1996)
hprt mutation analysis	CHO cells	2,500 ppm[a]	Mutation spectrum supports role of glutathione conjugate	Graves et al. (1996)
DNA SSBs and DNA-protein cross-links	CHO cells	3,000 and 5,000 ppm	Positive at concentration of 0.5% (v/v) for SSBs in presence of mouse liver cytosol, but increase in DNA-protein cross-links marginal; formaldehyde (in absence of mouse liver cytosol) was positive at 0.5 mM for both DNA SSBs and DNA-protein cross-links; CHO cell cultures were suspended	Graves and Green (1996)
Comet assay	Chinese hamster V79 lung fibroblast cells transfected with mouse GST-T1	2.5, 5, 10 mM	A significant, dose-dependent increase in DNA damage resulting from DNA-protein cross-links in V79 cells transfected with mouse GST-T1 compared to parental cells	Hu et al. (2006)
DNA-protein cross-links	Syrian golden hamster hepatocytes	0.5–5 mM	Negative	Casanova et al. (1997)
DNA-protein cross-links	CHO cells (K1)	60 mM	Positive only with mouse liver S9 added; formaldehyde positive at lower concentrations (0.5–4 mM)	Graves et al. (1994a)
Hamster without GST activity from mouse				
Chromosomal aberrations	CHO cells	Not provided	Positive, independent of rat liver S9	Thilagar and Kumaroo (1983)

Table 4-21. Results from in vitro genotoxicity assays of dichloromethane with mammalian systems, by type of test

Assay	Test system	Concentrations	Results	Reference
Forward mutation (*hgprt* locus)	Chinese hamster epithelial cells	10,000, 20,000, 30,000, 40,000 ppm	Negative	Jongen et al. (1981)
DNA SSBs by alkaline elution	Syrian golden hamster hepatocytes	0.4–90 mM	Negative. Cytotoxicity at 90 mM as measured by Trypan blue exclusion assay.	Graves et al. (1995)
Sister chromatid exchange	Chinese hamster V79 cells	10,000, 20,000, 30,000, 40,000 ppm	Weak positive with or without rat-liver microsomal system	Jongen et al. (1981)
Sister chromatid exchange	CHO cells	Not provided	Negative with or without rat liver S9	Thilagar and Kumaroo (1983)
DNA and protein synthesis	CHO cells	1,000 µg/mL	Negative	Garrett and Lewtas (1983)
Unscheduled DNA synthesis	Chinese hamster epithelial cells	5,000, 10,000, 30,000, 50,000 ppm	Negative	Jongen et al. (1981)
Calf				
DNA adducts	Calf thymus DNA	50 mM	Positive in the presence of bacterial GST DM11 and dichloromethane dehalogenase; adducts primarily formed with the guanine residues	Kayser and Vuilleumier (2001)
DNA adducts	Calf thymus DNA	Up to 60 mM	Positive in the presence of bacterial GST DM11, rat GST5-5, and human GSTT11; adducts primarily formed with the guanine residues	Marsch et al. (2004)
Human				
Micronucleus test	Human AHH-1, MCL-5, h2E1 cell lines	Up to 10 mM	Positive in MCL-5, h2E1 cell lines, increasing with increasing concentrations from 2 to 10 mM	Doherty et al. (1996)
DNA damage by comet assay	Primary human lung epithelial cells	10, 100, 1,000 µM	Weak trend, independent of GST activity (GST enzymatic activity not present in the cultured cells)	Landi et al. (2003)
DNA SSBs by alkaline elution	Human hepatocytes	5–120 mM	Negative. Cytotoxicity >90 mM as measured by Trypan blue exclusion assay.	Graves et al. (1995)
Sister chromatid exchange	Primary human peripheral blood mononuclear cells	0, 15, 30, 60, 125, 250, 500 ppm	Sister chromatid exchanges significantly increased at exposures of 60 ppm and higher, most strongly in the high GST-T1 activity group	Olvera-Bello et al. (2010)
DNA-protein cross-links	Human hepatocytes	0.5–5 mM	Negative	Casanova et al. (1997)
Unscheduled DNA synthesis	Human peripheral lymphocytes	250, 500, 1,000 ppm	Negative with or without rat liver S9	Perocco and Prodi (1981)

Table 4-21. Results from in vitro genotoxicity assays of dichloromethane with mammalian systems, by type of test

Assay	Test system	Concentrations	Results	Reference
Unscheduled DNA synthesis	Primary human fibroblast	5,000, 10,000, 30,000, 50,000 ppm	Negative	Jongen et al. (1981)

CHO = Chinese hamster ovary; *hprt* = hypoxanthine-guanine phosphoribosyl transferase
[a] Methods section described concentration as 3,000 ppm (0.3%v/v) but Table I describes it as 2,500 ppm (0.25% v/v).

The instability of the S-(chloromethyl)glutathione-DNA adducts presents considerable challenges to studies of these products (Hashmi et al., 1994). Kayser and Vuilleumier (2001), however, demonstrated the formation of DNA adducts with radiolabeled dichloromethane in calf thymus DNA in the presence of dichloromethane dehalogenase/GST purified from a bacterial source (Methylophilus sp. strain DM11) and GSH (Table 4-21). The type of adduct could not be identified because of low yield, but it was determined that guanine was more actively incorporated than cytosine, adenine, or thymine by at least twofold in the presence of GST-activated dichloromethane, indicating a base specificity for these adducts. Incubation of calf thymus DNA with formaldehyde and GSH, however, did not result in detectable DNA adduct formation. In another study, Marsch et al. (2004) further evaluated the presence of adducts in calf thymus DNA in the presence of dichloromethane and human (GST-T1), rat (GST5-5), or bacterial (DM11) GST (Marsch et al., 2004). This study found that all three enzymes yielded a similar pattern of adduct formation, forming primarily with guanine and to a lesser extent with cytosine, adenine, and thymine (two- to threefold less than guanine), consistent with the results reported by Kayser and Vuilleumier (2001). High levels of guanosine-specific adducts were also seen with S-(1-acetoxymethyl)glutathione, a compound that is structurally similar but more stable than S-(chloromethyl)glutathione (Marsch et al., 2001). These findings indicate that the S-(chloromethyl)glutathione intermediate formed by GSH conjugation has mutagenic potential and is likely responsible, at least in part, for the mutagenic response observed following dichloromethane exposure.

In studies with human cell lines or isolated cells, positive results were reported for chromosomal mutation assays (chromosomal aberrations and micronucleus test) (Olvera-Bello et al., 2010; Doherty et al., 1996), as well as for indicator assays of DNA damage (e.g., sister chromatid exchange) (Olvera-Bello et al., 2010; Thilagar et al., 1984). An increasing percentage of micronucleated cells was seen from 2 to 10 mM dichloromethane in MCL-5 and H2E1, but not in AHH-1 cell lines, with approximately a three- to fourfold increase seen above 6 mM (Doherty et al., 1996). Negative results with human cells were seen in the unscheduled DNA synthesis assays (Jongen et al., 1981; Perocco and Prodi, 1981), DNA SSBs, and DNA-protein cross-links (Casanova et al., 1997; Graves et al., 1995).

Dichloromethane-induced DNA damage (comet assay) was examined in primary cultures of human lung epithelial cells collected by brush biopsy from four healthy volunteers (Landi et al., 2003). This study was designed to assess the genotoxicity of four trihalomethanes (chloroform, bromodichloromethane, dibromochloromethane, and bromoform), with dichloromethane included for comparison because of its known activation by GST-T1. Two of the subjects were of the GST-T1^{+} genotype, and two were of the GST-T1^{-} genotype.[5] However,

[5]Landi et al. (2003) did not clearly describe their treatment of GST-T1$^{+/-}$ heterozygote genotypes; EPA considers it likely that they were included in the pool from which the GST-T1^{+} samples were drawn. In addition, there is a discrepancy in the paper regarding the coding of the GST-T1 genotypes. Samples A and C are noted to be the

the cells had been frozen, and no GST-T1 activity was detectable. Without GST activity, only a weak trend with dichloromethane is observed. The relative response pattern among the 5 compounds tested could be seen in the estimated slopes (beta coefficient for the change in tail extent moment per unit increase in μM concentration): 0.0, 0.003, 0.004, 0.006, and 0.02 for dibromochloromethane, dichloromethane, chloroform, bromoform, and bromodichloromethane, respectively (statistical significance not reported). Similar results are seen in analyses using categorical data: for dichloromethane, values of 9.4, 7.6, 12.6, and 12.9 were seen in the 0, 10, 100, and 1,000 μM groups, respectively. This pattern was similar to that seen with chloroform (9.4, 6.9, 11.4, and 12.7 in the 0, 10, 100, and 1,000 μM groups, respectively) but much weaker than the pattern for bromodichloromethane (9.4, 25.2, 28.5, and 39.1 in the 0, 10, 100, and 1,000 μM groups, respectively).[6]

Genotoxicity was examined in human peripheral blood mononuclear cells obtained from a total of 20 healthy, non-smoking, male volunteers; 4 of the 20 had low GST-T1 activity, 10 had medium GST-T1 activity, and 6 had high GST-T1 activity (Olvera-Bello et al., 2010). Mean GST-T1 activity was 0.077, 0.325, and 7.365 nmol HCOH/min/mg protein, respectively, in the low, medium, and high activity groups. The cultured cells were exposed to dichloromethane (15, 30, 60, 125, 250, or 500 ppm) for 72 hours and sister chromatid exchanges were evaluated as a measure of genotoxicity. Frequency of sister chromatid exchanges significantly increased beginning at 125 ppm. Compared with controls, the strongest increase in sister chromatid exchange was seen among the highest GST-T1 activity group, with a linear increase from a 1.5-fold increase at 30 ppm to a 2.5-fold increase at 500 ppm. The low activity group exhibited a more modest increase, up to a 1.5-fold increase at 500 ppm. Little change was seen in the medium activity group. Indices of cytoxicity (mitotic index) and cytostaticity (cell proliferation kinetics) were also examined. The mitotic index decreased significantly and in a dose-dependent manner at exposures of 60–500 ppm; this decrease was seen in each of the GST-T1 activity groups. The mitotic index was approximately 50% lower in the highest activity group compared to the medium and low activity groups at all concentrations. Similar patterns were seen with the cell proliferation kinetics measure. This study provides evidence of genotoxic and cytotoxic damage in human cells in vitro at relatively low exposure concentrations, with the strongest effects seen in samples with the highest GST-T1 activity.

Several studies have examined patterns of mutations or DNA damage with dichloromethane and formaldehyde to assess the relative role of S-(chloromethyl)glutathione and formaldehyde in the observed genotoxicity. In a study in CHO cells incubated with dichloromethane (0.3% plus mouse liver cytosol), 2.5-fold increases in DNA-protein cross-links that are indicative of formaldehyde exposure were observed, compared with a 25-fold increase

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GST-T1$^-$ samples in one part of the paper, and C and D are described as the GST-T1$^-$ samples in another part of the paper.

[6]These values are based on the mean of the GST-T1$^+$ and the GST-T1$^-$ samples from Table 1 of Landi et al. (2003).

when 1 mM formaldehyde was added directly to cultures. Both treatments induced a comparable degree of DNA SSBs (Graves and Green, 1996). In a subsequent study, Graves et al. (1996) compared the mutational spectra induced by dichloromethane to that induced by direct addition of formaldehyde or 1,2-dibromoethane (a chemical known to act through a glutathionyl conjugate metabolite) at the *hprt* locus in CHO cells. The mutations induced by dichloromethane and 1,2-dibromoethane were predominantly GC to AT transitions, while all six formaldehyde-induced mutants sequenced were single base transversions. This provided further evidence that the S-(chloromethyl)glutathione intermediate may be primarily responsible for dichloromethane genotoxicity. In contrast, Hu et al. (2006) found evidence of significant amounts of formaldehyde formation following dichloromethane exposure in the cytosol of V79 (hamster) cells transfected with the murine GST-T1 gene compared to the parent cell line. In accordance with this, they observed concentration-dependent increases in DNA-protein cross-links in the GST-T1 transfected cells using the comet assay with and without proteinase K treatment that frees DNA from cross-links and allows DNA migration. These findings are consistent with those by Casanova et al. (1997), who performed a comparison of the amounts of DNA-protein and RNA-formaldehyde cross-links formed following dichloromethane exposure in hepatocytes isolated from mice, rats, hamsters, and human GST-T1 genetic variants. DNA-protein cross-links were only observed in mouse hepatocytes, but RNA-formaldehyde cross-links were found in all species and were highest in the mouse hepatocytes (4-, 7-, and 14-fold higher than rats, humans, and hamsters, respectively). These results showed that human hepatocytes can metabolize dichloromethane to formaldehyde, resulting in RNA-formaldehyde cross-links. In addition, the results indicate that there is considerable variation among species and that the human variation in the GST-T1 gene can also affect the amount of formaldehyde produced. The authors also noted that comparing results following ectopic addition of formaldehyde directly to cells with results following dichloromethane metabolism in situ can be misleading, as the formaldehyde produced internally may reside in different locations intracellularly, potentially affecting the capability of interacting with DNA. These results show that, while most studies indicate the importance of the S-(chloromethyl)glutathione intermediate in mediating genotoxic damage following dichloromethane exposure, DNA damage resulting from formaldehyde formation should also be considered.

4.5.1.2. *In Vivo Genotoxicity Assays*

Genotoxicity findings in *Drosophila melanogaster* assays are mixed (Table 4-22). A study of gene mutation in *D. melanogaster* showed a marginal increase in sex-linked recessive deaths following oral exposure (Gocke et al., 1981). An additional feeding study (Rodriguez-Arnaiz, 1998) reported a positive response in the somatic w/w^+ assay. A third study of *D. melanogaster* (Kramers et al., 1991) found no evidence of increased sex-linked recessive death, somatic mutation, or recombination following exposure to airborne dichloromethane.

Table 4-22. Results from in vivo genotoxicity assays of dichloromethane in insects

Assay	Test system	Doses	Result	Reference
Gene mutation (sex-linked recessive lethal)	Drosophila	125, 620 mM	Positive (feeding exposure)	Gocke et al. (1981)
Gene mutation (sex-linked recessive lethal, somatic mutation and recombination)	Drosophila	6 hrs—1,850, 5,500 ppm 1 wk—2,360, 4,660 ppm 2 wks—1,370, 2,360 ppm (all approximate)	Negative (inhalation exposure)	Kramers et al. (1991)
Somatic w/w+ assay	Drosophila	50, 100, 250, 500 mM	Positive (feeding exposure)	Rodriguez-Arnaiz (1998)

Some in vivo studies investigating certain genotoxic endpoints in mice exposed to dichloromethane produced negative results (Table 4-23). Unscheduled DNA synthesis was not induced in hepatocytes from mice (and rats) after 2- or 6-hour inhalation exposures to concentrations that were carcinogenic in the NTP (1986) mouse bioassay (Trueman and Ashby, 1987) or after other exposure routes (Lefevre and Ashby, 1989). It is generally recognized that this assay is not sensitive for detecting genotoxic chemicals (Eastmond et al., 2009; Madle et al., 1994). Direct evidence of chromosomal mutation (e.g., induction of micronuclei or chromosomal aberrations) or indications that DNA damage has occurred (e.g., sister chromatid exchange) were not consistently found in bone marrow in several studies of mice after acute oral exposures (Sheldon et al., 1987) or parenteral exposures (Westbrook-Collins et al., 1990; Gocke et al., 1981). However, the lack of finding genotoxic effects in the bone marrow (a tissue with minimal GST activity) is not inconsistent with the observed tumorigenic effects in mice, which are generally localized to the liver (due to high GST activity) and the lung (due to increased availability of GST-mediated metabolism). Crebelli et al. (1999) stated that halogenated hydrocarbons (such as dichloromethane) are not very effective in inducing micronucleus formation in mouse bone marrow, and a negative bone marrow micronucleus assay should not offset the consistently positive in vitro results.

Table 4-23. Results from in vivo genotoxicity assays of dichloromethane in mice

Assay	Test system	Route and dose	Duration	Results	Reference
Kras and Hras oncogenes	Mouse liver and lung tumors ($B6C3F_1$)	0, 2,000 ppm	Up to 104 wks	No difference in mutation profile between control and dichloromethane-induced liver tumors; number of spontaneous lung tumors (n = 7) limits comparison at this site	Devereux et al. (1993)
p53 tumor suppressor gene	Mouse liver and lung tumors ($B6C3F_1$)	0, 2,000 ppm	Up to 104 wks	Loss of heterozygosity infrequently seen in liver tumors from exposed or controls; number of spontaneous lung tumors (n = 7) limits comparison at this site	Hegi et al. (1993)
Micronucleus test	Mouse bone marrow (NMRI)	425, 850, or 1,700 mg/kg	Two doses	Negative at all doses	Gocke et al. (1981)
Micronucleus test	Mouse bone marrow (C57BL/6J/A1pk)	Gavage, 1,250, 2,500, and 4,000 mg/kg	Single dose	Negative at all doses	Sheldon et al. (1987)
Micronucleus test	Mouse peripheral red blood cells ($B6C3F_1$)	Inhalation 6 hr/d, 5 d/wk, 0, 4,000, 8,000 ppm	2 wk	Positive at 4,000 and 8,000 ppm	Allen et al. (1990)
Micronucleus test	Mouse peripheral red blood cells ($B6C3F_1$)	Inhalation, 6 hr/d, 5 d/wk, 0, 2,000 ppm	12 wks	Positive at 2,000 ppm	Allen et al. (1990)
Chromosome aberrations	Mouse bone marrow (C57BL/6J)	Intraperitoneal, 100, 1,000, 1,500, 2,000 mg/kg	Single dose	Negative	Westbrook-Collins et al. (1990)
Chromosome aberrations	Mouse bone marrow ($B6C3F_1$)	Subcutaneous, 0, 2,500, 5,000 mg/kg	Single dose	Negative	Allen et al. (1990)
Chromosome aberrations	Mouse lung and bone marrow cells ($B6C3F_1$)	Inhalation, 6 hr/d, 5 d/wk, 0, 4,000, 8,000 ppm	2 wks	Increase beginning at 4,000 ppm in lung cells; increase only at 8,000 ppm in bone marrow cells	Allen et al. (1990)
DNA SSBs by alkaline elution	Mouse hepatocytes ($B6C3F_1$)	Inhalation, 2,000 and 4,000 ppm	3 or 6 hrs	Positive at 4,000 ppm at 3 and 6 hrs	Graves et al. (1994a)
DNA SSBs by alkaline elution	Mouse liver and lung homogenate ($B6C3F_1$)	Liver: inhalation, 2,000, 4,000, 6,000, 8,000 ppm Lung: inhalation, 1,000, 2,000, 4,000, 6,000 ppm	3 hrs 3 hrs	Liver: positive at 4,000–8,000 ppm Lung: positive at 2,000–4,000 ppm	Graves et al. (1995)

(Table 4-23; page 1 of 2)

Table 4-23. Results from in vivo genotoxicity assays of dichloromethane in mice

Assay	Test system	Route and dose	Duration	Results	Reference
DNA damage by comet assay	Mouse stomach, urinary bladder, kidney, brain, bone marrow (CD-1)	Gavage, 1,720 mg/kg; organs harvested at 0 (control), 3, and 24 hrs	Single dose	Negative 3 or 24 hr after dosing	Sasaki et al. (1998)
DNA damage by comet assay	Mouse liver and lung cells (CD-1)	Gavage, 1,720 mg/kg; organs harvested at 0 (control), 3, and 24 hrs	Single dose	Positive only at 24 hrs after dosing	Sasaki et al. (1998)
DNA adducts	Mouse liver and kidney cells ($B6C3F_1$)	Intraperitoneal, 5 mg/kg	Single dose	Negative	Watanabe et al. (2007)
DNA-protein cross-links	Mouse liver and lung cells ($B6C3F_1$)	Inhalation, 6 hr/d, 3 d, 4,000 ppm	3 d	Positive in mouse liver cells at 4,000 ppm; negative in mouse lung cells	Casanova et al. (1992)
DNA-protein cross-links	Mouse liver and lung cells ($B6C3F_1$)	Inhalation, 6 hr/d, 150, 500, 1,500, 3,000, 4,000 ppm	3 d	Positive in mouse liver cells at 500–4,000 ppm; negative in mouse lung cells	Casanova et al. (1996)
Sister chromatid exchange	Mouse bone marrow (C57BL/6J)	Intraperitoneal, 100, 1,000, 1,500, 2,000 mg/kg	Single dose	Negative	Westbrook-Collins et al. (1990)
Sister chromatid exchange	Mouse bone marrow ($B6C3F_1$)	Subcutaneous, 0, 2,500, 5,000 mg/kg	Single dose	Negative at all doses	Allen et al. (1990)
Sister chromatid exchange	Mouse lung cells and peripheral lymphocytes ($B6C3F_1$)	Inhalation 6 hr/d, 5 d/wk, 0, 4,000, 8,000 ppm	2 wks	Positive at 4,000 and 8,000 ppm for mouse lung cells and at 8,000 ppm for peripheral lymphocytes	Allen et al. (1990)
Sister chromatid exchange	Mouse lung cells ($B6C3F_1$)	Inhalation 6 hr/d, 5 d/wk, 0, 2,000 ppm	12 wks	Positive at 2,000 ppm	Allen et al. (1990)
DNA synthesis	Mouse liver ($B6C3F_1$)	Gavage, 1,000 mg/kg; inhalation, 4,000 ppm	Single dose; 2 hrs	Negative in both oral and inhalation studies	Lefevre and Ashby (1989)
Unscheduled DNA synthesis	Mouse hepatocytes ($B6C3F_1$)	Inhalation, 2,000 and 4,000 ppm.	2 or 6 hrs	Negative	Trueman and Ashby (1987)

(Table 4-23; page 2 of 2)

When genotoxic endpoints were examined in the cancer target tissues (liver and lung) in mice exposed to dichloromethane, positive results were consistently reported (Table 4-23). Increased chromosomal aberrations in lung and bone marrow cells and increased micronuclei in peripheral red blood cells were found in mice exposed by inhalation for 2 weeks to 8,000 ppm or for 12 weeks to 2,000 ppm (Allen et al., 1990). Under the same exposure conditions, increased sister chromatid exchanges were also found in lung cells and peripheral lymphocytes (Allen et al., 1990). DNA-protein cross-links were detected in mouse hepatocytes but not in lung cells after a 3-day inhalation exposure to 4,000 ppm (Casanova et al., 1992) and between 500 and 4,000 ppm (Casanova et al., 1996). DNA damage, detected as increased DNA SSBs, was observed in liver and lung tissue of $B6C3F_1$ mice immediately following 3-hour exposures (Graves et al., 1995). The DNA damage was not detectable 2 hours after in vivo exposure, indicating that DNA repair occurs rapidly. Pretreatment of mice with buthionine sulphoximine, a GSH depletor, caused a decrease compared to levels seen in controls in the amount of DNA damage detected immediately after in vivo exposure in liver and lung tissue, indicating GSH involvement in the genotoxic process. DNA damage (detected by the comet assay) was also reported in liver and lung tissues from male CD-1 mice sacrificed 24 hours after administration of a single oral dose of 1,720 mg/kg of dichloromethane (Sasaki et al., 1998). In this study, no DNA damage in lung or liver was detected 3 hours after dose administration, and no DNA damage occurred at either time point in several other tissues in which a carcinogenic response was absent in chronic animal cancer bioassays (e.g., stomach, kidney, bone marrow).

Formation of DNA adducts was evaluated in male and female $B6C3F_1$ mice as well as in male F344 rats (Watanabe et al., 2007). Animals were administered 5 mg/kg intraperitoneally of radiolabeled dichloromethane and sacrificed at 1 or 8 hours after administration. The kidneys and livers were removed, and the DNA was isolated from these tissues to evaluate formation of DNA adducts. At the administered dose, DNA adducts were not detected.

Devereux et al. [(1993) (also reported in Maronpot et al., (1995)] analyzed liver tumors in female $B6C3F_1$ mice for the presence of activated H-*ras* oncogenes. Fifty dichloromethane-induced and 49 spontaneous liver tumors were screened for H-*ras* mutations. There was a relatively high frequency of activated H-*ras* among the nonexposed $B6C3F_1$ mice: 67% of the spontaneous tumors and 76% of the dichloromethane-induced tumors contained mutations in the H-*ras* gene. Overall, the mutation profile of the dichloromethane-induced tumors did not significantly differ from the spontaneous tumors. Hegi et al. (1993) analyzed the liver tumors from female $B6C3F_1$ mice for inactivation of the tumor suppressor genes, *p53* and *Rb-1*. Half of the liver tumors used for this study had an activated H-*ras* oncogene. Twenty liver tumors (15 carcinomas and 5 adenomas) were screened for loss of heterozygosity (LOH) on chromosome 11 and 14, which is associated with malignant conversion of the *p53* gene (chromosome 11) and the *Rb-1* gene (chromosome 14). Only one tumor out of 20 contained a LOH at chromosome 14, and no dichloromethane-induced liver tumors contained a LOH at chromosome 11.

These studies also analyzed the mutation profiles of the lung tumors in $B6C3F_1$ mice. In the analysis of K-*ras* mutations in 54 dichloromethane-induced and 17 spontaneous lung tumors reported by Devereux et al. (1993), 20% of the dichloromethane-induced tumors and 24% of the spontaneous tumors contained mutations in the K-*ras* gene. Devereux et al. (1993) stated that there may be a significant difference in the incidence of K-*ras* activation between spontaneous and dichloromethane-induced tumors. However, the small number of the spontaneous tumors available for the study limits the conclusions that can be made from the results. In the analysis of LOH in tumor suppressor genes by Hegi et al. (1993), 14% (n = 7) of the 49 dichloromethane-induced lung carcinomas exhibited LOH at chromosome 11. No *p53* mutations were detected in the seven spontaneous lung tumors or the five dichloromethane-induced lung adenomas. Only three dichloromethane-induced tumors exhibited LOH at chromosome 14.

Results from in vivo studies in other mammals (i.e., rats and hamsters) of hepatocyte sensitivity to dichloromethane induction of DNA SSBs (Table 4-24) are consistent with interspecies differences in the induction of liver tumors in the inhalation cancer bioassays. A gavage study in rats reported the presence of DNA SSBs with a dose of 1,275 mg/kg (Kitchin and Brown, 1989). The other available studies, however, did not find any genotoxicity following dichloromethane exposure in mammals other than mice. No increase in unscheduled DNA synthesis in rat hepatocytes was seen following inhalation of dichloromethane for 2–6 hours at 2,000 or 4,000 ppm (Trueman and Ashby, 1987), exposure by gavage up to 1,000 mg/kg (Trueman and Ashby, 1987), or intraperitoneal exposure of 400 mg/kg (Mirsalis et al., 1989). DNA adducts were not detected in the livers and kidneys of male F344 rats dosed with 5 mg/kg dichloromethane intraperitoneally (Watanabe et al., 2007). DNA SSBs were significantly increased in hepatocytes isolated from $B6C3F_1$ mice exposed to 4,831 ppm (4,000 ppm nominal) for 6 hours but were not increased in hepatocytes from Sprague-Dawley rats exposed to 4,527 ppm (4,000 ppm nominal) for 6 hours (Graves et al., 1994a). Results from in vivo interspecies comparisons of dichloromethane induction of DNA-protein cross-links in hepatocytes (expected products of the GSH pathway) are also consistent with the hypothesis that the greater activity of the GST pathway in the mouse contributes to the increased sensitivity to genotoxic effects. DNA-protein cross-links were formed in the liver of mice but not hamsters following in vivo exposure to air concentrations ranging from 500 to 4,000 ppm, 6 hours/day for 3 days (Casanova et al., 1996). The absence of a genotoxic response in the rat and hamster is consistent with considerably lower GST activity in these mammalian systems, and therefore would be expected to be less sensitive at detecting genotoxic effects than the studies conducted in mice.

Table 4-25 compares results from studies of mice and rats in which comparable tissue-specific endpoints were examined. Several of the endpoints that were positive in mice (e.g., sister chromatid exchange, DNA-protein cross-links, comet assay) have not been examined in the

rat. In contrast to the positive results seen in mouse inhalation exposure studies, DNA SSB induction was not seen in rat inhalation studies but was seen in a gavage study.

In summary, the available data provide evidence for the mutagenic potential of dichloromethane. Most of the in vitro bacterial assays showed positive results when there was GST activity; nonpositive results were reported only in bacterial assays with low GST activity. Evaluation of the in vitro mammalian studies also demonstrates the influence of GST activity on the observation of genotoxic effects. In rat and hamster cell lines in which GST activity is significantly less than in mouse cells, primarily negative results were reported following dichloromethane exposure. However, when mouse liver cytosol or transfected mouse GST were included in these same cell lines, genotoxic effects were reported. In mouse cell lines, positive results were obtained in Clara cells. In vitro studies using human cells reported effects of dichloromethane on frequency of micronuclei, DNA damage (comet assay), and sister chromatid exchanges, but no effects on unscheduled DNA synthesis, DNA SSBs, or DNA-protein cross-links. The results of in vivo genotoxicity in mice also support the site-specificity of the observed tumors. With the exception of one study of unscheduled DNA synthesis in hepatocytes, numerous studies in either the liver or lung were also positive at various doses. These liver and lung studies included chromosomal aberrations, indicating mutagenic potential, as well as SSBs, sister chromatid exchanges, and DNA-protein cross-links that provide further evidence of the genotoxicity of dichloromethane and correspond to genotoxic and mutagenic effects associated with metabolites from the GST pathway.

Table 4-24. Results from in vivo genotoxicity assays of dichloromethane in rats and hamsters

Assay	Test system	Route and dose	Duration	Results	Reference
DNA SSBs by alkaline elution	Rat hepatocytes	Inhalation, 3 or 6 hrs, 2,000 and 4,000 ppm	3 or 6 hrs	Negative at all concentrations and time points	Graves et al. (1994a)
DNA SSBs by alkaline elution	Rat liver homogenate	Gavage, 2 doses, 425 mg/kg and 1,275 mg/kg, administered 4 and 21 hrs before liver harvesting	4 or 21 hrs (time between dosing and liver harvesting)	Positive at 1,275 mg/kg	Kitchin and Brown (1989)
DNA SSBs by alkaline elution	Rat liver and lung homogenate	Liver: inhalation, 4,000, 5,000 ppm Lung: inhalation, 4,000 ppm	3 hrs 3 hrs	Negative for both liver and lung at all concentrations	Graves et al. (1995)
DNA adducts	Rat liver and kidney cells	Intraperitoneal, 5 mg/kg	Single dose	Negative	Watanabe et al. (2007)
DNA-protein cross-links	Hamster liver and lung cells	Inhalation, 6 hr/d, 500, 1,500, 4,000 ppm	3 d	Negative at all concentrations	Casanova et al. (1996)
Unscheduled DNA synthesis	Rat hepatocytes	Gavage, 100, 500, 1,000 mg/kg	Liver harvested 4 and 12 hrs after dosing	Negative 4 or 12 hrs after dosing	Trueman and Ashby (1987)
Unscheduled DNA synthesis	Rat hepatocytes	Inhalation, 2 or 6 hrs, 2,000 and 4,000 ppm	2 or 6 hrs	Negative at both concentrations and exposure durations	Trueman and Ashby (1987)
Unscheduled DNA synthesis	Rat hepatocytes	Intraperitoneal, single dose, 400 mg/kg	Single dose	Negative 48 hrs after dosing	Mirsalis et al. (1989)

Table 4-25. Comparison of in vivo dichloromethane genotoxicity assays targeted to lung or liver cells, by species

Assay	Studies in B6C3F$_1$ mice				Studies in rats			
	Test system	Route, dose (duration)	Results	Reference	Test system	Route, dose (duration)	Results	Reference
Chromosome aberrations	Lung cells	Inhalation, 6 hr/d, 5 d/wk, 0, 4,000, 8,000 ppm (2 wks)	Positive at 8,000 ppm	Allen et al. (1990)				No studies
DNA SSBs by alkaline elution	Hepatocytes	Inhalation, 2,000 and 4,000 ppm (3 or 6 hrs)	Positive at 4,000 ppm	Graves et al. (1994a)	Hepatocytes	Inhalation, 3 or 6 hrs, 2,000 and 4,000 ppm	Negative at all concentrations and time points	Graves et al. (1994a)
DNA SSBs by alkaline elution	Liver and lung homogenate	Liver: inhalation, 2,000, 4,000, 6,000, 8,000 ppm (3 hrs) Lung: inhalation, 1,000, 2,000, 4,000, 6,000 ppm (3 hrs)	Liver: Positive at 4,000–8,000 ppm Lung: Positive at 2,000–4,000 ppm	Graves et al. (1995)	Liver and lung homogenate	Liver: inhalation, 4,000, 5,000 ppm Lung: inhalation, 4,000 ppm	Negative in liver and lung at all concentrations and time points	Graves et al. (1995)
DNA SSBs by alkaline elution				No studies	Liver homogenate	Gavage, 425 mg/kg and 1,275 mg/kg	Positive at 1,275 mg/kg	Kitchin and Brown (1989)
DNA damage by comet assay	Liver and lung cells	Gavage, 1,720 mg/kg; organs harvested at 0 (control), 3, and 24 hrs	Positive only at 24 hrs after dosing	Sasaki et al. (1998)				No studies
DNA-protein cross-links	Liver and lung cells	Inhalation, 6 hr/d, 3 d, 4,000 ppm (3 d) Inhalation, 6 hr/d, 150, 500, 1,500, 3,000, 4,000 ppm (3 d)	Positive in liver 4,000 ppm Positive in liver at 500–4,000 ppm; both studies negative in lung	Casanova et al. (1992)				No studies
DNA adducts	Liver and kidney cells	Intraperitoneal, 5 mg/kg	Negative	Watanabe et al. (2007)	Liver and kidney cells	Intraperitoneal, 5 mg/kg	Negative	Watanabe et al. (2007)
Sister chromatid exchange	Lung cells	Inhalation 6 hr/d, 5 d/wk, 0, 4,000, 8,000 ppm (2 wks) Inhalation 6 hr/d, 5 d/wk, 0, 2,000 ppm (12 wks)	Positive at 8,000 ppm Positive at 2,000 ppm	Allen et al. (1990)				No studies
DNA synthesis	Liver	Gavage, 1,000 mg/kg; inhalation, 4,000 ppm (2 hrs)	Negative in oral and inhalation studies	Lefevre and Ashby (1989)				No studies

(Table 4-25; page 1 of 2)

Table 4-25. Comparison of in vivo dichloromethane genotoxicity assays targeted to lung or liver cells, by species

Assay	Studies in B6C3F$_1$ mice				Studies in rats			
	Test system	Route, dose (duration)	Results	Reference	Test system	Route, dose (duration)	Results	Reference
Unscheduled DNA synthesis	Hepatocytes	Inhalation, 2,000 and 4,000 ppm (2 or 6 hrs)	Negative	Trueman and Ashby (1987)	Hepatocytes	Inhalation, 2,000 and 4,000 ppm (2 or 6 hrs)	Negative	Trueman and Ashby (1987)
Unscheduled DNA synthesis				No studies	Hepatocytes	Intraperitoneal, 400 mg/kg	Negative	Mirsalis et al. (1989)

(Table 4-25; page 2 of 2)

4.5.2. Mechanistic Studies of Liver Effects

One of the major target organs from dichloromethane exposure is the liver, and several studies have focused on examining the potential mechanisms producing liver tumors. This section summarizes the primary mechanistic studies that were conducted to examine the hepatic tumors produced by dichloromethane in mice. A parallel set of studies, discussed in the next section, focus on potential mechanisms that produce lung tumors. Briefly, dichloromethane-induced liver tumors first appeared in mice after 52 weeks of exposure (Maronpot et al., 1995; Kari et al., 1993), which was when tumors began to appear in control mice, indicating a similar time course in tumor formation between treated and untreated groups. The results of the six "stop-exposure" protocols of differing durations and sequences used in the study suggest that 52 weeks of exposure was required to increase the incidence of liver tumors in mice, that early exposure was more effective than late exposure, and that the increased risk continued after cessation of exposure. Onset of liver tumor formation is not preceded by liver cell proliferation (Casanova et al., 1996; Foley et al., 1993). A second subset of mechanistic studies was conducted to elucidate the reason that mice are the most sensitive species to liver tumors and if other species exhibited changes in liver function (Thier et al., 1993; Reitz et al., 1989a). It was found that mice have the highest level of GST-T1 catalytic activity but that humans, rats, and hamsters, among other species, also metabolize dichloromethane in the liver to a GST conjugate. In contrast, there has been little research focusing on the mechanisms through which noncancer hepatic effects (seen most strongly in the rat) are produced, and the role of the parent material, metabolites of the CYP2E1 pathway, metabolites of the GST pathway, or some combination of parent material and metabolites is not known. Summaries of these studies are provided in Appdendix E, Section E.5.

4.5.3. Mechanistic Studies of Lung Effects

The finding of increased lung tumors in $B6C3F_1$ mice exposed to dichloromethane (Mennear et al., 1988; NTP, 1986) has stimulated a number of studies designed to examine the mechanism for dichloromethane-induced lung tumors in this animal. The lung tumor mechanism studies were conducted with $B6C3F_1$ mice, and the frequency of lung tumors in control animals was very low. Time-course studies for lung tumor development were conducted, and it was found that the onset of lung tumor development was much shorter than liver tumors (Kari et al., 1993) (reported in Maronpot et al. (1995)). As a result, it is hypothesized that a potential common mechanism independent of liver metabolism is producing tumors in the lung. As with the liver tumor data from the same series of experiments, these data suggest that early exposure was more effective than late exposure and that the increased risk continued after cessation of exposure. Additionally, the Clara cells, which are nonciliary secretory cells found in the primary bronchioles of the lung, are selectively targeted after dichloromethane exposure. Acute dichloromethane exposure produces Clara cell vacuolization, which is not sustained with long-

term exposure (Foster et al., 1992). There is a correlation between the acute effects on the Clara cell and the lung tumors from chronic exposure to dichloromethane (Kari et al., 1993). However, the exact mechanism for producing these lung effects is not completely understood. Summaries of these studies are provided in Appdendix E, Section E.6.

4.5.4. Mechanistic Studies of Neurological Effects

Several neurobehavioral studies (see Section 4.4.2) have demonstrated that dichloromethane exposure results in decreased spontaneous motor activity with pronounced lethargy at high concentrations (≥1,000 ppm). These effects, combined with the observation that dichloromethane impairs learning and memory (Alexeeff and Kilgore, 1983) and affects production of evoked responses to sensory stimuli (Herr and Boyes, 1997; Rebert et al., 1989), indicate that dichloromethane produces significant neurological effects. The mechanisms behind these changes have been examined by measuring changes in neurotransmitter levels and changes in neurotransmitter localization. Specific brain regions (e.g., hippocampus, caudate nucleus, cerebellum) were analyzed to determine if dichloromethane-induced behavioral effects, such as learning and memory (hippocampus, caudate nucleus) and movement (cerebellum), are resulting from pathological changes in these regions. Changes in neurotransmitter levels were also monitored to see if there was any correlation in behavior and neurochemical changes. Summaries of these studies are provided in Appdendix E, Section E.7. It is not known if dichloromethane directly interacts with neuronal receptors, as has been demonstrated for toluene and ethanol, two other solvents with neurobehavioral and neurophysiological profiles that are similar to those of dichloromethane (for a review see Bowen et al. (2006)).

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

4.6.1.1. *Summary of Human Data*

Human studies involving oral exposure to dichloromethane are limited to case reports of neurological impairment, liver and kidney effects (as severe as organ failure), and gastrointestinal irritation in individuals who ingested amounts ranging from about 25 to 300 mL (Chang et al., 1999; Hughes and Tracey, 1993). Neurological effects with these individuals consisted of general CNS depressive symptoms, such as drowsiness, confusion, headache, and dizziness. Hemoglobinuria has been noted as a kidney effect associated with ingestions.

4.6.1.2. *Summary of Animal Data*

Acute oral or intraperitoneal administration of dichloromethane in animals has resulted in several significant effects. General activity and function were affected as evidenced by decreased neuromuscular activity (Moser et al., 1995). Additionally, decreased sensorimotor function was detected through measurement of evoked potentials (Herr and Boyes, 1997) and by

using the FOB (Moser et al., 1995). Neurochemical changes (e.g., acetylcholine, dopamine, norepinephrine, serotonin) were detected 2 hours after oral dosage of dichloromethane within specific parts of the brain. It should be noted that the acute effects observed after oral or intraperitoneal administration occurred within 5 hours after dosage. No other significant organ effects were noted after a single acute oral exposure, but in oral pharmacokinetic studies, it is known that dichloromethane is primarily distributed to the liver, lungs, and kidneys (Angelo et al., 1986a).

Results from short-term, subchronic, and chronic oral toxicity studies in laboratory animals are summarized in Table 4-26. The data indicate that rats may be more sensitive than mice to nonneoplastic liver effects from orally administered dichloromethane, as evidenced by lower NOAELs and LOAELs with more severe liver effects in rats. The most frequently observed liver effect was hepatocyte vacuolation, seen with drinking water exposure (90 days) in F344 rats at ≥166 mg/kg-day and B6C3F$_1$ mice at 586 mg/kg-day (Kirschman et al., 1986) and with gavage exposure (14 days) in CD-1 mice at 333 mg/kg-day (Condie et al., 1983). Hepatocyte degeneration or necrosis was observed in female F344 rats exposed by drinking water for 90 days to 1,469 mg/kg-day (Kirschman et al., 1986) and in female F344 rats exposed by gavage for 14 days to 337 mg/kg-day (Berman et al., 1995) but was not seen in a 90-day drinking water study in B6C3F$_1$ mice exposed to doses as high as 2,030 mg/kg-day (Kirschman et al., 1986). In the chronic-duration (2-year) study, liver effects were described as foci and areas of alteration in F344 rats exposed to drinking water doses between 50 and 250 mg/kg-day; an increased incidence of fatty changes in the liver was also noted but the incidence was not provided (Serota et al., 1986a). These effects were considered to be nonneoplastic for several reasons. Serota et al. (1986a) observed a dose-related increased incidence of 0, 65, 92, 97, 98, and 100% in male rats and 51, 41, 73, 89, 91, and 85% in female rats for the 0, 5, 50, 125, 250 mg/kg-day, and 250 mg/kg-day with recovery groups, respectively. Evidence for liver tumors has been reported in female rats only. Specifically, evidence for liver tumors in rats includes a small number of hepatocellular carcinomas observed in female rats at 50 and 250 mg/kg-day, which reached statistical significance (for trend and for individual pairwise comparisons) only with the combined grouping of neoplastic nodules and hepatocellular carcinomas. In male rats, only one hepatocellular carcinoma was observed in all of the exposure groups (compared to 4 in the controls), and the incidence of neoplastic nodules and hepatocellular carcinomas was higher in controls (16%) than in any exposure group (16, 3, 0, 6, 5, and 13% for the 0, 5, 50,125, 250 mg/kg-day, and 250 mg/kg-day with recovery groups, respectively). The authors (Serota et al., 1986a) did not elaborate on the characterization of the altered foci. However, the characterization of altered foci could range from a focal change in fat distribution (nonneoplastic effect) to enzyme altered foci which are generally considered a precursor to tumor formation (Goodman et al., 1994). As noted previously, Serota et al. (1986a) reported an increased incidence of fatty change in the liver at doses of ≥50 mg/kg-day, but the

incidence was not reported. In addition, a 90-day study (Kirschman et al., 1986) demonstrated that increased fatty deposits were present in the hepatocyte vacuoles. Therefore, the altered foci (i.e., change in fat distribution) observed by Serota et al. (1986a) may represent a precursor to fatty liver changes, which is considered a nonneoplastic effect. Taken together, the data support the conclusion that the altered foci were nonneoplastic.

The NOAEL and LOAEL, 101 and 337 mg/kg-day, respectively, for altered neurological functions in female F344 rats [as reported by Moser et al. (1995)] were identical to those reported by Berman et al. (1995) for hepatocyte necrosis in female F344 rats. In the 90-day (Kirschman et al., 1986) and 104-week (Serota et al., 1986a, b) drinking water studies, no obvious clinical signs of neurological impairment were observed in rats or mice at exposure levels that induced liver effects (see Table 4-26), but these studies did not include standardized neurological testing batteries.

Results from the available studies do not provide evidence for effects on reproductive or developmental endpoints (Table 4-26). No effects on pup survival, resorptions, or pup weight were found following exposure of pregnant F344 rats to doses as high as 450 mg/kg-day on GDs 6–19, a dose that depressed maternal weight gain (Narotsky and Kavlock, 1995), and no effects on reproductive performance endpoints (fertility index, number of pups per litter, pup survival) were found in studies in male and female Charles River CD rats (General Electric Company, 1976) or in male Swiss-Webster mice (Raje et al., 1988). There are no oral two-generation exposure studies or oral exposure studies focusing on neurobehavioral effects or other developmental outcomes.

Table 4-26. NOAELs and LOAELs in selected animal studies involving oral exposure to dichloromethane for short-term, subchronic, or chronic durations

Type of effect and exposure, reference	Species and exposure details	Results	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)
Hepatic, 14-d gavage				
Berman et al. (1995)	F344 rat, female, 8/group 0, 34, 101, 337, 1,012 mg/kg-d	Hepatocyte necrosis	101	337
Condie et al. (1983)	CD-1 mouse, male, 5/group for histological examinations; 8/group for blood urea nitrogen, serum creatinine, and serum glutamate-pyruvate transaminase; 0, 133, 333, 665 mg/kg-d	Hepatocyte vacuolation (minimal to mild in 3/5)	133	333
Hepatic, 90-d drinking water				
Kirschman et al. (1986)	F344 rat, male and female; 15/sex/group; males 0, 166, 420, 1,200 mg/kg-d females 0, 209, 607, 1,469 mg/kg-d	Hepatic vacuolation (generalized, centrilobular, or periportal, at lowest dose, in 10/15 males and 13/15 females compared with 1/15 males and 6/15 females in controls)	Not identified	166
Kirschman et al. (1986)	$B6C3F_1$ mouse, male and female, 15/sex/group males 0, 226, 587, 1,911 mg/kg-d females 0, 231, 586, 2,030 mg/kg-d	Hepatic vacuolation (increased severity of centrilobular fatty change in mid- and high-dose groups compared with controls)	226	587
Hepatic, 104-wk drinking water				
Serota et al. (1986a)	F344 rat, male and female, 85–135/sex/group 0, 5, 50, 125, 250 mg/kg-d	Liver foci/areas of alteration (considered nonneoplastic histologic changes); fatty liver changes also seen at same doses but incidence data not reported; no evidence that increased altered foci progresses to liver tumor formation	5	50
Serota et al. (1986b); Hazleton Laboratories (1983)	$B6C3F_1$ mouse, male and female, 0, 60, 125, 185, 250 mg/kg-d	Some evidence of fatty liver; marginal increase in the Oil Red-O-positive material in the liver	185	250
Neurologic, 14 d				
Moser et al. (1995)	F344 rat, female, 8/group 0, 34, 101, 337, 1,012 mg/kg-d	FOB from 4 d postexposure: altered autonomic, neuromuscular, and sensorimotor and excitability measures	101	337

(Table 4-26; page 1 of 2)

Table 4-26. NOAELs and LOAELs in selected animal studies involving oral exposure to dichloromethane for short-term, subchronic, or chronic durations

Type of effect and exposure, reference	Species and exposure details	Results	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)
Reproductive				
General Electric Company (1976)	Charles River CD rat, male and female, gavage for 90 d before mating (10 d between last exposure and mating period); 0, 25, 75, 225 mg/kg-d; F1 offspring received same treatment as parents for 90 d	Reproductive performance of F0 and histologic examination of tissues from F1 offspring	225	Not identified
Raje et al. (1988)	Swiss-Webster mouse, male, 0, 250, 500 mg/kg (subcutaneous injection), 3 × per wk, 4 wks prior to mating with nonexposed females (1 wk between last exposure and mating period)	No statistically significant effects on testes, number of litters, live fetuses/litter, percent dead fetuses/litter, percent resorbed/litter, or fertility index	500	Not identified
Developmental				
Narotsky and Kavlock (1995)	F344 rat, pregnant female, gavage on GDs 6–19; 0, 337.5, 450 mg/kg-d	Maternal: weight gain depression	338	450
		Fetal: no effects on pup survival, resorptions, pup weight	450	Not identified

4.6.2. Inhalation

4.6.2.1. *Summary of Human Data*

As discussed in Section 4.1.2, acute inhalation exposure of humans to dichloromethane has been associated with decreased oxygen availability from COHb formation and neurological impairment from interaction of dichloromethane with nervous system membranes. Results from studies of acutely exposed human subjects indicate that acute neurobehavioral deficits measured, for example, by psychomotor tasks, tests of hand-eye coordination, visual evoked response changes, and auditory vigilance, may occur at concentrations >200 ppm with 4–8 hours of exposure (Bos et al., 2006; ACGIH, 2001; ATSDR, 2000; Cherry et al., 1983; Putz et al., 1979; Gamberale et al., 1975; Winneke, 1974).

The clinical and workplace studies of noncancer health effects of chronic dichloromethane exposure have examined markers of disease and specific clinical endpoints relating to cardiac disease, neurological disease, hepatic function, and reproductive health. As summarized in Section 4.1.2.5, the limited available data do not provide evidence of cardiac damage related to dichloromethane exposure in occupationally exposed workers (Hearne and Pifer, 1999; Gibbs et al., 1996; Lanes et al., 1993; Ott et al., 1983e; Cherry et al., 1981; Tomenson, In Press). Limitations in these studies that would result in a reduced ability to detect cardiovascular effects include the presence of the healthy worker effect in these worker cohorts, and the absence of data pertaining to workers who died before the establishment of the analytic cohort (Gibbs et al., 1996; Gibbs, 1992).

Relatively little is known about the long-term neurological effects of chronic exposures, although there are studies that provide some evidence of an increased prevalence of neurological symptoms among workers with average exposures of 75–100 ppm (Cherry et al., 1981), long-term effects on some neurological measures (i.e., possible detriments in attention and reaction time in complex tasks) in workers whose past exposures were in the 100–200 ppm range (Lash et al., 1991), and an increased risk of suicide in worker cohort studies (Hearne and Pifer, 1999; Gibbs, 1992). Given the suggestions from these studies and their limitations (particularly with respect to sample size and power considerations), the statement that there are no long-term neurological effects of chronic exposures to dichloromethane cannot be made with confidence.

With respect to markers of hepatic damage, three studies measured serum enzyme and bilirubin levels in workers exposed to dichloromethane (Soden, 1993; Kolodner et al., 1990; Ott et al., 1983a). There is some evidence of increasing levels of serum bilirubin with increasing dichloromethane exposure (Kolodner et al., 1990; Ott et al., 1983a), but the patterns with respect to the other hepatic enzymes examined (serum γ-glutamyl transferase, serum AST, serum ALT) are difficult to interpret. Thus, to the extent that this damage could be detected by these serologic measures, these studies do not establish the presence of hepatic damage in dichloromethane exposed workers.

Only limited and somewhat indirect evidence pertaining to immune-related and infectious disease effects of dichloromethane in humans is available. No risk of the broad category of infection- and parasite-related mortality was reported by Hearne and Pifer (1999), but there was some evidence of an increased risk of influenza and pneumonia-related mortality at two cellulose triacetate fiber production work sites in Maryland and South Carolina (Gibbs, 1992), and an increased risk of bronchitis-related mortality based on only four exposed cases was seen in another cohort study (Radican et al., 2008).

Few studies have been conducted pertaining to reproductive effects (i.e., spontaneous abortion, low birth weight, or oligospermia) of dichloromethane exposure from workplace settings (Wells et al., 1989; Kelly, 1988; Taskinen et al., 1986) or environmental settings (Bell et al., 1991). Of these, the data pertaining to spontaneous abortion provide the strongest evidence of an adverse effect of dichloromethane exposure. The limitations of the only study pertaining to this outcome (Taskinen et al., 1986), however, do not allow firm conclusions to be made regarding dichloromethane and risk of spontaneous abortion in humans. The high exposure scenario, including the potential for substantial dermal exposure in the study of Kelly (1988), also suggests the potential for adverse male reproductive effects.

4.6.2.2. *Summary of Animal Studies*

Acute and short-term (up to 7 days) inhalational exposure to dichloromethane in animals has resulted in neurological and hepatocellular changes. Several neurological-mediated parameters were reported, including decreased activity (Kjellstrand et al., 1985; Weinstein et al., 1972; Heppel et al., 1944; Heppel and Neal, 1944), impairment of learning and memory (Alexeeff and Kilgore, 1983), and changes in responses to sensory stimuli (Rebert et al., 1989). Although learning and memory properties were impaired in one acute exposure (47,000 ppm until loss of righting reflex), it should be noted that this effect has not been characterized by using other learning and memory tasks nor any other exposure paradigms. In a 3-day exposure to dichloromethane (70, 300, or 1,000 ppm, 6 hours/day), there were changes in catecholamine (dopamine, serotonin, norepinephrine) in the rat hypothalamus and caudate nucleus (Fuxe et al., 1984). The catecholamine level changes did not affect hormonal release, which is a primary function of the hypothalamus.

Another acute exposure study examining immunological response reported an increase in Streptococcal pneumonia-related mortality and decrease in bactericidal activity of pulmonary macrophages in CD-1 mice following a single 3-hour exposure to dichloromethane at 100 ppm (Aranyi et al., 1986). No effects were seen at 50 ppm. A 4-week inhalation exposure to 5,000 ppm dichloromethane in rats did not result in changes in immune response as measured by the sheep red blood cell assay (Warbrick et al., 2003). These studies suggest a localized, portal-of-entry effect within the lung without evidence of systemic immunosuppression.

Mouse hepatocytes showed balloon degeneration (dissociation of polyribosomes and swelling of rough endoplasmic reticulum) within 12 hours of exposure to 5,000 ppm (Weinstein et al., 1972). A subacute exposure in Wistar rats to 500 ppm dichloromethane 6 hours/day for 6 days resulted in increased hemochrome content in liver microsomal CYP (Savolainen et al., 1977).

Results pertaining to liver, lung, and neurological effects from longer (>7 days) subchronic and chronic inhalation toxicity studies in laboratory animals are summarized in Table 4-27; reproductive and developmental studies are summarized in Table 4-28.

Table 4-27. NOAELs and LOAELs in animal studies involving inhalation exposure to dichloromethane for subchronic or chronic durations, hepatic, pulmonary, and neurologic effects

Type of effect, exposure period, reference	Species and exposure details	Results	NOAEL	LOAEL
			ppm	
Hepatic, subchronic (13–14 wks)				
Haun et al. (1971)	Beagle, female (n = 8) Rhesus monkeys, female (n = 4) Sprague-Dawley rat, male (n = 20) ICR mouse, female (n = 380) 0, 1,000, 5,000 ppm (continuous exposure; 14 wks)	Fatty liver at 1,000 ppm in dogs; "borderline" liver changes in monkey at 5,000 ppm; mottled liver changes in rats at 5,000 ppm; hepatocytes degeneration at 5,000 ppm in mice, no information about liver effects in mice at 1,000 ppm.	Not identified (dog) Not identified (monkey) 1,000 (rat) Not identified (Mouse)	1,000 (dog) 5,000 (monkey) 5,000 (rat) 5,000 (mouse)
Haun et al. (1972)[a]	Beagle, female (n = 16) Rhesus monkey, female (n = 4) Sprague-Dawley rat, male (n = 20) ICR mouse, female (n = 20); 0, 25, 100 ppm (continuous exposure; 14 wks)	Increased hepatic cytoplasmic vacuolation and decreased CYP levels in liver microsomes in mice at 100 ppm; increased fatty liver content at 25, 100 ppm in rats	100 (dog) 100 (monkey) Not identified (rat) 25 (mouse)	Not identified (dog) Not identified (monkey) 25 (rat) 100 (mouse)
Leuschner et al. (1984)	Sprague-Dawley rat, male and female, (20/sex/group); 0, 10,000 ppm (6 hrs/d, 90 d). Beagle, male and female (3/sex/group); 0, 5,000 ppm	No liver effects noted	10,000 (rat) 5,000 (dog)	Not identified (rat) Not identified (dog)
NTP (1986)	F344/N rat, male and female (10/sex/group); 0, 525, 1,050, 2,100, 4,200, 8,400 ppm (6 hrs/d, 5 d/wk, 13 wks)	Decreased lipid:liver weight ratios at 4,200 (females) and 8,400 (males); decreased BW by 23 and 11% in males and females at 8,400 ppm compared with controls; one male and one female died at 8,400 ppm before the end of the study	4,200	8,400
NTP (1986)	B6C3F$_1$ mouse, male and female (10/sex/group); 0, 525, 1,050, 2,100, 4,200, 8,400 ppm (6 hrs/d, 5 d/wk, 13 wks)	Hepatocyte centrilobular degeneration at 4,200 (females) and 8,400 (males); decreased lipid:liver weight ratios at 8,400 (females); at 8,400 ppm, 4/10 males and 2/10 females died before end of study	2,100	4,200
Hepatic, 2 yrs (6 hrs/d, 5 d/wk)				
Mennear et al. (1988); NTP (1986)	F344/N rat, male and female (50/sex/group); 0, 1,000, 2,000, 4,000 ppm	Hepatocyte vacuolation and necrosis; hemosiderosis in liver; renal tubular degeneration	Not identified Not identified 1,000	1,000 1,000 2,000

Table 4-27. NOAELs and LOAELs in animal studies involving inhalation exposure to dichloromethane for subchronic or chronic durations, hepatic, pulmonary, and neurologic effects

Type of effect, exposure period, reference	Species and exposure details	Results	NOAEL	LOAEL
			ppm	
Mennear et al. (1988); NTP (1986)	$B6C3F_1$ mouse, male and female (50/sex/group); 0, 2,000, 4,000 ppm	Hepatocyte degeneration; renal tubule casts	Not identified Not identified	2,000 2,000
Burek et al. (1984)	Syrian golden hamster, male and female (95/sex/group); 0, 500, 1,500, 3,500 ppm	No effects on histologic, clinical chemistry, urinalytic, and hematologic variables no obvious clinical signs of toxicity	3,500	Not identified
Burek et al. (1984)	Sprague-Dawley rat, male and female (92–97/sex/group); 0, 500, 1,500, 3,500 ppm	Hepatocyte vacuolation (males and females); hepatocyte necrosis (males only), no obvious clinical signs of toxicity)	Not identified 500	500 1,500
Nitschke et al. (1988a)	Sprague-Dawley rat, male and female (90/sex/group); 0, 50, 200, 500 ppm	Hepatocyte vacuolation significantly increased in females; nonsignificant increase in males at 500 ppm (31% in controls and 40% in 500 ppm group)	200	500
Pulmonary, 13 wks (6 hrs/d, 5 d/wk)				
NTP (1986)	F344 rat, male and female (10/sex/group); 0, 525, 1,050, 2,100, 4,200, 8,400 ppm	Foreign body pneumonia	4,200	8,400
NTP (1986)	$B6C3F_1$ mouse, male and female (10/sex/group); 0, 525, 1,050, 2,100, 4,200, 8,400 ppm	No nonneoplastic pulmonary lesions	8,400	Not identified
Foster et al. (1992)	$B6C3F_1$ mouse, male and female (5/group); 0, 4,000 ppm	Clara cell vacuolation (persistent)	4,000	Not identified
Neurological, 14 d				
Savolainen et al. (1981)	Wistar rat, male (15/group); 500, 1,000, 1,000 TWA (100 + 2,800 1-hr peaks[b]) ppm (6 hrs/d, 5 d/wk, 2 wks)	Increased RNA in cerebrum at 1,000 ppm; increased enzymatic activities[c] in cerebrum and cerebellum at 1,000 ppm TWA	500	1,000 for brain RNA concentration; 1,000 TWA for brain enzymatic activity
Neurological, 13–14 wks				
Mattsson et al. (1990)	F344 rat, male and female(12/sex/group); 0, 50, 200, 2,000 ppm (6 hrs/d, 5 d/wk)	No exposure-related effects on an observational battery, hind-limb grip strength, a battery of evoked potentials, or histology of brain, spinal cord, peripheral nerves; measured 64 hrs postexposure	2,000	Not identified

Table 4-27. NOAELs and LOAELs in animal studies involving inhalation exposure to dichloromethane for subchronic or chronic durations, hepatic, pulmonary, and neurologic effects

Type of effect, exposure period, reference	Species and exposure details	Results	NOAEL	LOAEL
			ppm	
Haun et al. (1971)	Beagle dog, female(n=8) Rhesus monkey, female(n=4) Sprague-Dawley rat, male (n=20) ICR mouse, female (n=20); 0, 1,000, 5,000 ppm (continuous exposure)	Clinical signs (incoordination, lethargy) of CNS depression most evident in dogs, monkeys, and mice	Not identified (dog) Not identified (monkey) 1,000 (rat) Not identified (mouse)	1,000 (dog) 1,000 (monkey) 5,000 (rat) 1,000 (mouse)
Karlsson et al. (1987) Briving et al. (1986) Rosengren et al. (1986)	Mongolian gerbil, male and female (10/sex/group); 210, 350, 700 ppm (continuous exposure, followed by 4 mo exposure-free period)	Astrogliosis in frontal and sensory motor cerebral cortex suggested by increases in astroglial proteins; cell loss in cerebellar regions; decreased DNA in hippocampus; neurochemical changes observed at all exposures	Not identified	210
Thomas et al. (1972)	ICR mouse, female (10/group); 0, 25, 100 ppm, continuous	Increased spontaneous activity observed at 25 ppm but not 100 ppm	Not identified	25
CoHb, 13–14 wks				
Haun et al. (1972)	Beagle (n = 16) Rhesus monkey (n = 4); 0, 25, 100 ppm (continuous exposure; 14 wks)	CoHb levels significantly higher at 25, 100 ppm for monkeys and 100 ppm for beagles	Not identified	25
COHb, 2 yrs (6 hrs/d, 5 d/wk)				
Burek et al. (1984)	Syrian golden hamster, male and female (95/sex/group); 0, 500, 1,500, 3,500 ppm	About 30% COHb in each exposed group	Not identified	500
Burek et al. (1984)	Sprague-Dawley rat, male and female (92–97/sex/group); 0, 500, 1,500, 3,500 ppm	About 12–14% COHb in each exposed group	Not identified	500
Nitschke et al. (1988a)	Sprague-Dawley rat, male and female (90/sex/group); 0, 50, 200, 500 ppm	COHb values at 2 yrs: about 2, 7, 13, 14%	Not identified	50[d]

[a] Strain and sex not specified but are assumed to be the same as indicated for Haun et al. (1971).
[b] Equivalent to 1,000 ppm TWA.
[c] Decreased GSH, γ-aminobutyric acid, and phosphoethanolamine in frontal cortex; GSH and γ-aminobutyric acid increased in posterior cerebellar vermis.
[d] Dose-related increase beginning at 50 ppm, but no duration-dependence seen in this effect.

(Table 4-27; page 3 of 3)

Table 4-28. NOAELs and LOAELs in selected animal studies involving inhalation exposure to dichloromethane, reproductive and developmental effects

Type of effect and exposure period, reference	Species and exposure details	Results	NOAEL	LOAEL
			ppm	
Reproductive				
Nitschke et al. (1988b)	F344 rat, male and female (30/sex/group) F0: 6 hr/d, 5 d/wk for 14 wk before mating and GDs 0 to 21; F1: 6 hr/d, 5 d/wk, beginning PND 4 for 17 wk before mating; 0, 100, 500, 1,500 ppm	No statistically significant effects on fertility or litter size, neonatal survival, growth rates, or histopathologic lesions in F1 or F2 weanlings	1,500	Not identified
Mennear et al. (1988); NTP (1986)	$B6C3F_1$ mouse (50/sex/group) 0, 2,000 or 4,000 ppm, 6 hrs/d, 5 d/wk for 2 yrs	Testicular atrophy; ovarian atrophy (considered secondary to hepatic effects)	2,000 Not identified	4,000 2,000
Raje et al. (1988)	Swiss-Webster mouse, male (20/group) 2 hr/d, 5 d/wk for 6 wk before mating with nonexposed females; 0, 100, 150, 200 ppm	No statistically significant effects on testes, number of litters, live fetuses/litter, percent dead fetuses/litter, percent resorbed/litter; Fertility index was lower in 150 and 200 ppm groups (80%) compared with controls and 100 ppm groups (95%) (statistical significance depends on test used)	200 100	Not identified 150
Developmental				
Schwetz et al. (1975)	Swiss-Webster mouse, pregnant female (30–40/group) 7 hr/d, GDs 6–15; 0, 1,250 ppm	Maternal effects: 9–10% COHb; increased absolute, not relative, liver weight, increased maternal weight (11–15%) Fetal effects: increased litters with extra center of ossification in sternum	Not identified Not identified	1,250 1,250
Schwetz et al. (1975)	Sprague-Dawley rat, pregnant female (20–35/group) 7 hr/d, GDs 6–15; 0, 1,250 ppm	Maternal effects: 9–10% COHb; increased absolute, not relative, liver weight Fetal effects: increased incidence of delayed ossification of sternebrae	Not identified Not identified	1,250 1,250
Other developmental				
Bornschein et al. (1980); Hardin and Manson (1980)	Long-Evans rat, female (16–20/group) 6 hr/d for 12–14 d before breeding and GDs 1–17; 6 hr/d; 0, 4,500 ppm	Maternal (both protocols) effects: increased absolute and relative liver weight (~10%); Fetal effects/offspring: decreased fetal BW (~10%); changed behavioral habituation to novel environments; no changes in gross, skeletal, or soft-tissue anomalies	Not identified Not identified	4,500 4,500

Hepatic centrilobular degeneration was observed in several studies containing different species and inhalational exposures. Monkeys, rats, and mice continuously exposed (24 hours/day) to 5,000 ppm dichloromethane for 14 weeks also had increased centrilobular degeneration (Haun et al., 1972; Haun et al., 1971). This effect was also observed at lower exposures when mice were exposed to 4,200 ppm (6 hours/day) for 13 weeks (NTP, 1986) and in dogs exposed to 1,000 ppm (24 hours/day) for up to 14 weeks (Haun et al., 1972; Haun et al., 1971).

Increased incidences of histologic hepatic lesions were not found in F344 rats exposed to 4,200 or 8,400 ppm (6 hours/day) for 13 weeks (NTP, 1986) or in Sprague-Dawley rats exposed to 10,000 ppm (6 hours/day) for 90 days (Leuschner et al., 1984). Hepatic lesions were also not observed in beagle dogs exposed to 5,000 ppm (6 hours/day) for 90 days (Leuschner et al., 1984) or in dogs, monkeys, rats, and mice exposed to 25 or 100 ppm (24 hours/day) for up to 14 weeks (Haun et al., 1972).

Gross neurological impairments were observed in several laboratory species with repeated exposure to 1,000 or 5,000 ppm, 24 hours/day for 14 weeks (Haun et al., 1972; Haun et al., 1971). Dogs exposed to 5,000 ppm, 6 hours/day for 90 days showed slight sedation during exposures, but Sprague-Dawley rats exposed to 10,000 ppm for 90 days did not (Leuschner et al., 1984). In F344 rats exposed to concentrations up to 2,000 ppm, 6 hours/day for 13 weeks, no effects were observed on an observational battery, hind-limb grip strength, a battery of evoked potentials, or histology of the brain, spinal cord, or peripheral nerves; these tests were conducted beginning ≥65 hours after the last exposure (Mattsson et al., 1990).

Exposure-related nonneoplastic effects on the lungs reported in the subchronic studies were restricted to foreign body pneumonia in rats exposed to 8,400 ppm, 6 hours/day for 13 weeks (NTP, 1986).

The chronic duration inhalation studies were conducted at lower exposure levels than the short-term and subchronic studies and provide results indicating that the liver is the most sensitive target for noncancer toxicity in rats and mice (Table 4-27). Life-time exposure was associated with hepatocyte vacuolation and necrosis in F344 rats exposed to 1,000 ppm for 6 hours/day (Mennear et al., 1988; NTP, 1986), hepatocyte vacuolation in Sprague-Dawley rats exposed to 500 ppm for 6 hours/day (Nitschke et al., 1988a; Burek et al., 1984), and hepatocyte degeneration in $B6C3F_1$ mice exposed to 2,000 ppm for 6 hours/day (lower concentrations were not tested in mice) (Mennear et al., 1988; NTP, 1986). As shown in Tables 4-27 and 4-28, other effects observed include renal tubular degenerations in F344 rats and $B6C3F_1$ mice at 2,000 ppm, testicular atrophy in $B6C3F_1$ mice at 4,000 ppm, and ovarian atrophy in $B6C3F_1$ mice at 2,000 ppm (considered secondary to hepatic effects). No exposure-related increased incidences of nonneoplastic lung lesions were found in any of the chronic studies (Table 4-27).

In comparison to rats and mice, Syrian golden hamsters are less sensitive to the chronic inhalation toxicity of dichloromethane. No exposure-related changes were found in

comprehensive sets of histologic, clinical chemistry, urinalytic, and hematologic variables measured in hamsters exposed for 2 years to 500, 1,500, or 3,500 ppm for 6 hours/day, with the exception that mean COHb percentages were about 30% in each of these groups compared with a mean value of about 3% for the controls (Burek et al., 1984).

The reproductive and developmental studies are limited in terms of the exposure regimen used. Nitschke et al. (1988b) used a noncontinuous exposure period (i.e., exposure of dams before mating and on GDs 0–21, and beginning again on PND 4), and two of the developmental studies using only a single, relatively high daily exposure over the gestational period [1,250 ppm, GDs 6–15 in Schwetz et al. (1975) and 4,500 ppm, GDs 1–17 in Bornschein et al. (1980) and Hardin and Manson (1980)]. No significant effects on reproductive performance variables were found in a two-generation reproduction assay with F344 rats exposed to concentrations as high as 1,500 ppm (Nitschke et al., 1988b). No effects on most of the measures of reproductive performance were observed in male mice exposed to 200 ppm, 2 hours/day for 6 weeks before mating to nonexposed females. In the study by Raje et al. (1988), fertility index was reduced in the 150 and 200 ppm groups, but the statistical significance of this effect varied considerably depending on the statistical test used in this analysis. No adverse effects on fetal development were found following exposure of pregnant Swiss-Webster mice or Sprague-Dawley rats to 1,250 ppm for 6 hours/day on GDs 6–15 (Schwetz et al., 1975). Following exposure of female Long-Evans rats to 4,500 ppm (6 hours/day) for 14 days before breeding, plus during gestation or during gestation alone, a 10% decrease in fetal BW and changed behavioral habituation of the offspring to novel environments were seen (Bornschein et al., 1980; Hardin and Manson, 1980). No exposure-related changes in gross, skeletal, or soft-tissue anomalies were found.

4.6.3. Mode-of-Action Information

4.6.3.1. *Mode of Action for Nonneoplastic Liver Effects*

Studies of chronically exposed rats, both by the oral and inhalation routes, identified liver changes as the most sensitive exposure-related noncancer effect associated with exposure to dichloromethane (Tables 4-26 to 4-28). The liver changes included increased incidence of liver foci/areas of alteration and hepatocyte vacuolation in rats and degenerative liver effects in rats, guinea pigs, monkeys, and mice.

The mode of action by which dichloromethane induces these nonneoplastic hepatic effects is not known. The determination of whether these effects are due to the parent material, metabolites of the CYP2E1 pathway, metabolites of the GST pathway, or some combination of parent material and metabolites has not been elucidated. The available data indicate that rats may be more sensitive than mice to the noncancer hepatotoxicity, but a mechanistic explanation of this possible interspecies difference is not currently available.

4.6.3.2. *Mode of Action for Nonneoplastic Lung Effects*

Single 6-hour inhalation exposures to concentrations ≥2,000 ppm dichloromethane produced a transient vacuolation of Clara cells in the bronchiolar epithelium of $B6C3F_1$ mice. Vacuolization of the Clara cells disappeared or was diminished with repeated exposure and was correlated with subsequent transient diminishment of CYP metabolic activity. CYP inhibition with piperonyl butoxide counteracted the vacuolation observed in the Clara cells (Foster et al., 1994; Foster et al., 1992). With repeated exposure to 4,000 ppm (up to 13 weeks), the Clara cell vacuolation did not appear to progress to necrosis, and no hyperplasia of the bronchiolar epithelium was found. Foster et al. (1994; 1992) proposed that the diminished severity or disappearance of Clara cell vacuolation with repeated exposure was due to the development of tolerance to dichloromethane, linked with a transient decrease of CYP metabolism of dichloromethane. The available data suggests that CYP metabolism of dichloromethane may be involved in the mode of action for the acute effects of dichloromethane on the bronchiolar epithelium of mice.

Nonneoplastic lung effects have received relatively little attention with respect to mode-of-action research. No exposure-related increased incidences of nonneoplastic lung lesions (including epithelial hyperplasia) were found in any of the chronic studies listed in Table 4-27. In a study that included interim sacrifices at 13, 26, 52, 68, 75, 78, 83, and 91 weeks of $B6C3F_1$ mice exposed to 2,000 ppm, hyperplasia of lung epithelium (the only nonneoplastic lung lesion found) was found in only three of the eight interim sacrifices (68, 78, and 91 weeks) and was only statistically significantly elevated at 91 weeks (5/30 versus 0/15 in controls) (Kari et al., 1993).

4.6.3.3. *Mode of Action for Neurological Effects*

Results from studies of acutely exposed human subjects indicate that mild neurobehavioral deficits may occur at air concentrations >200 ppm with 4–8 hours of exposure (Bos et al., 2006; ACGIH, 2001; ATSDR, 2000; Cherry et al., 1983; Putz et al., 1979; Gamberale et al., 1975; Winneke, 1974), or at lower exposures for longer durations (Cherry et al., 1981). Acute high-dose exposures also resulted in gross neurological impairments in several laboratory species (Haun et al., 1972; Haun et al., 1971; Heppel et al., 1944; Heppel and Neal, 1944). Exposure of F344 rats to concentrations up to 2,000 ppm, 6 hours/day for 13 weeks produced no effects on an observational battery, hind-limb grip strength, a battery of evoked potentials, or histology of the brain, spinal cord, or peripheral nerves (Mattsson et al., 1990). However, oral exposures have been shown to alter autonomic, neuromuscular, and sensorimotor functions in F344 rats exposed to gavage doses ≥337 mg/kg-day for 14 days (Moser et al., 1995).

Dichloromethane may be metabolized by the CYP2E1 enzyme to CO (Guengerich, 1997; Hashmi et al., 1994; Gargas et al., 1986). Many of the acute human exposure studies evaluated whether CO was the primary metabolite responsible for producing the CNS depressant effects

observed during dichloromethane exposure. Overall, at lower exposures and acute durations, it appears that CO may be a major mediator of the neurobehavioral effects, although unmetabolized dichloromethane may also contribute to these effects. Putz et al. (1979) demonstrated that similar neurobehavioral deficits were present in humans when an equivalent COHb blood level (and CO exposure) was achieved between CO and dichloromethane exposures; however, after a longer duration, neurobehavioral deficits are more pronounced with dichloromethane exposure in comparison to CO exposure alone. This additional increase in the CNS depressive effects is most likely due to the saturation of the CYP2E1 metabolic pathway. CYP2E1 pathway saturation with dichloromethane has also been noted in hamsters (Burek et al., 1984) and rats (Nitschke et al., 1988a; McKenna et al., 1982). It is probable that CYP2E1 is initially metabolizing dichloromethane to CO, either of which can result in neurological effects. At higher concentrations and for longer durations, however, the CYP2E1 pathway is most likely saturated. The resulting effects would thus be due to either the remaining dichloromethane compound or to metabolites generated through the GST pathway.

Neurological changes associated with COHb levels have primarily represented neurobehavioral endpoints. Neurophysiological and neurochemical changes could also be related to dichloromethane, CO, or potentially other metabolites. Based on the available literature on other solvents, such as toluene and perchloroethylene [for a review see Bowen et al. (2006)], it can be hypothesized that dichloromethane or a metabolite may interact directly with excitatory and inhibitory receptors such as the NMDA, GABA, dopamine, and serotonin receptors, among other targets, to produce the resulting neurobehavioral effects. This hypothesis is supported by the evidence that changes in relation to dichloromethane exposures in glutamate, GABA, dopamine, serotonin, acetylcholine, and other neurotransmitters are found in the brain (Kanada et al., 1994; Briving et al., 1986; Fuxe et al., 1984). Additionally, several neurobehavioral effects such as decreased spontaneous motor activity, deficits in learning and memory, and deficits in FOB parameters are similar to other more characterized solvents such as toluene. However, more comprehensive studies specifically designed to determine the mode of action for dichloromethane-induced impairment of neurological functions have not been conducted.

4.6.3.4. *Mode of Action for Neurodevelopmental Effects*

The mode of action for neurodevelopmental effects observed in Bornschein et al. (1980) and Hardin and Manson (1980) can be hypothesized to involve dichloromethane or the production of CO from the CYP2E1 pathway. The placental transfer of dichloromethane has been demonstrated with inhalation exposure (Withey and Karpinski, 1985; Anders and Sunram, 1982). CO is a known developmental neurotoxicant. Demonstrated effects include neurobehavioral deficits and neurochemical changes (Giustino et al., 1999; Cagiano et al., 1998; De Salvia et al., 1995; Fechter, 1987). In humans, CYP2E1 activity in the brain occurs earlier in

gestation than it does in the liver, with activity in the brain seen in the first trimester (Johnsrud et al., 2003; Brzezinski et al., 1999). Thus, the direct effects of dichloromethane in fetal circulation, as well as the effects of CO and the effects of the CYP2E1-related metabolism in the fetal liver and the fetal brain, may be relevant to the risk of developmental effects in humans.

4.6.3.5. *Mode of Action for Immunotoxicity*

Evidence of a localized immunosuppressive effect in the lung resulting from inhalation dichloromethane exposure was seen in an acute exposure (3 hours, 100 ppm) study in CD-1 mice (Aranyi et al., 1986). The lung infectivity assay used in this study examined response to bacterial challenges (i.e., risk of Streptococcal-pneumonia-related mortality and clearance of Klebsiella bacteria). The innate immune response plays an important role in limiting the initial lung burden of bacteria through the activity of macrophages, neutrophils, and dendritic cells, and alveolar macrophages are particularly important in the response to respiratory infections (Marriott and Dockrell, 2007). The adaptive response develops from several days up to several weeks following infection so that an effective immune response in a lung infectivity assay requires multiple immune mechanisms and, in particular, cooperation of macrophages, neutrophils, and T cells along with the appropriate cytokines (Selgrade and Gilmour, 2006). Although immunosuppression in the Streptococcal and Klebsiella infectivity models has been reported in the acute exposure scenarios tested in Aranyi et al. (1986), mechanistic studies of dichloromethane or its metabolites that would provide mode of action information on the immune system cells or function have not been performed.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight-of-Evidence

Following U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, dichloromethane is "likely to be carcinogenic in humans," based predominantly on evidence of carcinogenicity at two sites in 2-year bioassays in male and female $B6C3F_1$ mice (liver and lung tumors) with inhalation exposure (NTP, 1986) and at one site in male $B6C3F_1$ mice (liver tumors) with drinking water exposure (Serota et al., 1986b; Hazleton Laboratories, 1983). The incidence rates for liver tumors in female mice in the drinking water exposure study were not presented (Serota et al., 1986b; Hazleton Laboratories, 1983), but it was reported that exposed female mice did not show increased incidences of proliferative hepatocellular lesions. Additional evidence of the tumorigenic potential of dichloromethane in rats comes from the observation of an increase in benign mammary tumors following inhalation exposure in rats (Nitschke et al., 1988a; NTP, 1986; Burek et al., 1984). A gavage study in female Sprague-Dawley rats reported an increased incidence of malignant mammary tumors, mainly adenocarcinomas (8, 6, and 18% in the control, 100, and 500 mg/kg dose groups, respectively), but the increase was not statistically significant; data were not provided to allow an analysis that

accounts for differing mortality rates (Maltoni et al., 1988). An inhalation study (exposures of 0, 50, 200, and 500 ppm) also reported the presence of another relatively rare tumor in rats, astrocytoma or glioma (mixed glial cell) tumors (Nitschke et al., 1988a). Taken together, the rat data provide supporting evidence of carcinogenicity. Studies in humans also observed evidence linking occupational exposure to dichloromethane and increased risk for some specific cancers, including brain cancer (Cocco et al., 1999; Hearne and Pifer, 1999; Heineman et al., 1994; Tomenson, In Press), liver and biliary tract cancer (Lanes et al., 1993; Lanes et al., 1990), non-Hodgkin lymphoma (Barry et al., 2011; Wang et al., 2009; Seidler et al., 2007; Miligi et al., 2006), and multiple myeloma (Gold et al., 2010).

The proposed mode of action for dichloromethane-induced tumors is through a mutagenic mode of carcinogenic action (discussed in more detail in Section 4.7.3). In brief, mode-of-action data indicate that dichloromethane-induced DNA damage in cancer target tissues of mice (i.e., liver and lung) involves DNA-reactive metabolites produced via a metabolic pathway catalyzed by GST-T1. Evidence of mutagenicity includes in vitro bacterial assays in several strains (Demarini et al., 1997; Pegram et al., 1997; Oda et al., 1996; Thier et al., 1993; Dillon et al., 1992), and in vitro mutagenicity tests in mammalian systems, including the *hprt* gene mutation assay in CHO cells with added GST activity (Graves et al., 1996) and the micronucleus test in human MCL-5 and h2E1 cell lines (Doherty et al., 1996). In in vivo studies using mouse red blood cells, the micronucleus test and assays for chromosome aberrations were also positive at inhalation doses consistent with the doses inducing mouse tumors (Allen et al., 1990). Additional in vivo evidence of genotoxicity as evidenced by sister chromatid exchanges and DNA damage (comet assay) is also seen in mouse liver and lung cells (Sasaki et al., 1998; Graves et al., 1995; Graves et al., 1994a; Casanova et al., 1992; Allen et al., 1990), although a dichloromethane distinctive mutational spectrum in critical genes (Kras, Hras, p53) leading to tumor initiation and tumor promotion has not been established (Devereux et al., 1993; Hegi et al., 1993). The GST-T1 metabolic pathway is found in human tissues, albeit at lower activities than in mouse tissues; therefore, the cancer results in animals are considered relevant to humans.

EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing toxicokinetic data that absorption does not occur by other routes. For dichloromethane, systemic tumors were observed in mice following inhalation and oral exposure. No animal cancer bioassay data following dermal exposure to dichloromethane are available. Based on the observance of systemic tumors following oral exposure and inhalation exposure, and in the absence of information to indicate otherwise, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, dichloromethane is "likely to be carcinogenic to humans" by all routes of exposure.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

Section 4.1.3 reviewed the results, strengths, and limitations of epidemiological research of dichloromethane and cancer, including cohort and case-control studies. (These studies are described in more detail in Appendix D). The available epidemiologic studies provide evidence of an association between dichloromethane and brain cancer, liver and biliary tract cancer, and some hematopoietic cancers (specifically non-Hodgkin lymphoma and multiple myeloma).

Two small cohort studies with relatively good exposure metrics and relatively long follow-up periods (mean over 25 years) reported an increased risk of brain cancer, with SMRs of 1.83 (95% CI 0.79–3.60) in Tomenson et al. and 2.2 (95% CI 0.79–4.69) in Cohort 1 of Hearne and Pifer (1999). Cohort 1 is an inception cohort, following workers from the beginning of employment, and thus is methodologically more robust than Cohort 2 of Hearne and Pifer (1999), which only included workers who were working between 1964 and 1970. In Hearne and Pifer (1999) and in Tomenson et al. , an increasing risk was seen with cumulative exposure in the middle exposure groups (e.g., 400 to 800 ppm-years), with a decrease in risk above 800 ppm-years; the small number of observations and resulting imprecision in relative risk estimates makes it difficult to interpret these patterns. These observations are supported by the data from a case-control study of brain cancer using lifetime job history data that reported relatively strong trends ($p < 0.05$) with increasing probability, duration, and intensity measures of exposure but not with a cumulative exposure measure (Heineman et al., 1994). This difference in results among different exposure measures could reflect a relatively more valid measure of relevant exposures in the brain from the intensity measure, as suggested by the study in rats reported by Savolainen et al. (1981) in which dichloromethane levels in the brain were much higher with a higher intensity exposure scenario compared with a constant exposure period with an equivalent TWA (see Section 3.2). The combination of high intensity of exposure and long (>20 years) duration of employment in exposed jobs was strongly associated with brain cancer risk (OR 6.1, 95% CI 1.5–28.3) in the Heineman et al. (1994) study; similar associations were seen with the measure combining high probability with long duration. In a case-control study of female brain cancer cases, Cocco et al. (1999), using more limited occupation data obtained from death certificates, observed a weak overall association with dichloromethane exposure, and no trends with probability or intensity. A statistically significant increased incidence of brain or CNS tumors has not been observed in any of the animal cancer bioassays, but a 2-year study using relatively low exposure levels (0, 50, 200, and 500 ppm) in Sprague-Dawley rats observed a total of six astrocytoma or glioma (mixed glial cell) tumors in the exposed groups (in females, the incidence was 0, 0, 0, and 2 in the 0, 50, 200, and 500 ppm exposure groups, respectively; in males, the incidence was 0, 1, 2, and 1 in the 0, 50, 200, and 500 ppm exposure groups, respectively; sample size of each group was 70 rats). These tumors are exceedingly rare in rats, and there are few examples of statistically significant trends in animal bioassays (Sills et al.,

1999). These cancers were not seen in two other studies in rats, both involving higher doses (1,000–4,000 ppm) (NTP, 1986; Burek et al., 1984), or in a high dose (2,000–4,000 ppm) study in mice (NTP, 1986).

With respect to epidemiologic studies of liver and biliary duct cancer, the highest exposure cohort, based in the Rock Hill, South Carolina, triacetate fiber production plant, suggested an increased risk of liver and biliary tract cancer with an SMR of 2.98 (95% CI 0.81–7.63) in the latest study update (Lanes et al., 1993). This observation was based on four cases (three of which were biliary tract cancers); an earlier analysis in this cohort reported an SMR of 5.75 (95% CI 1.82–13.8), based on these same four cases but with a shorter follow-up period (and thus a lower number of expected cases) (Lanes et al., 1990). The authors estimated a total of 0.15 expected cases of biliary tract cancer in the first of the follow-up studies (Lanes et al., 1990); this subset of cancers may represent a particularly relevant form of cancer with respect to dichloromethane exposure based on localization of GST-T1 in the nuclei of bile duct epithelial cells seen in human samples (Sherratt et al., 2002). No other cohort study has reported an increased risk of liver cancer mortality, although it should be noted that there is no other inception cohort study of a population with exposure levels similar to those of the Rock Hill plant, and no data from a case-control study of liver cancer are available pertaining to dichloromethane exposure.

The primary limitation of all of the available dichloromethane cohort studies is the limited statistical power for the estimation of effects relating to relatively rare cancers (such as brain cancer, liver cancer, and leukemia). Limitations with respect to studies of other cancers can also be noted. With respect to breast cancer, the only cohort that included a significant percentage of women had limited exposure information (analysis was based on a dichotomous exposure variable) and exposures to other solvents that also exhibited associations of similar magnitude to that seen with dichloromethane (Radican et al., 2008). Thus, in this situation, potential confounding by these other exposures should be considered. The only breast cancer case-control study available used death certificate data to classify disease and occupational exposure (Cantor et al., 1995), which is likely to result in significant misclassification; exposure misclassification in particular would be expected to result in an attenuated measure of association (Rothman and Greenland, 1998). The available epidemiologic studies do not provide a definitive evaluation of non-Hodgkin lymphoma, but the consistent observations of associations seen in three large case-control studies in Germany (Seidler et al., 2007), Italy (Miligi et al., 2006), and the United States (Barry et al., 2011; Wang et al., 2009) provide evidence of an increased risk of specific types of hematopoietic cancers in humans. These studies are limited by relatively small number of exposed cases, resulting in imprecise effect estimates.

In addition to the epidemiologic studies, several dichloromethane cancer bioassays in animals are available. In the only oral exposure cancer bioassay involving lifetime exposure,

increases in incidence of liver adenomas and carcinomas were observed in male but not female $B6C3F_1$ mice exposed for 2 years (Table 4-29 for males; female data not presented in the summary reports) (Serota et al., 1986b; Hazleton Laboratories, 1983). The authors concluded that these increases were "within the normal fluctuation of this type of tumor incidence," noting that there was no dose-related trend and that there were no significant differences comparing the individual dose groups with the combined control group.[7] Serota et al. (1986b) state that a two-tailed significance level of $p = 0.05$ was used for all tests, but that statement does not appear to accurately represent the p-value used in their statistical analysis. Each of the p-values for the comparison of the 125, 185, and 250 mg/kg-day dose groups with the controls was $p < 0.05$. (These p-values were found in the full report of this study, see Hazleton Laboratories (1983), but were not included in the Serota et al. (1986b) publication.) Hazleton Laboratories (1983) indicated that a correction factor for multiple comparisons was used specifically for the liver cancer data, reducing the nominal p-value to 0.0125 rather than the value of 0.05 that was reported to be used by Serota et al. (1986b); none of these individual group comparisons are statistically significant when a p-value of 0.0125 is used. EPA did not consider the use of a multiple comparisons correction factor for the evaluation of the liver tumor data (a primary a priori hypothesis) to be warranted. Thus, based on the Hazleton Laboratories (1983) statistical analysis, EPA concluded that dichloromethane induced a carcinogenic response in male $B6C3F_1$ mice as evidenced by a marginally increased trend test ($p = 0.058$) for combined hepatocellular adenomas and carcinomas, and by small but statistically significant ($p < 0.05$) increases in hepatocellular adenomas and carcinomas at dose levels of 125 ($p = 0.021$), 185 ($p = 0.019$), and 250 mg/kg-day ($p = 0.036$).

With respect to comparisons with historical controls, the incidence in the control groups was almost identical to the mean seen in the historical controls from this laboratory (17.8% based on 354 male $B6C3F_1$ mice), so there is no indication that the observed trend is being driven by an artificially low rate in controls and no indication that the experimental conditions resulted in a systematic increase in the incidence of hepatocellular adenomas and carcinomas. Although the occurrence of one elevated rate in an exposed group may be within the normal fluctuations of this type of tumor incidence (described for this laboratory as 5–40%, with a mean of 17.8%, based on 354 male controls), the pattern of incidence rates (increased incidence in all four dose groups, with three of these increases significant at a p-value < 0.05) suggest a treatment-related increase.

[7]Two control groups were used because of the potential for high and erratic liver tumor incidence in $B6C3F_1$ mice. The incidence of hepatocellular adenomas or carcinomas was 18 and 20% in the two control groups, and the combined group is used for the subsequent analysis because of the improved statistical precision of estimates based on the larger sample size (n = 125 compared with n = 60 and 65 for the individual control groups).

Table 4-29. Incidence of liver tumors in male B6C3F$_1$ mice exposed to dichloromethane in a 2-year oral exposure (drinking water) study[a]

Estimated mean intake (mg/kg-d)[a]	0 (Controls)	61	124	177	234	Trend p-value[c]
Number of male mice	125[b]	200	100	99	125	
	Number of cancers (%)					
Hepatocellular adenoma or carcinoma						
Mortality-adjusted percent[d]	24 (19)	51 (26)	30 (30)	31 (31)	35 (28)	0.058
Mortality-adjusted p-value[d]	(22)	(29) $p = 0.071$	(34) $p = 0.023$	(35) $p = 0.019$	(32) $p = 0.036$	

[a]Target doses were 60, 125, 185, and 250 mg/kg-d from the lowest dose group (excluding controls) to the highest dose group, respectively.
[b]Two control groups combined (n = 60 and 65 in the individual groups). The mortality-adjusted incidence in control groups 1 and 2 were 20 and 23%, respectively. Two additional sets of analyses using the individual control groups were also presented in Hazleton Laboratories (1983).
[c]Cochran-Armitage trend test (Hazleton Laboratories, 1983).
[d]Mortality-adjusted percent calculated based on number at risk, using Kaplan-Meier estimation, taking into account mortality losses; p-value for comparison with control group using asymptotic normal test [source: Hazleton Laboratories (1983)].

Sources: Serota et al. (1986b); Hazleton Laboratories (1983).

In a similar study in F344 rats (Serota et al., 1986a), no increased incidence of liver tumors was seen in male rats, and the pattern in female rats was characterized by a jagged stepped pattern of increasing incidence of hepatocellular carcinoma or neoplastic nodule (Table 4-30). Information was not provided which would allow characterization of the nodules as benign or malignant. Statistically significant increases in incidences were observed in the 50 and 250 mg/kg-day groups (incidence rates of 0, 3, 10, 3, and 14%, respectively, for the 0, 5, 50, 125, and 250 mg/kg-day groups) and in the group exposed to 250 mg/kg-day for 78 weeks followed by a 26-week period of no exposure (incidence rate 10%). A similar pattern, but with more sparse data, was seen for hepatocellular carcinomas, with two incidences in the 50 mg/kg-day and two in the 250 mg/kg-day groups. The authors concluded that dichloromethane exposure did not result in an increased incidence of liver tumors because the increase was based on a low rate (0%) in the controls and because of a lack of monotonicity. EPA agrees with this evaluation of the data.

Gavage exposure studies in Sprague-Dawley rats and in Swiss mice provide limited data concerning cancer incidence because the study was terminated early (at 64 weeks) due to high treatment-related mortality (Maltoni et al., 1988). Exposure groups included controls (olive oil), 100, or 500 mg/kg-day, 4–5 days/week. High-dose female rats showed an increased incidence of malignant mammary tumors, mainly adenocarcinomas (8, 6, and 18% in the control, 100, and 500 mg/kg dose groups, respectively), but the increase was not statistically significant. Data were not provided to allow an analysis accounting for differing mortality rates. A dose-related increase, although not statistically significant, in pulmonary adenomas was observed in male

mice (5, 12, and 18% in control, 100, and 500 mg/kg-day groups, respectively). When mortality was taken into account, high-dose male mice that died in the period ranging from 52 to 78 weeks were reported to show a statistically significantly ($p < 0.05$) elevated incidence for pulmonary tumors (1/14, 4/21, and 7/24 in control, 100, and 500 mg/kg-day groups, respectively). Details of this analysis were not provided. EPA applied a Fisher's exact test to these incidences and determined a p-value of 0.11 for the comparison of the 500 mg/kg-day group (7/24) to the controls (1/14).

Table 4-30. Incidences of liver tumors in male and female F344 rats exposed to dichloromethane in drinking water for 2 years

	Target dose (mg/kg-d)						
	0[a] (Controls)	5	50	125	250	Trend p-value[b]	250 with recovery[c]
Males							
Estimated mean intake (mg/kg-d)	0	6	52	125	235		232
Total n	135	85	85	85	85		25
n at terminal kill[d]	76	34	38	35	41		17
Number (%) with neoplastic nodules	9 (12)	1 (3)	0 (0)	2 (6)	1 (2)	Not reported	2 (13)
Number (%) with hepatocellular carcinoma	3 (4)	0 (0)	0 (0)	0 (0)	1 (2)	Not reported	0 (0)
Number (%) with neoplastic nodules and hepatocellular carcinoma	12 (16)	1 (3)	0 (0)	2 (6)	2 (5)	Not reported	2 (13)
Females							
Estimated mean intake (mg/kg-d)	0	6	58	136	263		269
Total n	135	85	85	85	85		25
n at terminal kill[d]	67	29	41	38	34		20
Number (%) with neoplastic nodules	0 (0)	1 (3)	2 (5)	1 (3)	3 (9)		2 (10)[e]
Number (%) with hepatocellular carcinoma	0 (0)	0 (0)	2 (5)	0 (0)	2 (6)	Not reported	0 (0)
Number (%) with neoplastic nodules and hepatocellular carcinoma	0 (0)	1 (3)	4 (10)[e]	1 (3)	5 (14)[e]	$p < 0.01$	2 (10)[e]

[a]Two control groups combined.
[b]Cochran-Armitage trend test was used for trend test of liver foci/areas of alteration. For tumor mortality-unadjusted analyses, a Cochran-Armitage trend test was used, and for tumor mortality-adjusted analyses, a tumor prevalence analytic method by Dinse and Lagakos (1982) was used. Similar results were seen in these two analyses.
[c]Recovery group was exposed for 78 weeks and then had a 26-week period without dichloromethane exposure; n = 17 for neoplastic lesions.
[d]Excludes 5, 10, and 20 per group sacrificed at 25, 52, and 78 weeks, respectively, and unscheduled deaths, which ranged from 5 to 19 per group.
[e]Significantly ($p < 0.05$) different from controls with Fisher's exact test, mortality-unadjusted and mortality-adjusted analyses.

Source: Serota et al. (1986a).

As discussed in Section 4.2, inhalation exposure to concentrations of 2,000 or 4,000 ppm dichloromethane produced increased incidences of lung and liver tumors in B6C3F$_1$ mice (Mennear et al., 1988; NTP, 1986). The incidence of mortality-adjusted liver tumors across dose groups of 0, 2,000, and 4,000 ppm increased from 48 to 67 and 93%, respectively, in male mice (trend p-value = 0.013) and from 10 to 48 and 100% in female mice (trend p-values < 0.001) (Table 4-31). For lung tumors, the mortality-adjusted incidence was 12, 74, and 100% in males and 11, 83, and 100% in females in the 0, 2,000, and 4,000 ppm groups, respectively (trend p-values < 0.001). Elevated incidences of lung and liver tumors in B6C3F$_1$ mice were observed with 52 weeks of exposure to 2,000 ppm, and lung tumors were also elevated after only 26 weeks of exposure to 2,000 ppm (Maronpot et al., 1995; Kari et al., 1993).

Table 4-31. Incidences of selected neoplastic lesions in B6C3F$_1$ mice exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Sex and neoplastic lesion	Exposure (ppm)[a]									Trend p-value[d]
	0 (Controls)			2,000			4,000			
	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	
Males										
Liver—hepatocellular adenoma or carcinoma	22	(44)	(48)	24	(49)	(67)	33[e]	(67)	(93)	0.013
Lung—bronchoalveolar adenoma or carcinoma	5	(10)	(12)	27[e]	(54)	(74)	40[e]	(80)	(100)	< 0.001
Females										
Liver— hepatocellular adenoma or carcinoma	3	(6)	(10)	16[e]	(33)	(48)	40[e]	(83)	(100)	< 0.001
Lung—bronchoalveolar adenoma or carcinoma	3	(6)	(11)	30[e]	(63)	(83)	41[e]	(85)	(100)	< 0.001

[a]2,000 ppm = 6,947 mg/m^3, 4,000 ppm = 13,894 mg/m^3.
[b]Total sample size was 50 per sex and dose group. Percentages based on the number of tissues examined microscopically per group; for male mice, 49 livers were examined in the 2,000 and 4,000 ppm groups; for female mice, 48 liver and lungs were examined. For comparison, incidences in historical controls reported in NTP (1986) were 28% for male liver tumors, 31% for male lung tumors, 5% for female liver tumors, and 10% for female lung tumors.
[c]Mortality-adjusted percentage.
[d]Life-table trend test, as reported by NTP (1986).
[e]Life-table test comparison dose group with control < 0.05, as reported by NTP (1986).

Sources: Mennear et al. (1988); NTP (1986).

A moderate trend of increasing incidence of neoplastic nodules or hepatocellular carcinoma was seen in female F344 rats (trend p-value = 0.08) but not males in the NTP (1986) study (Table 4-32). The nodules were not characterized as benign or malignant and there was no evidence of an increasing trend in incidence when hepatocellular carcinomas only were considered.

Table 4-32. Incidences of selected neoplastic lesions in F344/N rats exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Sex and neoplastic lesion	Exposure (ppm)[a]												Trend *p*-value[d]
	0 (Controls)			1,000			2,000			4,000			
	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	n	(%)[b]	(%)[c]	
Males													
Liver—Neoplastic nodule or hepatocellular carcinoma	2	(4)	(6)	2	(4)	(9)	4	(8)	(19)	1	(2)	(6)	0.43
Lung—bronchoalveolar adenoma or carcinoma	1	(2)	–	1	(2)	–	2	(4)	–	1	(2)		–
Mammary gland													
Adenoma, adenocarcinoma, or carcinoma	0	(0)		0	(0)		0	(0)		1	(2)		
Subcutaneous tissue fibroma or sarcoma	1	(2)	(6)	1	(2)	(6)	2	(4)	(9)	5	(10)	(23)	0.008
Fibroadenoma	0	(0)	(0)	0	(0)	(0)	2	(4)	(12)	1	(2)	(8)	< 0.001
Mammary gland or subcutaneous tissue adenoma, fibroadenoma, or fibroma	1	(2)	(6)	1	(2)	(6)	4	(8)	(21)	9[d]	(18)	(49)	< 0.001
Females													
Liver—neoplastic nodule or hepatocellular carcinoma	2	(4)	(7)	1	(2)	(2)	4	(8)	(14)	5	(10)	(20)	0.08
Lung—bronchoalveolar adenoma or carcinoma	1	(2)	–	1	(2)	–	0	(0)	–	0	(0)		–
Mammary gland													
Adenocarcinoma or carcinoma	1	(2)	–	2	(4)	–	2	(4)	–	0	(0)	–	–
Adenoma, adenocarcinoma, or carcinoma	1	(2)	–	2	(4)	–	2	(4)	–	1	(2)	–	–
Fibroadenoma	5	(10)	(16)	11[d]	(22)	(41)	13[d]	(26)	(44)	22[d]	(44)	(79)	< 0.001
Mammary gland adenoma, fibroadenoma, or adenocarcinoma	6	(12)	(18)	13	(26)	(44)	14[d]	(28)	(45)	23[e]	(46)	(86)	< 0.001

[a]1,000 ppm = 3,474 mg/m^3, 2,000 ppm = 6,947 mg/m^3, 4,000 ppm = 13,894 mg/m^3.
[b]Total sample size was 50 per sex and dose group. Percentages based on the number of tissues examined microscopically per group (varying between 49 and 50); for comparison, incidence in historical controls reported in NTP (1986) were 1% for female liver tumors and 16% for female mammary fibroadenomas.
[c]Mortality-adjusted percentage.
[d]Life-table trend test, as reported by NTP (1986)
[e]Life-table test comparison dose group with control < 0.05, as reported by NTP (1986).

NR = not reported

Sources: Mennear et al. (1988); NTP (1986).

Female F344 rats exposed by inhalation to 2,000 or 4,000 ppm showed significantly increased incidences of benign mammary tumors (adenomas or fibroadenomas) (Table 4-32); the number of benign mammary tumors per animal also increased with dichloromethane exposure in studies in Sprague-Dawley rats at levels of 50–500 ppm (Nitschke et al., 1988a) and 500–3,500 ppm (Burek et al., 1984) (Table 4-33). Male rats in two of these studies (Nitschke et al., 1988a; NTP, 1986) also exhibited a low rate of sarcoma or fibrosarcoma in mammary gland or subcutaneous tissue around the mammary gland.

In Syrian golden hamsters exposed to 500, 1,500, or 3,500 ppm for 2 years, no statistically significantly increased incidences of tumors were found in any tissues (Burek et al., 1984).

Supporting evidence for the carcinogenicity of dichloromethane comes from the results of genotoxicity and mode of action studies discussed in Section 4.5 and summarized below in Section 4.7.3. Since there are limited data on mutagenic events following oral exposure, EPA conducted a pharmacokinetic analysis to evaluate how comparable the internal doses to the liver in the oral bioassay (Serota et al., 1986b; Hazleton Laboratories, 1983) were to the internal doses to the liver in the inhalation bioassay (Mennear et al., 1988; NTP, 1986). The PBPK model of Marino et al. (2006) predicted that the average daily amount of dichloromethane metabolized via GST/L liver tissue was about 14-fold lower in mice exposed to the highest dose of 234 mg/kg-day in the drinking water bioassay than in mice exposed to the lowest inhalation exposure of 2,000 ppm inducing liver tumors (Table 4-34). Thus, the lower incidence of liver tumors induced by oral doses of 234 mg/kg-day compared with the higher incidence induced by inhalation exposure to 2,000 ppm is consistent with the predicted lower liver dose of GST metabolites (and hence lower probability of DNA modification) with oral exposure.

Table 4-33. Incidences of mammary gland tumors in two studies of male and female Sprague-Dawley rats exposed to dichloromethane by inhalation (6 hours/day, 5 days/week) for 2 years

Study, lesion	0 (Controls)	Exposure (ppm)[a] 50	200	500	Late 500[b]	Early 500[b]	1,500	3,500
Nitschke et al. (1988a)								
Males—n per group	57	65	59	64	[c]	[c]	[c]	[c]
Number (%) with:								
Mammary gland tumors								
Adenocarcinoma or carcinoma	0 (0)	0 (0)	0 (0)	0 (0)				
Fibroadenoma	2 (4)	0 (0)	2 (3)	2 (3)				
Fibroma	6 (11)	1 (6)	6 (11)	10 (16)				
Fibrosarcoma	0 (0)	1 (6)	1 (6)	0 (0)				
Undifferentiated sarcoma	0 (0)	2 (4)	0 (0)	0 (0)				
Fibroma, fibrosarcoma, or undifferentiated sarcoma[d]	6 (11)	4 (6)	7 (12)	10 (16)				
Females—n per group	69	69	69	69	25	25	[c]	[c]
Number (%) with:								
Mammary gland tumors								
Adenocarcinoma or carcinoma	6 (9)	5 (7)	4 (6)	4 (6)	3 (12)	2 (8)		
Adenoma	1 (1)	1 (1)	2 (3)	1 (1)	2 (8)	0 (0)		
Fibroadenoma	51 (74)	57 (83)	60 (87)	55 (80)	22 (88)	23 (92)		
Fibroma	0 (0)	1 (1)	0 (0)	1 (1)	1 (4)	1 (1)		
Fibrosarcoma	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
Number with benign tumors[e]	52 (74)	58 (84)	61(87)[f]	55 (79)	23 (92)	23 (92)		
Number of benign tumors per tumor-bearing rat[e]	2.0	2.3	2.2	2.7	2.2	2.6		
Burek et al. (1984)								
Males—n per group	92	[c]	[c]	95	[c]	[c]	96	97
Number (%) with benign tumors	7 (8)			3 (3)			7 (7)	14 (14)
Total number of benign tumors	8			6			11	17
Number of tumors per tumor-bearing rat[g]	1.1			2.0			1.6	1.2
Females—n per group	96	[c]	[c]	95	[c]	[c]	96	97
Number (%) with benign tumors	79 (82)			81 (85)			80 (83)	83 (86)
Total number of benign tumors	165			218			245	287
Number of tumors per tumor-bearing rat[g]	2.1			2.7			3.1	3.5

[a]50 ppm = 174 mg/m^3, 200 ppm = 695 mg/m^3, 500 ppm = 1,737 mg/m^3, 1,500 ppm = 5,210 mg/m^3, 3,500 ppm = 12,158 mg/m^3.
[b]Late 500 = no exposure for first 12 month followed by 500 ppm for last 12 month; early 500 = 500 ppm for first 12 month followed by no exposure for last 12 month.
[c]No data for this exposure level in this study.
[d]EPA summed across these tumor types, assuming no overlap.
[e] n per group for this analysis was 70 for the 0, 50, 200 and 500 ppm groups. historical controls, percent with benign tumors reported was 79–82% and number per tumor-bearing rat was 2.1.
[f]Significantly ($p \leq 0.05$) higher than control incidence by Fisher's exact test (Nitschke et al., 1988a).
[g]Calculated by EPA.

Sources: Nitschke et al. (1988a); Burek et al. (1984).

Table 4-34. Comparison of internal dose metrics in inhalation and oral exposure scenarios in male mice and rats

External dose	Internal exposure in liver (mg metabolized through GST pathway/L liver tissue/d)[a]	
	Male	
	Mouse	Rat
Inhalation (ppm)		
2,000	2,364	1,509
4,000	4,972	3,124
Oral (mg/kg-d)[b]		
61	17.5	77.1
124	63.3	233.6
177	112.0	385.7
234	169.5	559.0

[a]Mouse values derived by EPA from the PBPK model of Marino et al. (2006); rat values derived from EPA based on the modified PBPK model of Andersen et al. (1991) (see Appendix C for model details).
[b]Actual doses administered to mice (Serota et al., 1986a); BWs not given for males and females, so simulation results only provided for one gender.

While the amount metabolized by the GST pathway for inhalation exposure shown in Table 4-34 is lower in the rat than the mouse (as one would expect based on the enzyme expression level) for oral exposure, a higher amount of GST metabolism is predicted for the rat than the mouse. This difference occurs because, for oral exposure, 100% of the dose is absorbed, rather than limited by metabolism as it is for inhalation, and because the ratio of GST to CYP activity is higher in the rat than in the mouse. Specifically k_{fC}/Vmaxc is 0.626 for the rat and 0.152 for the mouse. Accordingly, in the rat, the fraction of the absorbed dose going to GST is approximately four times that in the mouse. Thus, for the same oral dose per kg BW per day (with 100% absorbed), approximately four times more dichloromethane is metabolized by GST in the rat than in the mouse.

Another interspecies difference is the localization of GST-T1 in the nuclei of hepatocytes and bile duct epithelial cells in the mouse, while the rat liver does not show preferential nuclear localization of GST-T1. In human liver tissue, some hepatocytes show nuclear localization of GST-T1 and others show localization in cytoplasm, as well as in nuclei of bile duct epithelial cells (Sherratt et al., 2002; Mainwaring et al., 1996).

Carcinogenicity information that could decrease support for selecting a cancer descriptor for dichloromethane of "likely to be carcinogenic to humans" was also considered. This information includes the high incidence of spontaneous liver tumors in male $B6C3F_1$ and the relevance of these tumors to humans, and the greater GST metabolic activity in the mouse that could result in greater susceptibility of the mouse to dichloromethane-induced hepatocarcinogenicity than humans. Other differences between mice and humans include a

higher alveolar ventilation rate, cardiac output, and dichloromethane blood:air partition coefficient in the mouse that would lead to a greater systemic absorption of inhaled dichloromethane, and thus higher internal doses in mice compared with rats and in rats compared with humans. A higher prevalence of Clara cells, which contain relatively high levels of CYP2E1 and GST-T1, is also seen the bronchioles of the mouse compared with rats and humans.

Male $B6C3F_1$ mice are relatively susceptible to liver tumors and mouse liver tumors can occur with a relatively high background. For these reasons, use of mouse liver tumor data in risk assessment has been a subject of controversy (King-Herbert and Thayer, 2006). With respect to dichloromethane, increased incidence of liver tumors was also seen in female as well as in male $B6C3F_1$ mice in the NTP inhalation study (Mennear et al., 1988; NTP, 1986). The background rate of these tumors in females is low. In the absence of mode-of-action data or other information that establishes lack of human relevance, EPA considers mouse liver tumors (as well as other rodent tumors) to be relevant to humans. Interspecies differences in the metabolic activity of GST (which is relatively higher in the mouse compared with humans) do not indicate a lack of human relevance; the PBPK modeling accounts for interspecies differences in the amount of the relevant metabolite(s) formed (which is relatively higher in the mouse). Similarly, the net difference in total lung CYP activity (versus liver) in humans and rats versus mice, which is due at least in part to differences in Clara cell prevalence, is accounted for by the PBPK model. The role of Clara cells per se in the development of mouse lung tumors is not known and reliably quantifying or predicting the micro-dosimetry in and around individual Clara cells is beyond the current state of knowledge.

Other issues that could influence the selection of the cancer descriptor, that is the interpretation of the chronic oral exposure study (Hazleton Laboratories, 1983; Serota et al., 1986a) and the interpretation of the epidemiological studies, have been discussed previously in this section. In brief, EPA considers it reasonable to conclude, based on the Hazleton Laboratories (1983) statistical analysis, that dichloromethane induced a carcinogenic response in male B6C3F1 mice as evidenced by a marginally increased trend test ($p = 0.058$) for combined hepatocellular adenomas and carcinomas, and by small but statistically significant ($p < 0.05$) increases in hepatocellular adenomas and carcinomas at the three highest dose levels in the study (i.e, $p = 0.021$ at 125 mg/kg-day, $p = 0.019$ at 185 mg/kg-day, and $p = 0.036$ at 250 mg/kg-day). The epidemiological data do not provide a picture of "negative" data, but rather indicate specific types of cancers with suggestive evidence, most notably liver (and biliary) cancer, brain cancer, and specific types of hematopoietic cancers.

4.7.3. Mode-of-Action Information

4.7.3.1. *Hypothesized Mode of Action*

The hypothesized mode of action for dichloromethane-induced lung and liver tumors is through a mutagenic mode of carcinogenic action. Key events within this mode of action are (1)

dichloromethane metabolized via GST metabolism, with increasing metabolism through this pathway as exposure levels increase above the saturation of CYP2E1; (2) reaction of GST-pathway metabolites with DNA, leading to (3) mutations in critical genes resulting in tumor initiation; and (4) tumor growth promoted by unidentified molecular or cellular events. Metabolism by CYP2E1, which is more predominant at lower exposures (or tissue concentrations) than metabolism by GST, is considered a protective mechanism against the formation of putatively carcinogenic metabolites from the GST pathway.

Much of the experimental mode of action research has focused on the liver and lung, the sites of tumor formation in chronic bioassays in mice (Mennear et al., 1988; NTP, 1986; Serota et al., 1986b; Hazleton Laboratories, 1983). The studies examining measures of chromosomal instability (e.g., *hprt* mutations, micronucleus tests, chromosomal aberrations) and the positive indicator assays of DNA damage in the database of available studies of dichloromethane, specifically in in vivo studies focusing on tissues that are the site of carcinogenic response, support the conclusion that a mutagenic mode of action is operative in dichloromethane-induced tumors. The mode of action is potentially relevant to other sites, particularly those in which GST-T1 is expressed (i.e., GSH conjugation), such as mammary tissue (Lehmann and Wagner, 2008) and the brain (Juronen et al., 1996) in humans. While the extent of GSH conjugation in these other tissues may not be significant to the overall dosimetry of dichloromethane, it can be significant in understanding the sites of action of dichloromethane and differences in tumor sites observed between species. The role of specific mutations in mouse or human cancers has not been established.

4.7.3.1.1. ***Experimental support for the hypothesized mode of action.*** Support for the first of the key events in the hypothesized mutagenic mode of action, the importance of GST metabolism, can be found in several types of studies (i.e., in vitro, in vivo using different system assays). Enhanced dichloromethane mutagenicity in bacterial and mammalian (i.e., CHO) in vitro assays with the introduction of GST metabolic capacity has been observed in numerous studies (summarized in Section 4.5.1.1 and Tables 4-20 and 4-21). In bacterial strains where GST activity was not present (e.g., TA1535, TA1538), mutagenic effects were not reported following dichloromethane exposure (Oda et al., 1996; Simula et al., 1993; Osterman-Golkar et al., 1983; Gocke et al., 1981); other studies demonstrated that the mutagenicity of dichloromethane is enhanced in the presence of GSH (Demarini et al., 1997; Graves et al., 1996; Graves and Green, 1996; Graves et al., 1995; Graves et al., 1994a; Thier et al., 1993). In an in vivo genotoxicity study examining liver and lung tissue in $B6C3F_1$ mice following acute inhalation exposure to 4,000 ppm dichloromethane, the formation of DNA SSBs was suppressed to the levels seen in controls when the mice were pretreated with a GSH depletor (Graves et al., 1995); Graves et al., 1994b), providing additional support for the involvement of GST metabolism.

The second key event involves the reaction of GST-pathway metabolites with DNA. GST-mediated metabolites of dichloromethane include S-(chloromethyl)glutathione and formaldehyde hydrate; the high lability of S-(chloromethyl)glutathione makes it difficult to detect in experimental conditions (Hashmi et al., 1994). DNA adducts have been observed in in vitro studies in which calf thymus DNA was incubated with dichloromethane and GST or with S-(1-acetoxymethyl)glutathione, a compound structurally similar to S-(chloromethyl)glutathione (Marsch et al., 2004; Kayser and Vuilleumier, 2001). These findings indicate that the S-(chloromethyl)glutathione intermediate formed by GSH conjugation is likely responsible, at least in part, for the mutagenic response observed following dichloromethane exposure. However, other studies (Hu et al., 2006; Casanova et al., 1996) provide evidence of formaldehyde-related DNA-protein cross-links in relation to dichloromethane exposure, suggesting that DNA damage resulting from formaldehyde formation should also be considered. DNA adducts produced by GST metabolites, (S-(chloromethyl)glutathione or formaldehyde), have not been identified in in vivo studies (Watanabe et al., 2007); the authors speculated that these results are due to the instability of the reaction products.

Several other lines of evidence support the ability of GST-mediated metabolites of dichloromethane to interact with DNA resulting in chromosomal instability (Table 4-35). In vivo chromosomal aberration and micronucleus assays in the $B6C3F_1$ mouse lung (a site of tumor response in this species) were predominantly positive (Allen et al, 1990), with dose-response patterns seen in the two-week inhalation exposure studies examining a range of doses (Allen et al., 1990). These observations occurred in the absence of evidence of cytotoxicity, as measured by mitotic index. Similar results were seen in these in vivo studies using peripheral red blood cells (Allen et al., 1990), but not in the studies of the bone marrow, which were predominately negative (Allen et al., 1990; Westbrook-Collins et al., 1990; Sheldon et al., 1987; Gocke et al., 1981). This difference likely reflects the extent of GST-mediated metabolism and subsequent generation of reactive metabolites at these various tissue sites, as noted by Crebelli et al. (1999). The liver is the other site of tumor response, but this tissue has not been examined in in vivo chromosomal instability assays in the mouse. The in vitro tests of chromosomal instability in other types of cells also indicates the influence of GST-T1 metabolism; the only negative study in this set of studies (Doherty et al., 1996; Graves et al., 1996; Graves and Green, 1996; Thilagar and Kumaroo, 1983; Jongen et al., 1981) was the study by (Jongen et al., 1981) using Chinese hamster epithelial cells without any GST activation.

Table 4-35. Results from dichloromethane chromosomal instability assays (in vivo and in vitro), by species

Test system, assay	Route, exposure, duration[a]	Results	Reference
In vivo, mouse ($B6C3F_1$ unless otherwise noted)			
Chromosome aberrations	Inhalation, 0, 4,000, 8,000 ppm, 2 wk	+, dose-response in lung cells	Allen et al. (1990)
Chromosome aberrations	Inhalation, 0, 4,000, 8,000 ppm, 2 wks	+ at 8,000 ppm in bone marrow cells	Allen et al. (1990)
Chromosome aberrations	Subcutaneous, 0, 2,500, 5,000 mg/kg, single dose	– in bone marrow cells	Allen et al. (1990)
Chromosome aberrations	Intraperitoneal, 100, 1,000, 1,500, 2,000 mg/kg, single dose	– in bone marrow cells (C57BL/6J/A1pk)	Westbrook-Collins et al. (1990)
Micronucleus test	Inhalation 0, 2,000 ppm, 12 wks	– in lung cells	Allen et al. (1990)
Micronucleus test	Inhalation 0, 4,000, 8,000 ppm, 2 wk	+, dose-response in peripheral red blood cells	Allen et al. (1990)
Micronucleus test	Inhalation, 0, 2,000 ppm, 12 wks	+ in peripheral red blood cells	Allen et al. (1990)
Micronucleus test	Intraperitoneal, 425, 850, or 1,700 mg/kg, two doses	– in bone marrow (NMRI)	Gocke et al. (1981)
Micronucleus test	Gavage, 1,250, 2,500, 4,000 mg/kg, single dose	– in bone marrow (C57BL/6J/A1pk)	Sheldon et al. (1987)
In vitro, other species			
hprt mutation analysis, CHO cells with mouse liver cytosol (20% S100)	3,000 ppm[b]	+ (mutation spectrum supports role of glutathione conjugate)	Graves and Green (1996); Graves et al. (1996)
Chromosomal aberrations, CHO cells with and without rat liver cytosol	0, 2, 5, 10 ppm	+, dose-response in both protocols ; stronger response with presence of rat liver cytosol	Thilagar and Kumaroo (1983)
Micronucleus test, human cell lines	0, 1.0, 2.5, 5.0, 10 mM	+, dose-response in MCL-5 and h2E1 (but not AHH-1)	Doherty et al. (1996)
Forward mutation, Chinese hamster V79 lung cells	10,000, 20,000, 30,000, 40,000 ppm	– (hgprt locus)	Jongen et al. (1981)

[a] In vivo inhalation studies are 6 hr/d, 5 d/wk unless otherwise noted
[b] Graves et al. (1996) is an extension of the Graves and Green (1996) study, in which 3,000 ppm was used; Graves et al. (1996) note in the methods section that 3,000 ppm was used but in Table 1 the dose is given as 2,500 ppm.

The chromosomal mutation results described above are further supported by the in vivo and in vitro data from indicator assays of DNA damage. Within this set of assays, negative results in studies of unscheduled DNA synthesis are given comparatively less weight because of the relative insensitivity of this assay, and thus a negative result cannot be interpreted with confidence as indicating a lack of response (Kirkland and Speit, 2008; Parton and Yount, 1995). In the in vivo studies (Table 4-36), DNA damage is observed in all of the studies of liver cells with two exceptions: 1) one very low exposure (single dose, 5 mg/kg) study in which inconsistent results were seen in the duplicate assays conducted (Watanabe et al., 2007) and 2) the studies using DNA synthesis and unscheduled DNA synthesis (Lefevre and Ashby, 1989;

Trueman and Ashby, 1987). In vivo studies of mouse lung cells reported positive results for the comet assay, DNA SSBs by alkaline elution and sister chromatid exchange (Sasaki et al., 1998; Graves et al., 1995; Allen et al., 1990), but not for DNA-protein cross-links (Casanova et al., 1996; 1992). The type of tissue specificity that was seen with the chromosomal instability studies was also seen with the mouse in vivo indicator assays, with predominately negative results at sites other than lung or liver, and in the rodents other than mice (Table 4-36).

Table 4-36. Results from dichloromethane in vivo DNA damage indicator assays, by species and tissue

Test system, assay	Route, dose, duration[a]	Results	Reference
Mouse liver and lung ($B6C3F_1$ unless otherwise noted)			
Comet assay	Gavage, 1,720 mg/kg, single dose; organs harvested at 0, 3, 24 hrs	+ at 24 hrs in liver cells + at 24 hrs in lung cells (CD-1 mice)	Sasaki et al. (1998)
DNA adducts	Intraperitoneal, 5 mg/kg, single dose (low exposure study)	± in liver and/or kidney cells[b]	Watanabe et al. (2007)
DNA-protein cross-links	Inhalation, 150, 500, 1,500, 3,000, 4,000 ppm, 3 d	+, dose-response in liver cells (beginning at 500 ppm); – in lung cells	Casanova et al. (1996) (1992)
DNA SSBs by alkaline elution	Inhalation, 2,000, 4,000 ppm, 3 or 6 hrs	+ at 4,000 ppm in hepatocytes	Graves et al. (1994a)
DNA SSBs by alkaline elution	Liver: inhalation, 2,000, 4,000, 6,000, 8,000 ppm, 3 hrs Lung: inhalation, 1,000, 2,000, 4,000, 6,000 ppm, 3hrs	+, dose-response beginning at 4,000 ppm in liver cells; + beginning at 2,000 ppm in lung cells	Graves et al. (1995)
Sister chromatid exchange	Inhalation 0, 4,000, 8,000 ppm, 2 wks	+, dose-response in lung cells	Allen et al. (1990)
Sister chromatid exchange	Inhalation 0, 2,000 ppm, 12 wks	+ in lung cells	Allen et al. (1990)
DNA synthesis	Gavage, 1,000 mg/kg, single dose; inhalation, 4,000 ppm, 2 hrs	–, both studies, in liver cells	Lefevre and Ashby (1989)
Unscheduled DNA synthesis	Inhalation, 2,000, 4,000 ppm, 2 or 6 hrs	– in liver cells	Trueman and Ashby (1987)
Mouse other tissues ($B6C3F_1$ unless otherwise noted)			
Comet assay	Gavage, 1,720 mg/kg; organs harvested at 0, 3, and 24 hrs, single dose	– in stomach, bladder, kidney, brain, bone marrow (CD-1)	Sasaki et al. (1998)
Sister chromatid exchange	Inhalation 0, 4,000, 8,000 ppm, 2 wks	+ at 8,000 ppm for peripheral lymphocytes	Allen et al. (1990)
Sister chromatid exchange	Intraperitoneal, 100, 1,000, 1,500, 2,000 mg/kg, single dose	– in bone marrow cells (C57BL/6J/A1pk)	Westbrook-Collins et al. (1990)
Sister chromatid exchange	Subcutaneous, 0, 2,500, 5,000 mg/kg, single dose	– in bone marrow cells	Allen et al. (1990)

Table 4-36. Results from dichloromethane in vivo DNA damage indicator assays, by species and tissue

Test system, assay	Route, dose, duration[a]	Results	Reference
Rat or hamster			
DNA adducts	Intraperitoneal, 5 mg/kg, single dose (low exposure study)	– in rat liver and kidney cells	Watanabe et al. (2007)
DNA-protein cross-links	Inhalation, 6 hr/d, 500, 1,500, 4,000 ppm, 3 d	– in hamster liver and lung cells	Casanova et al. (1996) (1992)
DNA SSBs by alkaline elution	Gavage, 39, 425, 1,275 mg/kg, 4 and 21 hrs before harvesting	+ at 1,275 mg/kg in rat liver homogenate	Kitchin and Brown (1989)
DNA SSBs by alkaline elution	Inhalation, 2,000 and 4,000 ppm, 3 or 6 hrs	– at all concentrations and time points in rat hepatocytes	Graves et al. (1994a)
DNA SSBs by alkaline elution	Liver: inhalation, 4,000, 5,000 ppm, 3 hrs Lung: inhalation, 4,000 ppm, 3 hrs	– for both liver and lung homogenates	Graves et al. (1995)
Unscheduled DNA synthesis	Gavage, 100, 500, 1,000 mg/kg, single dose	– 4 or 12 hrs after dosing in rat hepatocytes (in medium with serum)	Trueman and Ashby (1987)
Unscheduled DNA synthesis	Inhalation, 2,000 and 4,000 ppm, 2 or 6 hrs	– in rat hepatocytes (in medium with serum)	Trueman and Ashby (1987)
Unscheduled DNA synthesis	Intraperitoneal, 10, 50, 200, 400 mg/kg, single dose	– 48 hrs after dosing in rat hepatocytes (in medium with serum)	Mirsalis et al. (1989)

[a] In vivo inhalation studies are 6 hr/d, 5 d/wk unless otherwise noted
[b] Reported as positive in 3 or 4 animals in one assay but not duplicated in second assay; unclear whether results pertained to only liver, only kidney, or both tissues.

As with the other sets of studies, the in vitro DNA damage indicator studies report generally positive results in mouse liver and lung cells and in cells from other species with GST-T1 activation, and generally negative studies in cells from other species with low levels of GST-mediated metabolic activity relative to the mouse (e.g., rat, hamster) (Table 4-37).

With respect to the third key event in the hypothesized mutagenic mode of action, mutagenic data in critical genes leading to the initiation of dichloromethane-induced tumors are not available. In in vivo assays of mutations in tumor suppressor genes and oncogenes, similar frequencies of activated H-*ras* genes and inactivation of the tumor suppressor genes, *p53* and *Rb-1*, in the liver tumors were seen in nonexposed and dichloromethane-exposed $B6C3F_1$ mice (Devereux et al., 1993; Hegi et al., 1993). There were too few lung tumors (n = 7) in controls to support the comparison of mutation patterns between exposed and nonexposed tumors.

Table 4-37. Results from dichloromethane in vitro DNA damage indicator assays, by species and tissue

Test system, assay	Administered dose	Results	Reference
Mouse, liver or lung (high GST metabolism)			
DNA-protein cross-links	0.5–5 mM	+, dose-response in liver cells	Casanova et al. (1997)
DNA SSBs by alkaline elution	0, 0.4, 3.0, 5.5 mM	+, dose-response with plateau in liver cells	Graves et al. (1994a)
DNA SSBs by alkaline elution	0, 5, 10, 30, 60 mM	+, dose-response in lung Clara cells; damage reduced with GSH depletion	Graves et al. (1995)
Other species with mouse liver cytosol or other GST-T1 activation			
DNA adducts	50 mM	+ in calf thymus DNA with bacterial GST DM11	Kayser and Vuilleumier (2001)
DNA adducts	5 to 60 mM	+ in calf thymus DNA with bacterial GST DM11, rat GST5-5, and human GST-T1	Marsch et al. (2004)
DNA-protein cross-links	3,000 ppm	Marginal increase in CHO cells with mouse liver cytosol	Graves and Green (1996)
DNA-protein cross-links	60 mM	+ in CHO cells with mouse liver cytosol	Graves et al. (1994a)
DNA-protein cross-links	2.5, 5, 10 mM	+, dose-response in Chinese hamster V79 cells transfected with mouse GST-T1, with proteinase K	Hu et al. (2006)
DNA SSBs by alkaline elution	3,000 ppm	+ in CHO cells with mouse liver cytosol	Graves and Green (1996)
DNA SSBs by alkaline elution	60 mM	+ in CHO cells with mouse liver cytosol	Graves et al. (1994a)
DNA SSBs by comet assay	2.5, 5, 10 mM	– in Chinese hamster V79 cells transfected with mouse GST-T1	Hu et al. (2006)
Sister chromatid exchange	10,000, 20,000, 30,000, 40,000 ppm	Weak positive in Chinese hamster V79 lung cells	Jongen et al. (1981)
Sister chromatid exchange	0, 2, 5, 10 ppm	– in CHO cells with or without rat liver S9	Thilagar and Kumaroo (1983)
Sister chromatid exchange	15–500 ppm	+, dose-response in human peripheral red blood cells, strength of response related to degree of GST-T1activity	Olvera-Bello et al. (2010)
Other species or conditions			
Comet assay	10, 100, 1,000 µM	Very weak trend in human lung epithelial cells (GST enzyme activity not present)	Landi et al. (2003)
DNA-protein cross-links	0.5–5 mM	– in Syrian golden hamster hepatocytes and in rat hepatocytes	Casanova et al. (1997)
DNA SSBs by alkaline	0, 30, 60, 90 mM	+, dose-response in rat hepatocytes	Graves et al. (1994a)
DNA SSBs by alkaline elution	5–90 mM	– in hamster hepatocytes	Graves et al. (1995)
DNA SSBs by alkaline elution	5–120 mM	– in human hepatocytes	Graves et al. (1995)
DNA and protein synthesis	1,000 µg/mL	– in CHO cells	Garrett and Lewtas (1983)
Unscheduled DNA synthesis	1, 3, 10, 30 mM	– in rat hepatocytes (in serum free medium)	Andrae and Wolff (1983)
Unscheduled DNA synthesis	5,000, 10,000, 30,000, 50,000 ppm	– in Chinese hamster V79 lung cells (in medium with serum)	Jongen et al. (1981)
Unscheduled DNA synthesis	5,000, 10,000, 30,000, 50,000 ppm	– in primary human fibroblasts (in medium with serum)	Jongen et al. (1981)
Unscheduled DNA synthesis	250, 500, 1,000 ppm	– with or without rat liver S9 in human peripheral lymphocytes	Perocco and Prodi (1981)

Despite the absence of relevant data with respect to critical genes in dichloromethane-induced tumorigenesis, the preponderance of other data from experimental studies support the mutagenicity of dichloromethane and the key role of GST metabolism and the formation of DNA-reactive GST-pathway metabolites. The data pertaining to chromosomal instability provide greater weight to this collection of evidence than the genotoxicity data that are useful as indicators of DNA damage but which may not accurately predict changes in the genetic sequence; within each of these sets of studies, in vivo evidence provides greater weight than in vitro evidence. The database for dichloromethane provides support along each of these lines: 1) in vivo evidence of chromosomal mutations (chromosomal aberrations and micronuclei) in the mouse lung and peripheral red blood cells in the absence of evidence of cytoxicity; these endpoints have not been examined in the liver. These observations were not seen in the mouse bone marrow, a much more limited site in terms of degree of dichloromethane metabolism; 2) in vitro chromosomal instability evidence in human cells, other mammalian cells (i.e., CHO), and in bacterial systems; and 3) positive DNA damage indicator assays in numerous in vivo and in vitro studies. As noted previously, unscheduled DNA synthesis is generally a relatively insensitive measure of genotoxicity, and is given little weight in this synthesis of the data. Specific details regarding the studies within each of these lines of evidence were described in the preceding tables (Tables 4-35 to 4-37), and the body of evidence supporting the mode of action is summarized in Table 4-38. Evidence pertaining to tissue site specificity, dose-response concordance, and temporality is also summarized in Table 4-38.

One limitation of the hypothesized mutagenic mode of carcinogenic action for dichloromethane is that the available data are generally limited to relatively high exposure studies (i.e., ≥2,000 ppm, the exposure levels that induced liver and lung tumors in $B6C3F_1$ mice (Mennear et al., 1988; NTP, 1986). Although the probability of events induced through the GST-T1 pathway is reduced at lower exposures, there is no evidence indicating that this probability is eliminated and that the proposed mode of action could not operate at lower exposures. In the absence of such data, EPA considers the high-exposure data to be relevant.

Another limitation of the hypothesized mutagenic mode of carcinogenic action for dichloromethane is the lack of data demonstrating binding of the reactive GST metabolite, S-(chloromethyl)glutathione, to DNA. DNA reaction products (e.g., DNA adducts) produced by GST metabolites, such as S-(chloromethyl)glutathione, have not been identified in in vivo studies (Watanabe et al., 2007). The authors speculated that these results are due to the instability of the reaction products (Hashmi et al., 1994). DNA adducts, however, have been observed in studies in which calf thymus DNA was incubated with dichloromethane and GST or with S-(1-acetoxymethyl)glutathione, a compound structurally similar to S-(chloromethyl)glutathione (Marsch et al., 2004; Kayser and Vuilleumier, 2001).

Table 4-38. Experimental evidence supporting a mutagenic mode of action for dichloromethane

Criteria	Evidence for dichloromethane	Selected references
Evidence of GST involvement	• Presence or activation of GST is necessary for or increases genotoxicity and mutagenicity in bacterial and mammalian cell systems • Formation of DNA SSBs suppressed when mice were pretreated with a GSH depletor	• Simula et al. (1993); Osterman-Golkar et al. (1983); Gocke et al. (1981); Graves and Green (1996); Graves et al. (1996) • Graves et al. (1995)
Strength, consistency of genotoxicity data [weight of evidence for a mutagenic mode of action, in decreasing order of weight]	• Positive in vivo evidence of chromosomal instability (micronucleus test; chromosomal aberrations) in mouse lung and peripheral red blood cells (liver not tested) • Positive in vitro evidence of chromosomal damage (micronucleus test) in 2 human cell lines and positive in vitro evidence of mutation in mammalian cells (*hprt* mutation in CHO cells with added GST activity) • Positive in multiple bacterial (Ames assay) systems • Positive genotoxicity indicator assays (DNA damage) in mouse liver and lung cells in vivo and in vitro; mixed results in human cells	• Allen et al. (1990) • Doherty et al. (1996); Graves et al. (1996); Graves and Green (1996); • Oda et al. (1996); Simula et al. (1993); Osterman-Golkar et al. (1983); Gocke et al. (1981) • Casanova et al. (1997; 1996); Graves et al. (1996; 1995; 1994a); Sasaki et al. (1998); Hu et al. (2006); Olvera-Bello et al. (2010)
Target-tissue specificity	• In vivo mammalian studies demonstrate site-specific effects: chromosomal aberrations, DNA-protein cross-links, DNA SSBs, and sister chromatid exchanges in liver and /or lung cells of B6C3F$_1$ mice following acute inhalation exposure to concentrations producing liver and lung tumors with chronic exposure • DNA damage (detected by comet assay) after dichloromethane exposure enhanced in liver tissue but not stomach, kidney, brain or bone marrow in CD-1 mice	• Allen et al. (1990); Casanova et al. (1996; 1992); Graves et al. (1995; 1994a) • Sasaki et al. (1998)
Dose-response concordance [Do the key events increase with dose?]	• Dose-dependent increase in chromosomal aberrations, DNA SSBs or sister chromatid exchange in B6C3F1 mouse hepatocytes or lung cells; evidence of DNA instability in the absence of cytotoxicity • Dose-dependent increase in formation of DNA adducts in vitro calf thymus DNA when treated with dichloromethane (5–60 mM) and bacterial, rat, or human GST	• Hu et al. (2006); Casanova et al. (1997); Graves et al. (1995; 1994a); Allen et al. (1990) • Marsch et al. (2004)
Temporal relationship [Does the key event precede tumor appearance?]	• Tumor formation seen only with early exposure (i.e., exposure only during the first 26 weeks for lung tumors or 52 weeks for liver tumors of a 2-year bioassay) • Formation of DNA-protein cross-links and SSBs in B6C3F1 mouse liver and lung following short (3- or 6-hour) inhalation exposure to 2,000–8,000 ppm dichloromethane • Positive comet assay in liver in CD-1 mice, 24 hours after oral administration of 1,720 mg/kg dichloromethane	• Kari et al. (1993) • Casanova et al. (1996; 1992); Graves et al. (1995; 1994a) • Sasaki et al. (1998)

Biological plausibility and coherence. Bioactivation of a parent compound into a mutagenic metabolite resulting in cancer is a plausible mode of action of carcinogenicity in humans and is a generally accepted mode of action. Dichloromethane-induced carcinogenicity is hypothesized to be due to metabolism of the parent compound by the GST pathway (GST-T1) to one or more metabolite that is tumorigenic. The GST metabolite, S-(chloromethyl)glutathione,

formed from dichloromethane, has been characterized as labile and highly reactive through in vitro evaluation of dichloromethane metabolism in hepatocytes using [^{13}C]-NMR techniques (Hashmi et al., 1994) and through an enzyme digestion assay using calf thymus DNA and GST-T1 enzyme (Marsch et al., 2004). The hypothesis that the formation of a mutagenic metabolite is a preliminary step resulting in carcinogenicity is based on evidence that malignant tumors are primarily located in areas where dichloromethane is highly metabolized by GST-T1, such as the liver and the lung, and on mutagenicity studies indicating the importance of the GST pathway and that the lung and liver are more prone to mutagenic effects of dichloromethane (Sasaki et al., 1998; Casanova et al., 1996; Graves et al., 1995; Graves et al., 1994a; Casanova et al., 1992). The site selectivity of the positive mutagenic response in liver and lung tissue as evidenced by several studies suggests that the GST reactive metabolite remains in the tissue where it is formed. Collectively, the studies support the hypothesis that dichloromethane-mediated carcinogenicity results from a GST metabolite that produces selective DNA damage in the tissues where the metabolite is formed, but this hypothesis is based in part on assumptions regarding metabolite clearance and reactivity. DNA damage in the liver and lung, as well as the increased incidence of tumor formation resulting from dichloromethane exposure, indicates coherence of the mutagenic and carcinogenic effects and is evidence supporting a mutagenic mode of action.

Differences in GST activity in mice compared with other species, and the interspecies variability in genotoxic effects corresponding to interspecies variability in tumor response, support the mode of action hypothesis. DNA SSBs were not detected in liver or lung cells in rats exposed to similar inhalation exposures that induce strand breaks in mice (Graves et al., 1995; Graves et al., 1994a) and were detected at much lower in vitro concentrations in isolated hepatocytes from B6C3F$_1$ mice (0.4 mM) than in hepatocytes from Alpk:APfSD rats (30 mM) (Graves et al., 1995). The difference in susceptibility to carcinogenic response between mice and rats likely reflects differences in GST metabolism and localization of GST-T1 within hepatic cells. Toxicokinetic studies indicate that with increasing exposure levels, increasing amounts of dichloromethane are metabolized via GST metabolism.

4.7.3.1.2. ***Other possible modes of action for liver or lung tumors in rodents.*** Data are not available to support other possible modes of action for the liver and lung tumors in rodents. Efforts to observe sustained cell proliferation in liver following dichloromethane exposure of B6C3F$_1$ mice have been unsuccessful. Groups of female B6C3F$_1$ mice that were exposed to 0 or 2,000 ppm dichloromethane 6 hours/day, 5 days/week for up to 78 weeks did not exhibit enhanced cell proliferation in the liver when assessed at various intervals during exposure (Foley et al., 1993).

Indices of enhanced cell proliferation have been measured in the lungs of male B6C3F$_1$ mice following acute duration exposure at concentrations of about 1,500, 2,500, or 4,000 ppm dichloromethane (6 hours/day for 2 days) but not at exposure concentrations of 150 or 500 ppm

and not in lungs of Syrian golden hamsters exposed to concentrations up to 4,000 ppm (Casanova et al., 1996). The numbers of bronchiolar cells undergoing DNA synthesis (thymidine incorporation labeling) were markedly increased (about 6- to 15-fold) in bronchiolar cells of $B6C3F_1$ mice exposed to 4,000 ppm dichloromethane 6 hours/day on days 5, 8, and 9 of exposure, but no evidence for increased cell proliferation was found after 89, 92, or 93 days of exposure (Foster et al., 1992). The results suggest that enhanced cell proliferation is not sustained in the lung with longer-term exposure to dichloromethane concentrations associated with lung tumor development in mice, and that this mode of tumor promotion is not important in the development of dichloromethane-induced lung tumors.

4.7.3.2. *General Conclusions About the Mode of Action for Tumors in Rodents and Relevance to Humans*

Is the hypothesized mode of action sufficiently supported in test animals? The mode of action for dichloromethane is hypothesized to involve mutagenicity via reactive metabolites. The extensive body of research examining the proposed mode of action was summarized in the previous section. Mechanistic evidence indicates that dichloromethane-induced DNA damage in cancer target tissues of mice involves DNA-reactive metabolites produced via a metabolic pathway initially catalyzed by GST. Although mutational events in critical genes leading to tumor initiation have not been established, evidence supporting a mutagenic mode of action includes the identification of mutagenic response (reverse mutations) in short-term bacterial assays (with microsomal activation) and induced DNA-protein cross-links and DNA SSBs in mammalian cell assays. There are numerous positive in vivo mutagenicity and genotoxicity studies specifically examining responses in the liver and/or lung; these studies included evidence of chromosomal aberrations, SSBs, sister chromatid exchanges, and DNA-protein cross-links. The negative assays are generally those that were either micronucleus tests using mouse bone marrow, which is expected, as halogenated hydrocarbons (such as dichloromethane) are not very effective in this type of assay (Dearfield and Moore, 2005; Crebelli et al., 1999)), or unscheduled DNA synthesis, a relatively insensitive indicator of DNA damage. In conclusion, there is sufficient evidence supporting a mutagenic mode of action and indicating the involvement of GST metabolism in the lung and liver carcinogenicity of dichloromethane in mice.

Is the hypothesized mode of action relevant to humans? The postulated mode of action that dichloromethane is metabolized by GST to reactive metabolites that induce mutations in DNA leading to carcinogenicity is possible in humans. Mutagenicity as a mode of action for carcinogenicity in humans is generally accepted and is a biologically plausible mechanism for tumor induction. The toxicokinetic and toxicodynamic processes that would enable reactive metabolites to produce mutations in animal models are biologically plausible in humans. Furthermore, the detection of the GST pathway in human tissues indicates that the hypothesized

mode of action involving reactive metabolites from this pathway, S-(chloromethyl)glutathione and formaldehyde, is relevant to humans.

Some investigators question the relevance of the proposed mode of action to humans in low-exposure scenarios given the high exposure conditions of the genotoxicity and bioassay studies in mice and the relatively high GST activity in this species (Green, 1997). Comparisons in mice, rats, humans, and hamsters of GST enzyme activity in liver and lung tissues have indicated the following rank order: mice > rats > or ≈ humans > hamsters (Thier et al., 1998b; Reitz et al., 1989a).

Underlying questions of human relevance of dichloromethane-induced mouse tumors is the assumption that, at very low exposures, the amount metabolized through the GST activity in humans is effectively zero, and so any risk to humans would thus effectively be zero. EPA considered this line of reasoning, but found that it was not supported by several pieces of evidence. As discussed in Section 3.3, based on enzyme kinetics, the rate of reaction below ~20% of the K_m becomes indistinguishable from a first-order reaction, as it depends on the probability that a substrate molecule collides with an unoccupied active site on the enzyme. Thus, at low concentrations (i.e., [substrate] << K_m) the rate of enzyme-catalyzed reactions becomes proportional to the concentration of the substrate(s) and enzyme, and the reaction will proceed at a non-zero rate as long as GSH, GST, and dichloromethane are present at non-zero concentrations. The linearity of this metabolism at very low concentrations is discussed in the section on uncertainties in low-dose extrapolation (Section 5.4.5). At very low exposures, the amount of dichloromethane metabolized in humans through the GST pathway, while very low, is not zero.

Another factor noted by Green (1997) that may play a role in the apparent species differences in carcinogenicity resulting from dichloromethane exposure is species differences in intracellular localization of GST-T1. Nuclear production of S-(chloromethyl)glutathione catalyzed by GST-T1 in the nucleus is more likely than cytoplasmic production to lead to DNA alkylation. Using immunostaining techniques, Mainwaring et al. (1996) demonstrated that in mouse liver tissue, GST-T1 was localized in the nuclei of hepatocytes and bile-duct epithelium, whereas the rat and human liver did not show preferential nuclear localization of GST-T1. A later study by Sherratt et al. (2002) reported that in human tissue samples, bile duct epithelial cells and some hepatocytes showed nuclear localization of GST-T1, and other hepatocytes showed localization in cytoplasm. Although the degree of GST-T1 localization in the mouse is greater than in humans, the finding of some nuclear localization of GST-T1 in human liver tissue and in the nuclei of bile duct epithelial cells, and the observation of three biliary tract cancers, a very rare cancer, in a small cohort of dichloromethane exposed workers (Lanes et al., 1993; Lanes et al., 1990) support the relevance of the hypothesized mode of action to humans.

Which populations or lifestages can be particularly susceptible to the hypothesized mode of action? As discussed in Section 3.3, a polymorphism of the GST-T1 gene is present in humans. People with two functional copies of the gene (+/+) readily conjugate GSH to dichloromethane. Individuals having only one working copy of the gene (+/-) display relatively decreased conjugation ability. Individuals with no functional copy of the gene (-/-) do not express active GST-T1 protein and do not metabolize dichloromethane via a GST-related pathway (Thier et al., 1998b). Thus, the GST-T1$^{+/+}$ (wild-type) genotype would be considered to be the more "at risk" population; this subgroup represents approximately 30% of the U.S. population (Haber et al., 2002) but would be expected to be more common among Caucasians and African-Americans than among Asians (Raimondi et al., 2006; Garte et al., 2001; Nelson et al., 1995) (see Table 3-3).

According to the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), children exposed to carcinogens with a mutagenic mode of action are assumed to have increased early-life susceptibility. The *Supplemental Guidance* (U.S. EPA, 2005b) recommends the application of age-dependent adjustment factors (ADAFs) for carcinogens that act through a mutagenic mode of action. Although the database is lacking in vivo evidence of specific mutagenic events following chronic exposure to dichloromethane, the weight of the available evidence indicates that dichloromethane is acting through a mutagenic mode of carcinogenic action. Application of ADAFs is recommended for both the oral and inhalation routes of exposure when risks are assessed that are associated with early-life exposure.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

In humans, hepatic CYP2E1 begins to be expressed in the second trimester. In a study of 73 fetal liver samples from 7 to 37 weeks gestation, CYP2E1 was not detected in the first trimester, ranged from 0 to 3 pmol per mg microsomal protein (median 0.35 pmol per mg microsomal protein) during the second trimester, and increased to a median of 6.7 pmol per mg microsomal protein during the third trimester (Johnsrud et al., 2003). CYP2E1 continues to increase during the first year of life, with adult levels reached after age 3 months (Hines, 2007; Johnsrud et al., 2003; Treluyer et al., 1996; Vieira et al., 1996). In a study using 10 fetal brain samples collected between 7 and 12 weeks gestation, CYP2E1 activity is seen as early as GD 53, with increasing levels (from 2.2 to 4.5 pmol produced per mg microsomal protein per hr) seen through at least GD 113 (Brzezinski et al., 1999). The relatively high activity of CYP2E1 in the brain compared to the liver of the developing human fetus raises the potential for neurodevelopmental effects from dichloromethane exposure. Results from a developmental toxicity study in rats also raise concern for possible neurodevelopmental effects. Decreased offspring weight at birth and changed behavioral habituation of the offspring to novel

environments were seen following exposure of adult Long-Evans rats to 4,500 ppm for 14 days prior to mating and during gestation (or during gestation alone) (Bornschein et al., 1980; Hardin and Manson, 1980). In the only other animal study examining possible early-life susceptibility to dichloromethane toxicity, Alexeeff and Kilgore (1983) found that exposure of young male mice to approximately 47,000 ppm for about 20 seconds significantly impaired the ability to learn using a passive-avoidance conditioning task. Three-week-old mice were more affected than 5- or 8-week-old mice. The broad issue of childhood susceptibility to chronic neurobehavioral effects of early life exposure represents a data gap in the understanding of the health effects of dichloromethane.

The relatively low CYP2E1 activity in the liver of young infants (<3 months of age) would tend to shift metabolism of dichloromethane to the GST pathway. This shift could affect cancer risk, given the evidence of genotoxicity through this metabolic pathway. However, the available data in humans are not sufficient to address the question of whether in utero or early life exposures represent a period of increased susceptibility to potential carcinogenic effects of dichloromethane. McCarver and Hines (2002) reviewed data pertaining to fetal expression of GST-M, GST-P, and other classes of enzymes, but information pertaining to GST-T1 was not included. GST-T1 was not detected in any tissue examined in an 8-week embryo or a 13-week fetus (Raijmakers et al., 2001), but several studies have reported an interaction between maternal smoking and fetal GST-T1 null genotype on risk of oral cleft (Shi et al., 2007; Lammer et al., 2005; van Rooij et al., 2001). These studies indicate the potential relevance of fetal GST-T1 genotype in relation to in utero exposures. A threefold increased risk of childhood leukemia (acute lymphoblastic leukemia) was seen in relation to maternal occupational exposure to dichloromethane in the year before and during pregnancy in one population-based case-control study (OR 3.22 [95% CI 0.88–11.7]) for ratings of "probable or definite" exposure compared with possible or no exposure (Infante-Rivard et al., 2005). The estimates for categories based on concentration and frequency were similar, but there was no evidence for an increasing risk with increasing exposure level. This study did not examine interactions with GST-T1 genotype.

Experiments comparing cancer responses from early-life exposures with those from adult exposures are not available for F344 rats or $B6C3F_1$ mice, the strains of animals in which carcinogenic responses to dichloromethane have been observed. Animal data evaluating the effect of age on the susceptibility to dichloromethane carcinogenicity are restricted to a bioassay in which 54 pregnant Sprague-Dawley rats were exposed starting on GD 12 to 100 ppm dichloromethane 4 hours/day, 5 days/week for 7 weeks, followed by 7 hours/day, 5 days/week for 97 weeks (Maltoni et al., 1988). Groups of 60 male and 69 female newborns continued to be exposed after birth to 60 ppm dichloromethane 4 hours/day, 5 days/week for 7 weeks, followed by exposure 7 hours/day, 5 days/week for 97 weeks. Additional groups of 60 male and 70 female newborns were exposed after birth to 60 ppm dichloromethane 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 8 weeks. Endpoints

monitored included clinical signs, BW, and full necropsy at sacrifice (when spontaneous death occurred). For each animal sacrificed, histopathologic examinations were performed on the following organs: brain and cerebellum, zymbal glands, interscapular brown fat, salivary glands, tongue, thymus and mediastinal lymph nodes, lungs, liver, kidneys, adrenals, spleen, pancreas, esophagus, stomach, intestine, bladder, uterus, gonads, and any other organs with gross lesions. There was no significant effect of exposure to dichloromethane on the incidence of benign or malignant tumors among adults or the progeny. The results provide no evidence that Sprague-Dawley rats would be more sensitive to potential carcinogenic activity of dichloromethane during early life stages. Further conclusions from these results are precluded because the study included only one exposure level, which was below the maximum tolerated dose for adult Sprague-Dawley rats.

4.8.2. Possible Gender Differences

The limited data available from studies in humans do not indicate that there are large differences by gender in sensitivity to cardiovascular, neurologic, cancer, or other effects; studies have not been conducted specifically to examine this question and so do not provide information pertaining to smaller or more subtle differences. There was no evidence of variation by gender in hepatic CYP2E1 activity and protein levels in humans based on 238 (136 male, 83 female) samples (Johnsrud et al., 2003), and the prevalence of the GST-T1 polymorphisms does not vary by gender (Garte et al., 2001). The available animal studies similarly do not establish whether either gender may be more susceptible to the toxic effects of dichloromethane. Studies of the carcinogenic effects of dichloromethane, either by inhalation or by the oral route, have not suggested an increased susceptibility of either male or female animals.

4.8.3. Other

As discussed in Section 3.3, a polymorphism exists within the GST-T1 gene in humans, resulting in individuals with diminished or a lack of ability to conjugate GSH to dichloromethane. While the possible effects of this polymorphism on the toxicity of dichloromethane have not been directly demonstrated, it can be inferred from the proposed mode of action that a decrease in the GST-T1 metabolic pathway would result in a decreased generation of reactive metabolites and a decrease in any chronic effects mediated through those metabolites (Jonsson and Johanson, 2001; El-Masri et al., 1999).

Interindividual variation in the ability to metabolize dichloromethane via GST-T1 is associated with genetic polymorphisms in humans. Estimated U.S. population prevalence of nonconjugators (-/- at the GST-T1 locus) is about 20%, but higher prevalences (47–64%) have been reported for Asians (Raimondi et al., 2006; Haber et al., 2002; Garte et al., 2001; Nelson et al., 1995). Although nonconjugators are expected to have negligible extra risk for dichloromethane-induced cancer, the U.S. prevalences for low (+/- at the GST-T1 locus) and

high (+/+) conjugators have been estimated at 48 and 32%, respectively (Haber et al., 2002). The liver and kidney are the most enriched tissues in GST-T1, but GST-T1 is seen at lower levels in other tissues including the brain and lung (Sherratt et al., 2002; Sherratt et al., 1997).

Individuals may vary in their ability to metabolize dichloromethane through the CYP2E1 pathway. Individuals with decreased CYP2E1 activity may experience decreased generation of CO and an increased level of GST-related metabolites following exposure to dichloromethane, which may result in increased susceptibility to the chronic effects of dichloromethane from GST-related metabolites. Conversely, individuals with higher CYP2E1 activity may experience relatively increased generation of CO at a given dichloromethane exposure level and therefore, may be more susceptible to the acute CO-related toxicity or other chronic effects of dichloromethane resulting from this metabolic pathway. These individuals would be expected to be at a lower risk of chronic effects of dichloromethane from GST-related metabolites. Several studies indicate a three- to sevenfold variability in CYP2E1 activity among humans, as assessed by various types of measurements among "healthy" volunteers (Sweeney et al., 2004; Haufroid et al., 2003; Lipscomb et al., 2003; Lucas et al., 2001; Bernauer et al., 2000; Lucas et al., 1999; Kim and O'Shea, 1995; Shimada et al., 1994). This variability is incorporated into the PBPK models for dichloromethane. Factors that may induce or inhibit CYP2E1 activity (e.g., obesity, alcohol use, diabetes) or other exposures (i.e., to various solvents or medications) (Lucas et al., 1999) may result in greater variation within segments of the population. This variation in CYP2E1 activity may result in earlier saturation of this pathway and greater exposure to the parent compound, which would be of particular relevance to neurological effects.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

As discussed in Section 4.6.1, human data for oral exposures to dichloromethane are limited to case reports involving intentional (i.e., suicidal) or accidental, acute ingestion exposures (Chang et al., 1999; Hughes and Tracey, 1993). Reported effects reflect frank toxicity from very high doses such as marked CNS depression, injury to the gastrointestinal tract, liver and kidney failure, coma, and death. No studies of human chronic oral exposures are available. In the absence of adequate studies evaluating possible health effects in humans repeatedly exposed to dichloromethane via the oral route, the results from the chronic laboratory animal studies are assumed to be relevant to humans.

The database of laboratory animal oral exposure studies includes 90-day (Kirschman et al., 1986) and 2-year drinking water toxicity studies in F344 rats (Serota et al., 1986a) and B6C3F$_1$ mice (Serota et al., 1986b; Hazleton Laboratories, 1983). A reproductive study exposed Charles River CD rats via gavage before mating (General Electric Company, 1976), and a developmental study exposed F344 rats via gavage during GDs 6–19 (Narotsky and Kavlock, 1995). A 14-day gavage study examined neurotoxicity in F344 rats (Moser et al., 1995).

Hepatic effects (hepatic vacuolation, liver foci) are the primary dose-dependent noncancer effects associated with oral exposure to dichloromethane (see Table 4-26). The 90-day drinking water toxicity study in F344 rats (Kirschman et al., 1986) reported significant increases in hepatocyte vacuolation and necrosis in animals dosed between 166 and 1,200 mg/kg-day (males) or 200 and 1,469 mg/kg-day (females). These doses were used to develop dosing levels for the 104-week drinking water study (Serota et al., 1986a). The 104-week drinking water study of F344 rats (Serota et al., 1986a) provides adequate data to describe dose-response relationships for liver lesions from chronic oral exposure to dichloromethane (e.g., includes four exposure levels and a control group). In this study, rats dosed at ≥50 mg/kg-day in both sexes had increased fatty livers, but quantitative data were not provided by the authors. Liver lesions, described as foci or areas of cellular alteration, were also seen in this study in the same dose groups in which the fatty changes had occurred. A limitation of this study is that Serota et al. (1986a) did not describe the evaluation of the altered foci in detail. However, increases in altered foci did not correspond to tumor rate incidences in either male or female rats. Instead, the altered foci correlated more closely to fatty liver incidence changes for both sexes in the rats. Altered foci could range from a focal fatty change (nonneoplastic) to an enzymatic altered foci change (neoplastic) (Goodman et al., 1994). Several lines of evidence were considered in determining whether the lesions should be characterized as nonneoplastic or neoplastic: (1) there is a congruence between the incidence of this lesion and

the incidence of the fatty liver in the study by Serota et al. (1986a); (2) at higher doses, hepatocyte vacuolation and hepatocyte necrosis were seen (Berman et al., 1995; Kirschman et al., 1986); and (3) there is no clear indication that these altered foci progress to liver tumors since the rate of increased foci did not correlate with liver tumor increases in either male or female rats. Based on these observations, EPA concluded that the altered foci were more likely to be representative of a focal fatty change (nonneoplastic) than a neoplastic event.

The LOAELs for the liver lesions in rodents following repeated oral exposure (50–586 mg/kg-day) (Table 4-26) are in the same range or below the NOAELs of 225 mg/kg-day for reproductive performance in Charles River CD rats exposed for 90 days before mating (General Electric Company, 1976) and 450 mg/kg-day for developmental toxicity in pregnant F344 rats exposed during gestation (Narotsky and Kavlock, 1995). The LOAEL (337 mg/kg-day) and NOAEL (101 mg/kg-day) for mild neurological impairment in a 14-day gavage exposure study of F344 rats (Moser et al., 1995) indicates that the threshold for neurological effects may be similar to the threshold for liver effects. A limitation of the Moser et al. (1995) study, however, is that the observed effects were limited to measures taken within 4 hours of exposure.

The subchronic (i.e., ≤90-day study) data were not considered in the selection of a principal study for deriving the chronic RfD because the database contains reliable dose-response data from a chronic study at lower doses than the 90-day study (Kirschman et al., 1986). The data from the subchronic studies are, however, used to corroborate the findings in the chronic studies with respect to relevant endpoints (i.e., hepatic and neurological effects). The neurotoxicity study was not selected as the principal study due to the limited measurements to inform the chronic exposure to dichloromethane. The rat rather than the mouse chronic bioassay was selected as the principal study for the RfD because of the consistent evidence that rats may be more sensitive than mice to noncancer liver effects from orally administered dichloromethane; available rat LOAELs for liver lesions are lower than mouse LOAELs (see Table 4-26). Liver foci/areas of alteration in the rat, considered an adverse effect by EPA, was identified as the critical effect for RfD derivation. Figure 5-1 is an exposure-response array that presents NOAELs, LOAELs, and the dose range tested, corresponding to selected health effects from the short-term (neurotoxicological) and subchronic studies, and from the chronic, reproductive, and developmental toxicity studies that were evaluated for use in the derivation of the RfD.

5.1.2. Derivation Process for Noncancer Reference Values

The toxicity values (oral RfD and inhalation RfC) for noncancer endpoints were derived by using rat and human PBPK models to calculate internal doses in rats from experimental exposures and extrapolate PODs to human equivalent exposures. Figure 5-2 illustrates the process of using the PBPK models for toxicity value derivation. The process for the RfD and RfC is summarized below, using the example of a noncancer liver effect.

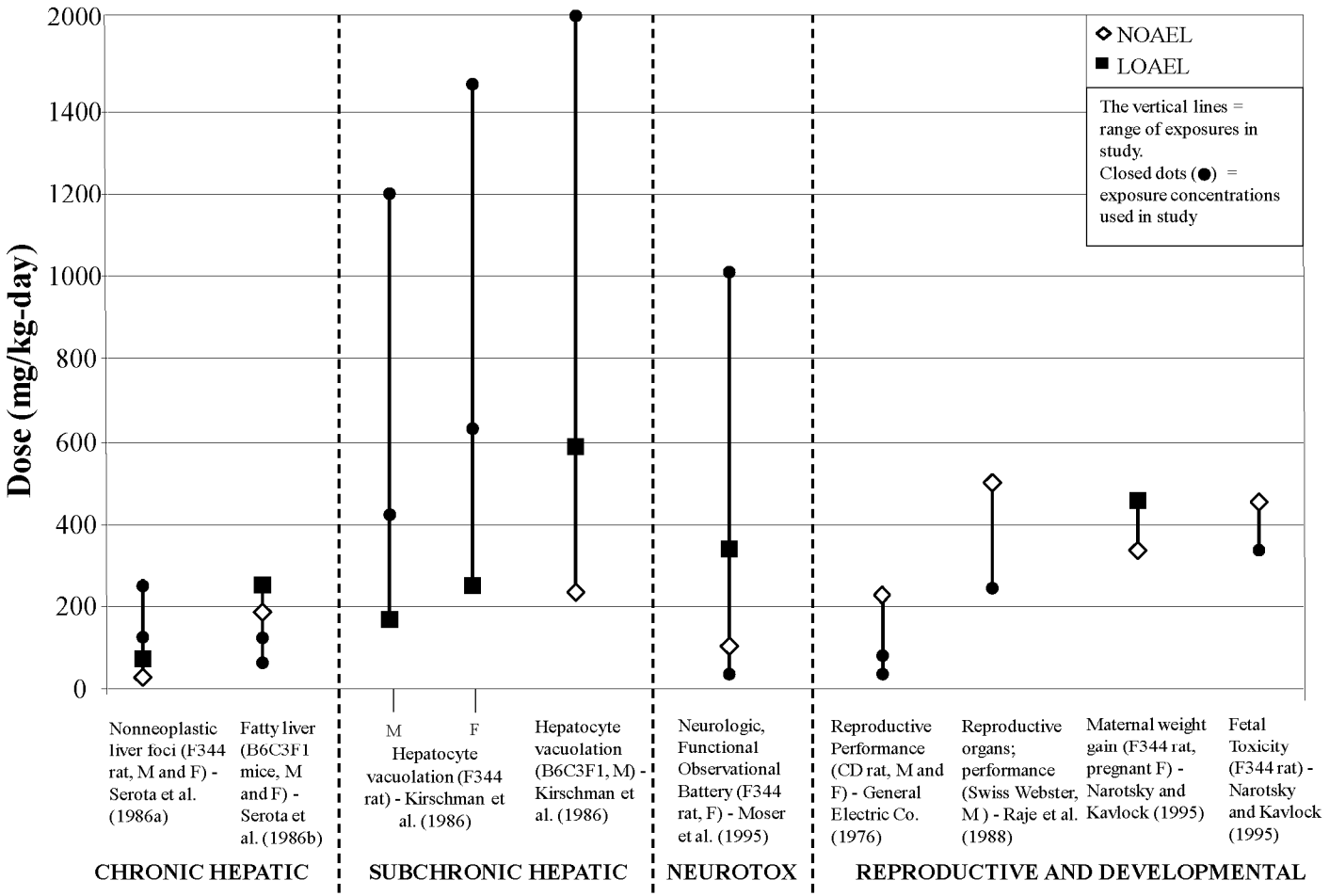


Figure 5-1. Exposure response array for oral exposure to dichloromethane (M = male; F = female).

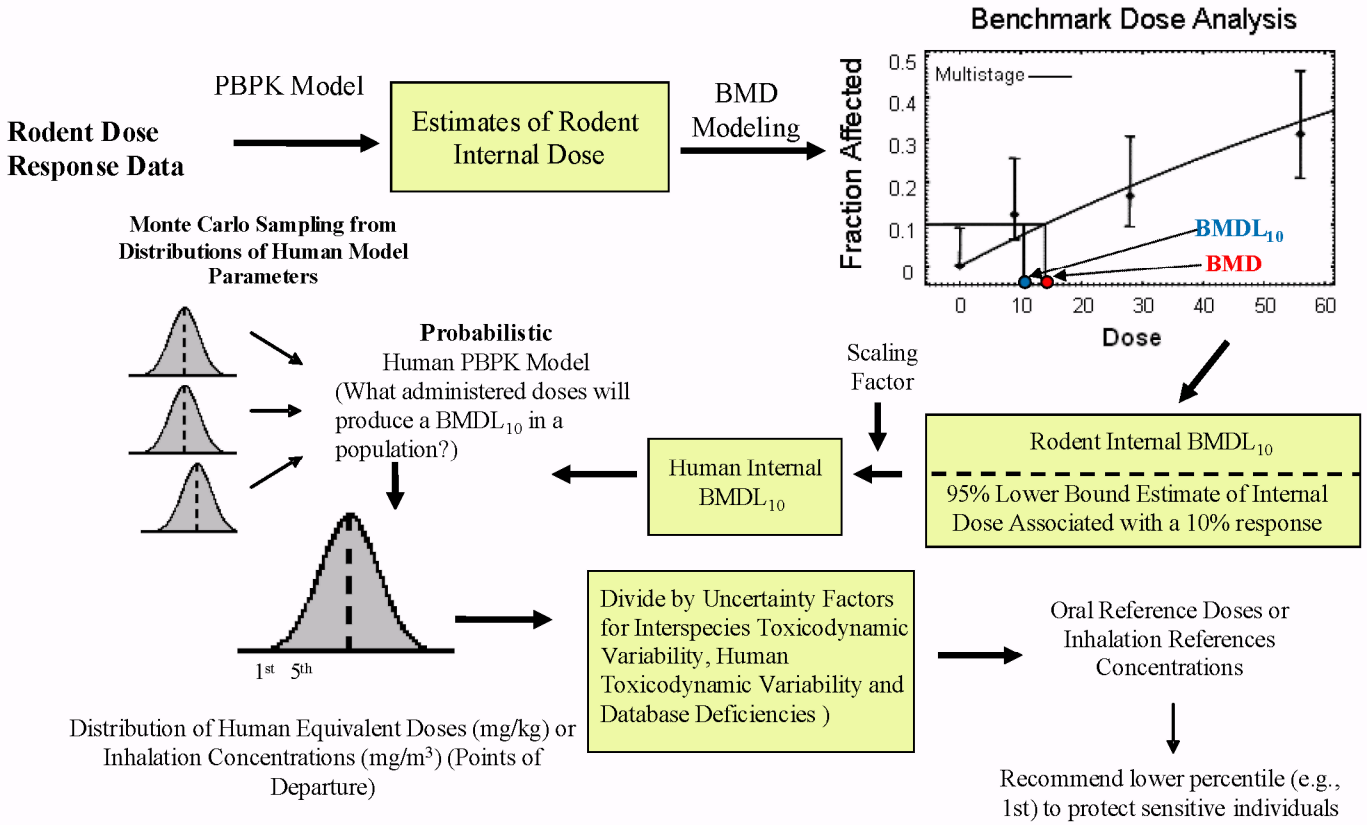


Figure 5-2. Process for deriving noncancer oral RfDs and inhalation RfCs using rodent and human PBPK models.

A deterministic PBPK model for dichloromethane in rats was first used to convert rat drinking water or inhalation exposures to values of an internal liver dose metric (see Appendix C for details of the rat PBPK model). Available models in EPA benchmark dose (BMD) software (BMDS) version 2.0 were then fit to the liver lesion incidence data, and internal liver dose data for rats and BMD_{10} values and their lower 95% confidence limits associated with a 10% extra risk ($BMDL_{10}$) were calculated from each of the models. Adequacy of model fit was assessed by overall χ^2 goodness of fit ($p > 0.10$) and examination of residuals, particularly in the region of the benchmark response (BMR). Among models with adequate fits, the choice of best-fitting model was based on the lowest Akaike's Information Criterion (AIC) value (i.e., a measure of the deviance of the model fit that allows for comparison across models for a particular endpoint) (U.S. EPA, 2000a).[8]

In some situations, the use of a PBPK model can replace the use of the $BW^{0.75}$ scaling factor to account for interspecies differences in toxicokinetics. Use of a scaling factor based on $BW^{0.75}$ is consistent with observed inter-species differences in overall metabolism, respiration, and cardiac output (Lindsted et al., 2002) and average scaling of xenobiotic clearance (Tang and Mayersohn, 2005). Whether or not to use a scaling factor depends on the dose metric that is used. Where PBPK models predict the concentration (in particular, the AUC) of the proximate causative agent, a scaling factor to account for interspecies differences is not typically used. That is, it is assumed that if the time-averaged (or steady-state) concentration of the proximate causative agent predicted by the PBPK model in the target tissue is the same in the test species as in humans, and the test species was exposed for an equivalent portion of its lifetime (2 years in rats and mice being equivalent to a 70-year lifetime in humans), then the resulting risks in the two species are the same. However, when the PBPK model predicts the rate of production of the agent rather than its concentration, then a $BW^{0.75}$ scaling factor may be appropriate, depending on what is known or expected regarding the rate of clearance of the agent or metabolite of interest. Two different scenarios can be considered. If the metabolite formed is considered to be highly reactive and is unlikely to involve processes or cofactors for which the rate or availability can be expected to scale allometrically, then it can be assumed that the rate of clearance (i.e., disappearance due to local reactivity) for this metabolite per volume tissue is equal in rodents and humans. Thus, in that situation as with the AUC dose metric, no $BW^{0.75}$ scaling factor is necessary, although differences in tissue volume fraction in humans versus rats (as occurs for liver) should be and are accounted for by the PBPK model. However, if the metabolite is removed by processes that scale allometrically (including enzymatic reactions or reactions with cofactors whose supply is limited by overall metabolism), then it is expected that interspecies

[8]If two or more models share the lowest AIC, $BMDL_{10}$ values from these models may be averaged to obtain a POD. However, this average is no longer a lower confidence bound that provides the stated coverage, and thus should be referred to only as an average of $BMDL_{10}$ values. U.S. EPA does not support averaging BMDLs in situations in which AIC values are similar, but not identical, because the level of stated coverage is lost and no consensus exists regarding a specific cut-off between similar and dissimilar AIC values.

differences in clearance or removal of the toxic metabolite follow the generally assumed $BW^{0.75}$ scaling for rates of metabolism and blood circulation. In this case, or in situations in which the reactivity or rate of removal of the metabolite has not been established, it is appropriate to use a scaling factor based on BW ratios to account for this difference. In the case of the noncancer liver effects of dichloromethane, very limited information is available on the mechanism(s) involved in creating the type of hepatic damage seen. The dose metric used in the PBPK modeling is a rate of metabolism rather than the concentration of putative toxic metabolites, and the clearance of these metabolites may be slower per volume tissue in the human compared with the rat. Thus, the rat internal dose metric for noncancer effects was adjusted by dividing by a pharmacokinetic scaling factor to obtain a human-equivalent internal $BMDL_{10}$.

A probabilistic PBPK model for dichloromethane in humans, adapted from the model of David et al. (2006) as described in Appendix B, was then used to calculate distributions of chronic exposures associated with the human equivalent internal $BMDL_{10}$, based on the responses in rats. Parameters in the human PBPK model are described by distributions that incorporate information about dichloromethane toxicokinetic and physiological variability and uncertainty among humans, with additional information on human variability for both the CYP2E1 and GST-T1 metabolic pathways (see Table 3-9 and Appendix B). Monte Carlo sampling was performed in which each human model parameter was defined by a value randomly drawn from its respective parameter distribution. The model was then executed by using the human internal $BMDL_{10}$ as input, and the resulting human equivalent dose (HED) or human equivalent concentration (HEC) was recorded. This process was repeated for 10,000 to 20,000 iterations to generate a distribution of HEDs or HECs.

As discussed in Section 3.5.2, the statistics reported for the fitted metabolic parameters by David et al. (2006) (Table 4 in that publication) only represent the population mean and uncertainty in that mean for each parameter. For the parameters other than V_{maxC} and k_{fC}, EPA considers it reasonable to assume that there is little true interindividual variability in the values, so the distributions were used as published in David et al. (2006). For the physiological parameters, the distributions presented by David et al. (2006) were supposed to represent a known range of interindividual variability, but EPA found that these did not adequately describe the full population, so many of the distributions were changed as discussed in Appendix B. For V_{maxC}, an independent data set (Lipscomb et al., 2003) where CYP2E1 levels were measured in vitro using liver samples from 75 human donors was used to estimate the degree of interindividual variability, and a "two-dimensional" sampling routine was used to incorporate the uncertainty as estimated by David et al. (2006) from those in vitro data. In addition, EPA concluded that the trivariate distribution (based on GST-T1 genotype), which David et al. (2006) used in place of the observed parameter uncertainty [based on ex vivo data from Warholm et al. (1994)], adequately represented interindividual variability but neglected the uncertainty in the population mean. Therefore, a two-dimensional sampling routine was used: first a specific

value for the population mean was sampled from the mean and variance (uncertainty) indicated for k_{fC} in Table 4 of David et al. (2006); second, given that value for the population mean, an individual value was sampled using the trivariate distribution, as indicated in Table 2 of David et al. (2006), but re-scaled to the (sampled) population mean.

The sampling routine used by EPA effectively assumes that the parameters are distributed independently, ignoring the covariance that was likely represented in the actual posterior chains, hence will tend to overestimate the overall range of parameters and distribution of dose metrics in the population compared to what one would obtain if the covariance were explicitly included. (This is offset to some extent by the assumption that there is no interindividual variability in the metabolic parameters other than V_{maxC} and k_{fC}.) Thus if the covariance (i.e., the variance-covariance matrix) for the set of parameters had been reported by David et al. (2006), it could have been used to narrow the predicted distribution of internal doses or equivalent applied doses. Lacking such information, the approach used will not underestimate risk or overestimate lower bounds on human equivalent exposure levels.

From these distributions of HEDs (or HECs), candidate RfDs (or RfCs) were derived by dividing the first percentile value (point of departure or POD) by uncertainty factors (UFs) to account for uncertainty about potential interspecies toxicodynamic variability, human toxicodynamic variability, and database deficiencies. The first percentile was chosen because it allowed generation of a stable estimate for the lower end of the distribution while being protective of the overall human population, including sensitive individuals. Choosing this lower point replaces the use of an additional UF to account for human toxicokinetic variability.

In summary, the PBPK model that is used only allows one to estimate the dose rate at which the metabolites are introduced to the target tissues (i.e., the oral or inhalation exposure rate; mg/kg-day inhaled or ingested) and not the rate of clearance of these metabolites from the tissue. The parent dichloromethane PBPK model is used to estimate the relationship between dichloromethane exposure and the metabolite exposure rate that occurs in the animal bioassay, and to back-estimate the human dichloromethane exposure (oral dose rate or inhalation concentration) distribution that gives rise to the internal human equivalent metabolite dose rate. Because the dose metric used in this model is an exposure rate, a scaling factor using $BW^{0.75}$ scaling is applied for animal-to-human extrapolation. Accordingly, only the toxicodynamic component of the UF for animal-to-human extrapolation ($UF_A = 3$) is applied in deriving the RfD (see Section 5.1.5) and RfC (see Section 5.2.4). Use of the first percentile of the distribution of the HED (or HEC) replaces the toxicokinetic component of the UF for human variability, and only the toxicodynamic component of this UF is applied ($UF_H = 3$). As discussed further in Section 5.1.5, the UFs for animal-to-human extrapolation and human variability in response do not include adjustment for toxicokinetic uncertainty.

5.1.3. Evaluation of Dose Metrics for Use in Noncancer Reference Value Derivations

There are no data to support the role of a specific metabolite in the development of the noncancer liver lesions seen in oral and inhalation exposure studies. Four dose metrics were examined as potential metrics for the internal dose of interest: rate of hepatic metabolism through the CYP pathway, rate of hepatic metabolism through the GST pathway, the combined rate of hepatic metabolism through the CYP and GST pathways, and the concentration (AUC) of dichloromethane (the parent compound) in the liver. The dose-response patterns for each of these metrics in the oral study in rats (Serota et al., 1986a) and in two inhalation studies in rats (Nitschke et al., 1988a; Burek et al., 1984) were examined for fit and congruence.

Using the oral exposure data, only one of the seven models, the log-logistic model, produced an adequate fit ($p > 0.10$) for the GST metabolism metric and the dichloromethane AUC metrics. Adequate model fit was seen in all of the models using the CYP dose metric with the oral data and using the GST, CYP, and AUC dose metrics for the inhalation data.

A limitation in using the GST metric can be observed when comparing the oral and inhalation responses at various exposure levels. At 200 ppm, where the GST metric is predicted by the PBPK model to be 93 mg metabolism/L liver/day, no liver effects were seen. In contrast, liver responses were elevated at an oral dose of 50 mg/kg-day, where the GST metric is predicted to be approximately 85 mg metabolism/L liver/day (see Tables 5-1 and 5-5, respectively, for the oral and inhalation internal metrics). Thus the liver GST metric produces an inconsistency in the dose-response relationship with different responses observed depending on the route of exposure. A similar inconsistency occurs with the AUC metric. These differences are not observed, however, when using the CYP metric. At the 200 ppm inhalation exposure, where no hepatoxicity was observed, the CYP metric is predicted to be 665 mg/L liver/day. This internal CYP metabolism metric is less than that predicted for the oral dose for the 50 mg/kg-day group (i.e., 724 and 801 mg metabolism/L liver/day in males and females, respectively) in which liver effects were observed. Thus, the CYP internal metric is consistent with the observed responses seen in the oral and inhalation exposure studies.

The GST metabolism and the AUC dose metrics did not present reasonable choices based on model fit and consistency of response across studies at comparable dose levels. Given these results, the combination of hepatic metabolism through the GST and the CYP pathways would not be expected to result in an improvement to a metric based only on CYP metabolism. Thus the CYP-metabolism dose metric is the most consistent with the data and this metric was selected for the subsequent RfD and RfC derivations. The lack of information on mechanisms with respect to noncancer health effects represents data gaps in the understanding of the health effects of dichloromethane.

5.1.4. Methods of Analysis—Including Models (PBPK, BMD, etc.)

PBPK models for dichloromethane in rats were described previously in Section 3.5. From the evaluation described in Appendix C, a modified model of Andersen et al. (1991) was selected for the calculation of internal dosimetry of ingested dichloromethane in the rats in the principal study (Serota et al., 1986a).

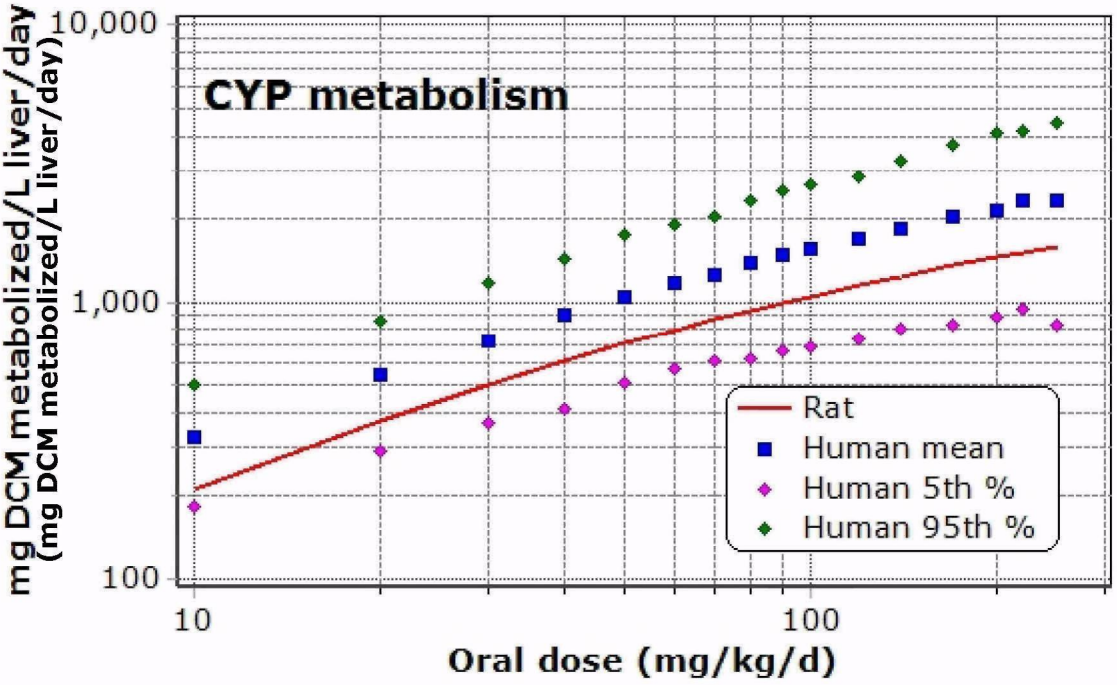
PBPK model simulations of the drinking water study of Serota et al. (1986a) (Table 5-1) were performed to calculate average lifetime daily internal liver doses in male and female F344 rats. In the absence of data for group- and sex-specific BWs, reference values were used for F344 rats in chronic studies (U.S. EPA, 1988b). The mode of action by which dichloromethane induces noncancer liver effects in rodents has not received research attention to determine the role of the parent material, metabolites of the CYP2E1 pathway, metabolites of the GST pathway, or some combination of parent material and metabolites. In the absence of this kind of knowledge, and considering the pattern of response seen in the oral and inhalation studies (as described in Section 5.1.3), an internal dose metric based on the amount of dichloromethane metabolized via the CYP pathway in the liver (mg dichloromethane metabolized via CYP pathway/L liver/day) was used. Figure 5-3 shows the comparison between oral external and internal doses using this dose metric for the rat and for the human.

Table 5-1. Incidence data for liver lesions and internal liver doses based on various metrics in male and female F344 rats exposed to dichloromethane in drinking water for 2 years

Sex	Nominal (actual) daily intake (mg/kg-d)	Rat liver lesion incidence[a]	Rat internal liver dose[b]			
			CYP	GST	GST and CYP	Parent AUC
Male (BW = 380 g)	0 (0)	52/76 (68%)	0	0	0	0
	5 (6)	22/34 (65%)	131.6	2.60	134.2	0.58
	50 (52)	35/38 (92%)[c]	723.9	81.5	805.3	18.1
	125 (125)	34/35 (97%)[c]	1,170.5	276.5	1,446.9	61.3
	250 (235)	40/41 (98%)[c]	1,548.0	612.1	2,160.1	135.7
Female (BW = 229 g)	0 (0)	34/67 (51%)	0	0	0	0
	5 (6)	12/29 (41%)	132.8	2.47	135.3	0.47
	50 (58)	30/41 (73%)[c]	801.4	93.3	894.7	17.8
	125 (136)	34/38 (89%)[c]	1,261.5	307.8	1,569.3	58.6
	250 (263)	31/34 (91%)[c]	1,672.4	705.6	2.378.0	134.4

[a]Liver foci/areas of cellular alteration; number affected divided by total sample size.
[b]Internal doses were estimated using a rat PBPK model from simulations of actual daily doses reported by the study authors. CYP dose is in units of mg dichloromethane metabolized via CYP pathway/L tissue/day; GST dose is in units of mg dichloromethane metabolized via GST pathway/L tissue/day; GST and CYP dose is in units of mg dichloromethane metabolized via CYP and GST pathways/L tissue/day; and parent AUC dose is in units of mg dichloromethane × hours/L tissue.
[c]Significantly ($p < 0.05$) different from control with Fisher's exact test.

Source: Serota et al. (1986a).



Six simulated daily drinking water episodes are described by Reitz et al. (1997). The human metabolism rates were estimated using a computational sample of 1,000 individuals per dose, including random samples of the three GST-T1 polymorphisms (+/+, +/-, -/-) in the current U.S. population based on data from Haber et al. (2002). Since a different set of samples was used for each dose, some stochasticity is evident as the human points (values) do not fall on smooth curves.

Figure 5-3. PBPK model-derived internal doses (mg dichloromethane metabolized via the CYP pathway/L liver/day) in rats and humans and their associated external exposures (mg/kg-day), used for the derivation of RfDs.

The seven dichotomous dose-response models in BMDS version 2.0 were fit to the rat liver lesion incidence data and PBPK model-derived internal dose data to derive a rat internal BMD_{10} and corresponding $BMDL_{10}$ associated with 10% extra risk (Table 5-2). A BMR of 10% was selected because, in the absence of information regarding the magnitude of change in a response that is thought to be minimally biologically significant, a BMR of 10% is generally recommended since it provides a consistent basis of comparison across assessments. There are no additional data to suggest that the severity of the critical effect or the power of the study would warrant a lower BMR. The male rats exhibited a greater sensitivity compared to the female rats (based on lower $BMDL_{10}$ values for all of the models examined), and thus the male data are used as the basis for the RfD derivation. The logistic model was the best fitting model for the male incidence data based on AIC value among models with adequate fit (U.S. EPA, 2000a). Modeling results are shown in detail in Appendix F, Section F.1.

Table 5-2. BMD modeling results for incidence of liver lesions in male and female F344 rats exposed to dichloromethane in drinking water for 2 years, based on liver-specific CYP metabolism dose metric (mg dichloromethane metabolism via CYP pathway/L liver tissue/day)

Sex and model[a]	BMD_{10}	$BMDL_{10}$	χ^2 goodness of fit *p*-value	AIC
Males				
Gamma[a]	138.12	41.28	0.58	185.46
Logistic[b]	**70.69**	**51.42**	**0.69**	**183.85**
Log-logistic[a]	188.81	38.74	0.80	184.85
Multistage (1)[a]	57.08	39.68	0.64	184.05
Probit	81.83	62.57	0.64	184.01
Log-probit[a]	176.71	66.89	0.77	184.93
Weibull[a]	105.85	40.69	0.52	185.67
Females				
Gamma[a]	287.41	81.95	0.49	233.19
Logistic	137.58	109.45	0.53	231.99
Log-logistic[a]	340.15	95.35	0.57	232.88
Multistage (1)[a]	100.41	74.33	0.40	232.72
Probit	145.33	118.47	0.53	231.97
Log-probit[a]	336.41	143.31	0.57	232.87
Weibull[a]	240.71	80.40	0.43	233.43

[a]These models in EPA BMDS version 2.0 were fit to the rat dose-response data shown in Table 5-1 by using internal dose metrics calculated with the rat PBPK model. Details of the models are as follows: Gamma and Weibull models restrict power ≥ 1; Log-logistic and Log-probit models restrict to slope >1, multistage model restrict betas ≥ 0; lowest degree polynomial with an adequate fit is reported (degree of polynomial noted in parentheses).
[b]Bolded model is the best-fitting model in the most sensitive sex (males), which is used in the RfD derivation.

Source: Serota et al. (1986a).

The $BMDL_{10}$ from the logistic model was used as the POD for the RfD calculations (Table 5-3). This rat internal dose metric for noncancer effects was adjusted to obtain a human-equivalent internal $BMDL_{10}$ by dividing by a pharmacokinetic scaling factor based on a ratio of BWs $(BW_{human}/BW_{rat})^{0.25} = 4.09$). This scaling factor was used because the metric is a rate of metabolism rather than the concentration of putative toxic metabolites, and the clearance of these metabolites may be slower per volume tissue in the human compared with the rat (that is, total rate of removal may scale as $BW^{0.75}$, while tissue volume scales as BW^{1}).

Table 5-3. RfD for dichloromethane based on PBPK model-derived probability distributions of human drinking water exposures extrapolated from liver lesion incidence data for male rats exposed via drinking water for 2 years, based on liver-specific CYP metabolism dose metric (mg dichloromethane metabolized via CYP pathway/L liver tissue/day)

Model[a]	Rat internal $BMDL_{10}$[b]	Human internal $BMDL_{10}$[c]	HED (mg/kg-d)[d]			Human RfD (mg/kg-d)[e]
			First percentile	Fifth percentile	Mean	
Logistic	51.42	13.31	0.189	0.225	0.350	6×10^{-3}

[a]Based on the best-fitting model from Table 5-2.
[b]Rat dichloromethane PBPK model-derived internal liver dose associated with the lower bound on 10% extra risk for developing liver foci/areas of cellular alteration. Units of $BMDL_{10}$: mg dichloromethane metabolized via CYP pathway/L liver tissue/day.
[c]Human dichloromethane internal liver dose, derived by dividing the rat internal $BMDL_{10}$ by a scaling factor of 4.09 ($[BW_{human}/BW_{rat}]^{0.25}$) to account for potential interspecies pharmacokinetic differences in the clearance of metabolites. Units of $BMDL_{10}$: mg dichloromethane metabolized via CYP pathway/L liver tissue/day.
[d]PBPK model-derived distributions of daily average dichloromethane drinking water doses predicted by the PBPK model to yield an internal dose in humans equal to the dichloromethane internal $BMDL_{10}$.
[e]Human RfD, based on male rat data, derived by dividing the first percentile of HED value by a total UF of 30: 3 ($10^{0.5}$) for possible toxicodynamic differences between species, 3 ($10^{0.5}$) for variability in human toxicodynamic response, and 3 ($10^{0.5}$) for database deficiencies. The first percentile POD is a stable estimate of the lower end of the distribution. Use of this value in the lower tail of the distribution instead of the mean HED replaces use of a default UF = 3 for human toxicokinetic variability with a lower, data-derived UF equal to the ratio of the mean:1st percentile HED (0.350/0.189 = 1.85). See Section 5.1.5 for discussion of UFs.

Source: Serota et al. (1986a).

The human PBPK model (adapted from David et al. (2006), as described in Appendix B), using Monte Carlo sampling techniques, was used to calculate quantiles of human equivalent administered oral daily doses (in mg/kg-day) associated with the internal $BMDL_{10}$ values (Table 5-3), as described above in Section 5.1.2. The human model used parameter values derived from Monte Carlo sampling of probability distributions for each parameter, including MCMC-derived distributions for the metabolic parameters for the metabolism through the CYP2E1 pathway (V_{max} and K_m) and a distribution of GST metabolic rate constants that is weighted to reflect the estimated frequency of GST-T1 genotypes (20% GST-$T1^{-/-}$, 48% GST-$T1^{+/-}$, and 32% GST-$T1^{+/+}$) in the current U.S. population based on data from Haber et al. (2002). All simulations also included a distribution of CYP activity based on data from Lipscomb et al. (2003). The drinking water exposures comprised six discrete drinking water episodes for specified times and percentages of total daily intake (Reitz et al., 1997). The mean and two lower points on the distributions of human equivalent doses derived from the Serota et al. (1986a) data for male rats, using the $BMDL_{10}$ from the logistic model, are shown in Table 5-3. Although a lower value in this distribution could be calculated, this would require proportionately greater iterations to achieve numerical stability.

5.1.5. RfD Derivation—Including Application of Uncertainty Factors (UFs)

The first percentile POD is a numerically stable estimate of the lower end of the distribution. Use of this value associated with a sensitive human population addresses the uncertainty associated with human toxicokinetic variability. To derive the candidate RfD based on data from male rats, the first percentile value of the distribution of HED associated with the male rat-derived $BMDL_{10}$ was divided by a composite UF of 30 (3 [$10^{0.5}$] to account for uncertainty about interspecies toxicodynamic equivalence, 3 [$10^{0.5}$] to account for uncertainty about toxicodynamic variability in humans, and 3 [$10^{0.5}$] for database deficiencies) (Table 5-3). The resulting RfD recommended for dichloromethane is 6×10^{-3} mg/kg-day.

The uncertainty factors, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), address five areas of uncertainty:

- Uncertainty in extrapolating from laboratory animals to humans (UF_A): The use of PBPK models to extrapolate internal doses from rats to humans reduces toxicokinetic uncertainty in extrapolating from the rat liver lesion data but does not account for the possibility that humans may be more sensitive than rats to dichloromethane due to toxicodynamic differences. An UF of 3 ($10^{0.5}$) to account for this toxicodynamic uncertainty was applied, as shown in Table 5-3.

- Uncertainty about variation from average humans to sensitive humans (UF_H): The probabilistic human PBPK model used in this assessment incorporates the best available information about variability in toxicokinetic disposition of dichloromethane in humans but does not account for humans who may be sensitive due to toxicodynamic factors. Thus, an UF of 3 ($10^{0.5}$) was applied to account for possible toxicodynamic differences in sensitive humans.

- Uncertainty in extrapolating from LOAELs to NOAELs (UF_L): An UF for extrapolation from a LOAEL to a NOAEL was not applied because BMD modeling was used to determine the POD, and this factor was addressed as one of the considerations in selecting the BMR. The BMR was selected based on the assumption that it represents a minimum biologically significant change.

- Uncertainty in extrapolating from subchronic to chronic durations (UF_S): The derived RfD is based on results from a chronic-duration drinking water toxicity study such that no UF is necessary.

- Uncertainty reflecting database deficiencies (UF_D): The oral database for dichloromethane includes well-conducted chronic drinking water studies in rats (Serota et al., 1986a) and mice (Serota et al., 1986b) and a supporting subchronic study in rats and mice (Kirschman et al., 1986). These studies provided dose-response data for the hepatic effects of dichloromethane. The database also includes one-generation oral reproductive toxicity (General Electric Company, 1976) and developmental toxicity (Narotsky and Kavlock, 1995) studies that found no reproductive or developmental effects at dose levels in the range of doses associated with liver lesions. A two-generation oral exposure study is not available; however, a

two-generation inhalation exposure study by Nitschke et al. (1988b) reported no effect on fertility index, litter size, neonatal survival, growth rates, or histopathologic lesions at exposures of ≥100 ppm. This study is limited in its ability to fully evaluate reproductive and developmental toxicity, however, because exposure was not continued throughout the gestation and nursing periods.

No oral exposure studies that evaluated neurobehavioral effects in offspring were identified. This is a relevant endpoint given the neurotoxicity associated with dichloromethane exposure after oral and inhalation exposures (see Section 4.4.2 for references) and the observed behavioral changes following inhalation developmental exposure to dichloromethane (Bornschein et al., 1980; Hardin and Manson, 1980). Dichloromethane is capable of crossing the placental barrier and entering fetal circulation (Withey and Karpinski, 1985; Anders and Sunram, 1982), and activity of CYP2E1 in the brain is relatively high compared to the liver of the developing human fetus (Hines, 2007; Johnsrud et al., 2003; Brzezinski et al., 1999). Dichloromethane, as well as the metabolite CO, has been implicated in neurological effects (reviewed in Section 4.6.3.3, Mode of Action for Neurological Effects). However, PBPK modeling predicts that CO levels from exposures around the RfD would not be high enough to result in neurodevelopmental toxicity.

There are no oral exposure studies that include functional immune assays; however, there is a 4-week inhalation study of potential systemic immunotoxicity that found no effect of dichloromethane exposure at concentrations up to 5,000 ppm on the antibody response to sheep red blood cells (Warbrick et al., 2003). The Warbrick et al. (2003) data suggest that systemic immunosuppression does not result from dichloromethane exposure. Because of concerns regarding the lack of an oral two-generation reproductive study, limitations in the available inhalation two-generation reproductive study, and the adequacy of available data pertaining to possible neurodevelopmental toxicity, an UF_D of 3 was applied.

5.1.6. Previous RfD Assessment

The previous IRIS assessment derived an RfD of 0.06 mg/kg-day based on the NOAELs of 5.85 and 6.47 mg/kg-day for liver toxicity (foci/areas of cellular alteration) in male and female rats, respectively, in a 2-year drinking water study (Serota et al., 1986a). The LOAELs associated with these NOAELs were 52.58 and 58.32 mg/kg-day for males and females, respectively. The RfD of 0.06 mg/kg-day was derived by dividing the average NOAEL of 6 mg/kg-day (for male and female rats) by a composite UF of 100 (10 for intraspecies variability and 10 for interspecies variability).

5.1.7. RfD Comparison Information

Use of the mean value (3.50×10^{-1} mg/kg-day) of the HED distribution instead of the first percentile, with an additional UF of 3 ($10^{0.5}$) to account for human toxicokinetic variability, would yield an RfD of 4×10^{-3} mg/kg-day.

Additional comparisons between the derived RfD and values developed from other endpoints or data sets using NOAEL/LOAEL methods are shown in Table 5-4 and Figure 5-4. NOAELs were used as comparison PODs and were not scaled allometrically.

Table 5-4. Potential PODs with applied UFs and resulting candidate RfDs

Endpoint	POD (mg/kg-d)	POD type and description	UFs applied[a]						Candidate RfD (mg/kg-d)	Reference
			Total UF	UF_A	UF_H	UF_L	UF_S	UF_D		
Alterations in liver foci, male rats[b]	**0.189**	**First percentile HED based on $BMDL_{10}$ for increase in incidence of liver lesion**	**30**	**3**	**3**	**1**	**1**	**3**	**6×10^{-3}**	**Serota et al. (1986a)**
Neurological changes (FOB), female rats	101	NOAEL; No effect at POD, approximate doubling of severity score of neuromuscular and sensorimotor domains	3,000	10	10	1	10	3	3.4×10^{-2}	Moser et al. (1995)
Maternal weight gain, female rats	338	NOAEL; No effect at POD, approximate 33% decrease in weight gain seen at next dose	300	10	10	1	1	3	1.1	Narotsky and Kavlock (1995)

[a]An UF for extrapolation from a LOAEL to NOAEL (UF_L) was not used for any of these studies. For the Serota et al. (1986a) study, the use of the first percentile of the HED distribution as the POD replaces the use of an UF_H for human toxicokinetic variability.
[b]Bolded value is the basis for the RfD of 6×10^{-3} mg/kg-day.

UF_A = uncertainty in extrapolating from laboratory animals to humans; UF_H = uncertainty about variation from average humans to sensitive humans; UF_L = uncertainty about extrapolating from LOAEL to NOAEL; UF_S = uncertainty in extrapolating from subchronic to chronic durations; UF_D = uncertainty reflecting database deficiencies

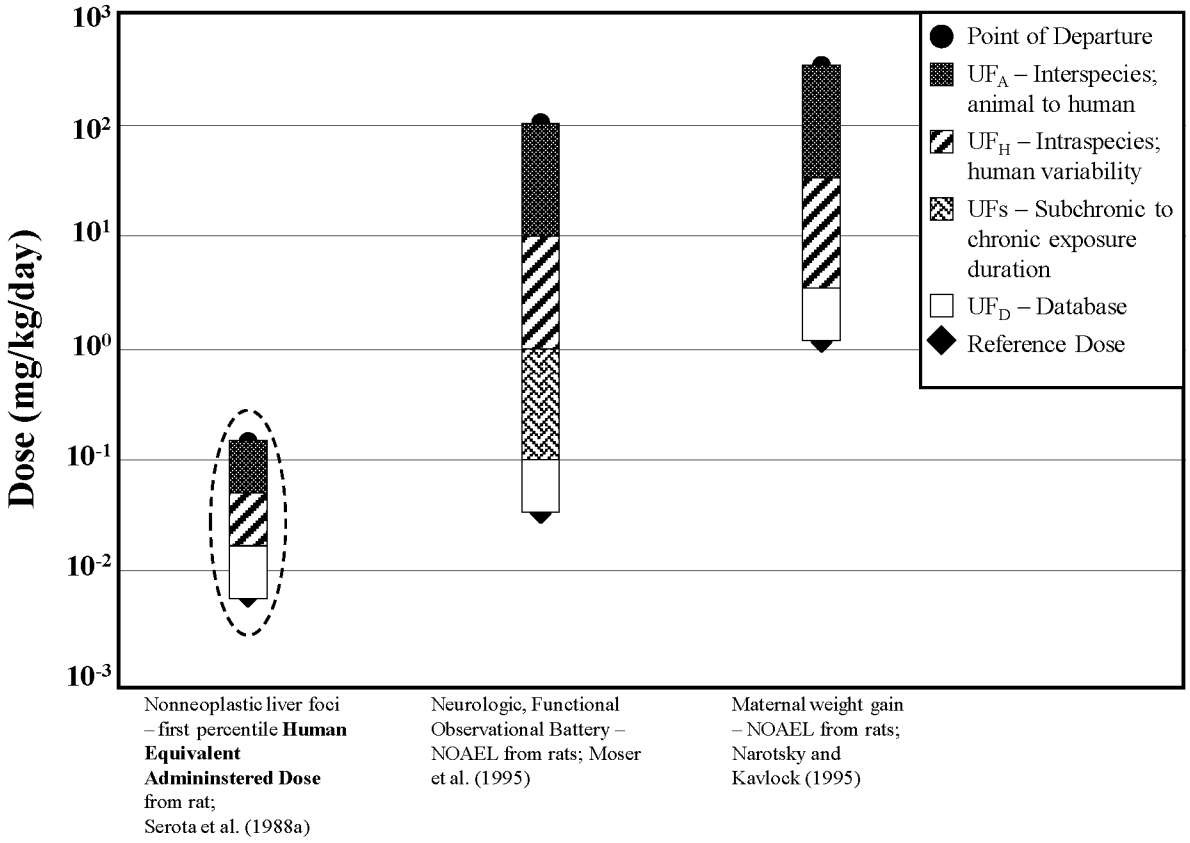


Figure 5-4. Comparison of candidate RfDs derived from selected PODs for endpoints presented in Table 5-4.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

Figure 5-5 includes exposure-response arrays from some of the human studies that were evaluated for use in the derivation of the RfC. Several acute-duration controlled exposure studies (Section 4.1.2.2) and cross-sectional occupational studies (Sections 4.1.2.3) in humans are available that show neurological effects from dichloromethane exposure. These effects include an increase in prevalence of neurological symptoms among workers (Cherry et al., 1981) and possible detriments in attention and reaction time in complex tasks among retired workers (Lash et al., 1991). However, these studies have inadequate power for the detection of effects with an acceptable level of precision. In addition, the Cherry et al. (1981) study is limited by the definition and documentation of neurological symptom history. The Lash et al. (1991) study has exposure measurements from 1974 to 1986, but the work histories of exposed workers go back to the 1940s. In addition, the outcome assessment was conducted a mean of 5 years after leaving the workplace, and so would underestimate health effects that diminish postexposure. Ott et al. (1983a) reported an increase in serum bilirubin among exposed workers, but there was no association seen with respect to the other hepatic enzymes examined (serum γ-glutamyl transferase, serum AST, serum ALT), and no evidence of hepatic effects was seen in a later study of the same cohort (Soden, 1993). Because of these limitations, these human studies of chronic exposures do not serve as an adequate basis for RfC derivation. As discussed in Section 5.2.6, however, the quantitative measures of neurological function from Cherry et al. (1983) and Lash et al. (1991) were used to derive a comparative RfC.

The database of experimental animal dichloromethane inhalation studies includes numerous 90-day and 2-year studies, with data on hepatic, pulmonary, and neurological effects, (see Table 4-27) and reproductive and developmental studies (Table 4-28) (see summary in Section 4.6.2). NOAELs, LOAELs, and the dose range tested corresponding to selected health effects from the chronic studies are shown in Figure 5-5, and effects seen in subchronic, reproductive, and developmental studies are shown in Figure 5-6. The subchronic (i.e., ≤90-day study) data were not considered in the selection of a principal study for deriving the RfC because the database contains reliable dose-response data from the chronic study at lower doses than the 90-day study. The data from the subchronic studies are, however, used to corroborate the findings with respect to relevant endpoints (i.e., hepatic and neurological effects).

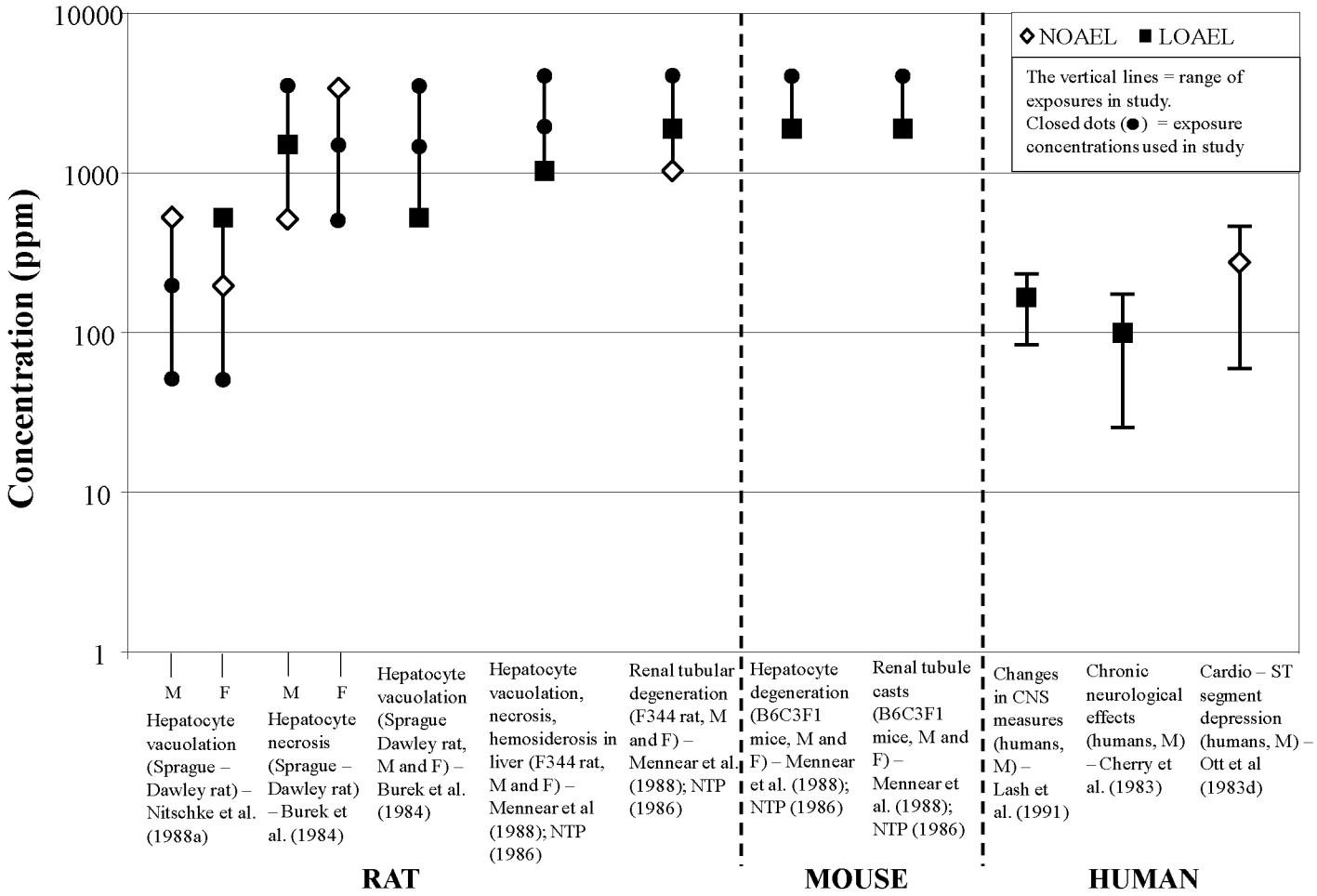


Figure 5-5. Exposure response array for chronic (animal) or occupational (human) inhalation exposure to dichloromethane (log Y axis) (M = male; F = female).

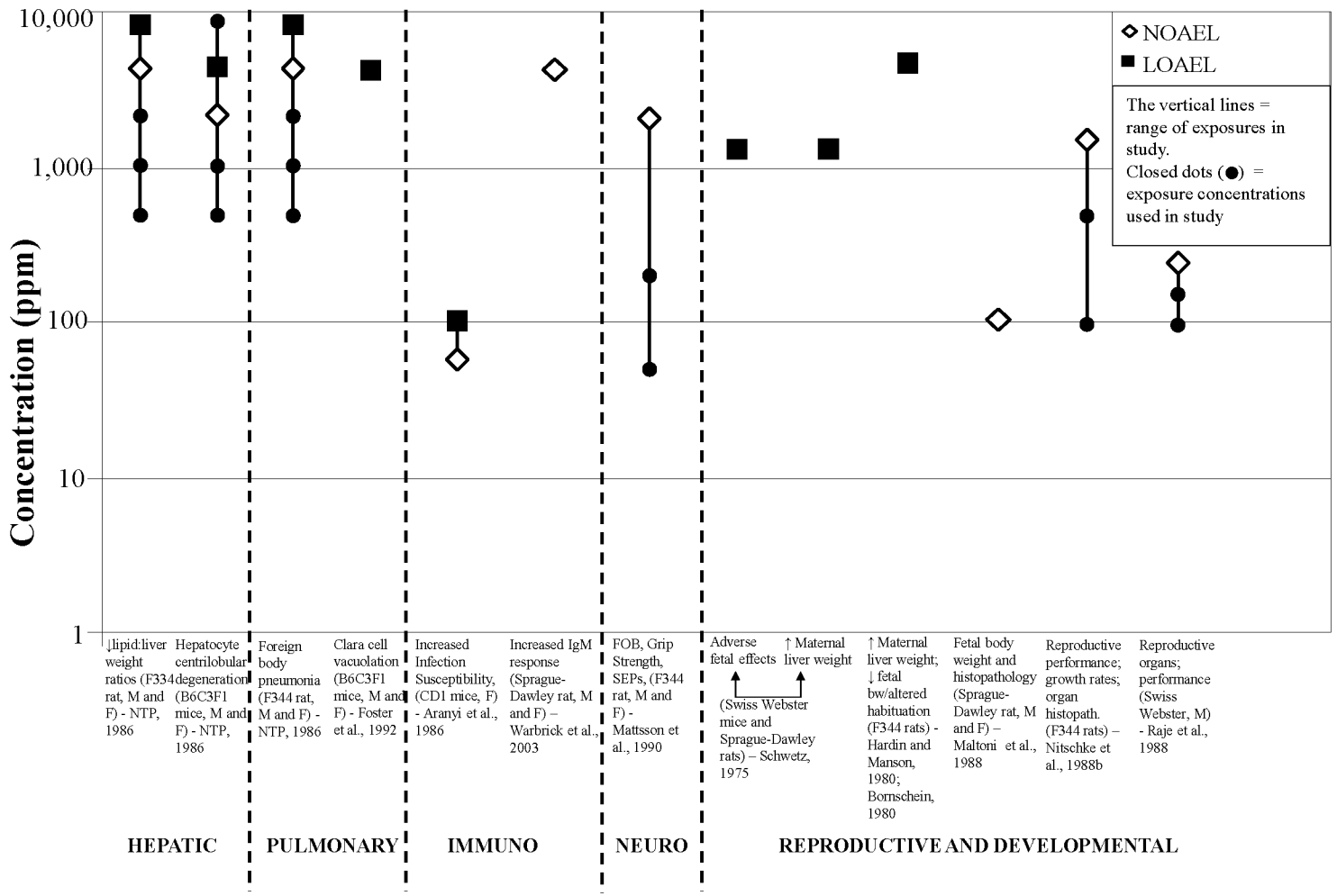


Figure 5-6. Exposure response array for subacute to subchronic inhalation exposure to dichloromethane (log Y axis) (M=male; F=female).

Hepatic effects (hepatic vacuolation and necrosis, hemosiderosis, hepatocyte degeneration) are the primary dose-dependent noncancer effects associated with inhalation exposure to dichloromethane. These effects were seen in mice (Mennear et al., 1988; NTP, 1986) and rats (Mennear et al., 1988; Nitschke et al., 1988a; NTP, 1986; Burek et al., 1984) but not in Syrian golden hamsters (Burek et al., 1984). Inhalation bioassays with Sprague-Dawley rats identified the lowest inhalation LOAEL for liver lesions in the database at 500 ppm (6 hours/day, 5 days/week for 2 years) (Nitschke et al., 1988a; Burek et al., 1984); Nitschke et al. (1988a) identified a NOAEL of 200 ppm in female rats.

Based on the results reviewed above, liver lesions (specifically, hepatic vacuolation) in rats are identified as the critical noncancer effect from chronic dichloromethane inhalation in animals. Vacuolization is defined as the process of accumulating vacuoles in a cell or the state of accumulated vacuoles and can reflect either a normal physiological response or an early toxicological process. As a normal physiological response, vacuolization is associated with the sequestration of materials and fluids taken up by cells, and also with secretion and digestion of cellular products (Henics and Wheatley, 1999). Robbins et al. (1976) characterized vacuolization (i.e., intracellular autophagy) as a normal cellular functional, homeostatic, and adaptive response. Vacuolization has also been identified as one of four principal types of chemical-induced injury (the other three being cloudy swelling, hydropic change, and fatty change) (Grasso, 2002). It is one of the most common responses of the liver following a chemical exposure; typically in the accumulation of fat in parenchymal cells, most often in the periportal zone (Plaa and Hewitt, 1998). The ability to detect subtle ultrastructural defects, such as vacuolization, early in the course of toxicity often permits identification of the initial site of the lesion and thus can provide clues to possible biochemical mechanisms involved in the pathogenesis of liver injury (Hayes, 2001). Given the range of underlying causes of hepatocellular vacuolation (from normal physiological response to indicator of chemically-induced toxicity), it is appropriate to take into consideration such factors as the characterization of vacuolization by the investigators (e.g., content of the vacuoles), changes in incidence, severity, or pattern of response with dose, other measures of toxicity in the target organ, and consistency of the observation across species and exposure routes when interpreting this response. In the case of dichloromethane, hepatocellular vacuolation was characterized by study authors as correlating with fatty change (Burek et al., 1984) or as a vacuolation of lipids in the hepatocyte (Nitschke et al., 1988a). Dose-related increases in the incidence of hepatocellular vacuolation have been observed in rats and mice following both inhalation (Mennear et al., 1988; Nitschke et al., 1988a; NTP, 1986; Burek et al., 1984; Haun et al., 1972) and oral exposure (Kirschman et al., 1986); these study investigators consistently identified vacuole content as lipid. Accumulation of lipids in the hepatocyte may lead to the more serious liver effects observed following dichloromethane exposure, such as hepatic steatosis (fatty liver) reported in dogs (Haun et al., 1971) and rats (Serota et al., 1986a). Given the liver findings for

dichloromethane in the database as a whole, the evidence is consistent with hepatic vacuolation as a precursor of toxicity. Accordingly, hepatic vacuolation is considered a toxicologically relevant and adverse effect.

The Burek et al. (1984) data support the observations from Nitschke et al. (1988a), with additional evidence of increasing risk at exposures higher than those used in the Nitschke et al. (1988a) study. In males in the Burek et al. (1984) study, the incidence of hepatic vacuolation increased from 17% in controls to 38%, 45%, and 54% in the 500, 1,500 and 3,500 ppm groups; in females, the baseline incidence was higher (34% in controls) but a similar pattern of increasing incidence was seen (52%, 58% and 65% in the 500, 1,500 and 3,500 ppm groups, respectively). Because Nitschke et al. (1988a) examined a range of exposures that included doses at the low end of the range compared with the range examined in Burek et al. (1984), the former study was selected as the principal study for derivation of a chronic inhalation RfC.

Reproductive performance (e.g., as assessed by number of litters, resorption rate, fetal survival, and growth) was not affected in two generations of F344 rats exposed to up to 1,500 ppm for 14 or 17 weeks before mating of the F0 and F1 generations, respectively (Nitschke et al., 1988b). Exposure in this study also continued from GD 0 to 21 and began again at PND 4. In addition, reproductive performance was not affected in a study of Swiss-Webster mice or Sprague-Dawley rats exposed to 1,250 ppm on GDs 6–15 (Schwetz et al., 1975). A decrease in fertility index was seen in the 150 and 200 ppm groups in a study of male Swiss-Webster mice exposed via inhalation for 6 weeks prior to mating (Raje et al., 1988), but the statistical significance of this effect varied considerably depending on the statistical test used in this analysis. Two types of developmental effects (decreased offspring weight at birth and changed behavioral habituation of the offspring to novel environments) were seen in Long-Evans rats following exposure to 4,500 ppm for 14 days prior to mating and during gestation (or during gestation alone) (Bornschein et al., 1980; Hardin and Manson, 1980). This dose was the only exposure dose used in this study. Schwetz et al. (1975) did not observe an adverse effect on gross development or soft tissue abnormalities in a study involving exposure to 1,250 ppm on GD 6 in Swiss-Webster mice or Sprague-Dawley rats, but an increase in delayed ossification of the sternebrae was seen.

Neurological impairment was not seen in lifetime rodent bioassays involving exposure to airborne dichloromethane concentrations of ≤2,000 ppm in F344 rats (Mennear et al., 1988; NTP, 1986), ≤3,500 ppm in Sprague-Dawley rats (Nitschke et al., 1988a; Burek et al., 1984), or ≤4,000 ppm in $B6C3F_1$ mice (Mennear et al., 1988; NTP, 1986). It should be noted, however, that these studies did not include standardized neurological or neurobehavioral testing. The only subchronic or chronic study in which neurobehavioral batteries were utilized found no effects in an observational battery, a test of hind-limb grip strength, a battery of evoked potentials, or brain, spinal cord, or peripheral nerve histology in F344 rats exposed to concentrations up to

2,000 ppm for 13 weeks, with the tests performed beginning 65 hours after the last exposure (Mattsson et al., 1990).

Other effects associated with lifetime inhalation exposure to dichloromethane include renal tubular degeneration and renal tubular casts in F344 rats exposed to ≥2,000 ppm (Mennear et al., 1988; NTP, 1986). No effects on histologic, clinical chemistry, urinalysis, or hematologic variables were found in Syrian golden hamsters exposed to concentrations up to 3,500 ppm for 2 years, with the exception that the mean COHb percentage of exposed hamsters was about 30% compared with values of about 3% in controls (Burek et al., 1984).

5.2.2. Derivation Process for RfC Values

The derivation process used for the RfC parallels the process described in Section 5.1.2 for RfD derivation; consideration of dose metrics was described in Section 5.1.3. As noted in the RfD discussion, the mechanistic issues with respect to noncancer health effects represent data gaps in the understanding of the health effects of dichloromethane.

5.2.3. Methods of Analysis—Including Models (PBPK, BMD, etc.)

The modified rat PBPK model of Andersen et al. (1991), described in Appendix C and also used in the derivation of the RfD (Figure 5-2), was used for calculating internal dosimetry of inhaled dichloromethane in Sprague-Dawley rats. As noted in Table 5-5, the male data were not used because the overall response (comparing the 500 ppm to the control group) was lower, and because no data pertaining to the response pattern in the lower exposure groups (50 and 200 ppm) were provided. Simulations of 6 hours/day, 5 days/week inhalation exposures used in the Nitschke et al. (1988a) study were performed to calculate average daily internal liver doses (Table 5-5). In the absence of data for group- and sex-specific BWs, reference values for Sprague-Dawley rats in chronic studies were used (U.S. EPA, 1988b).

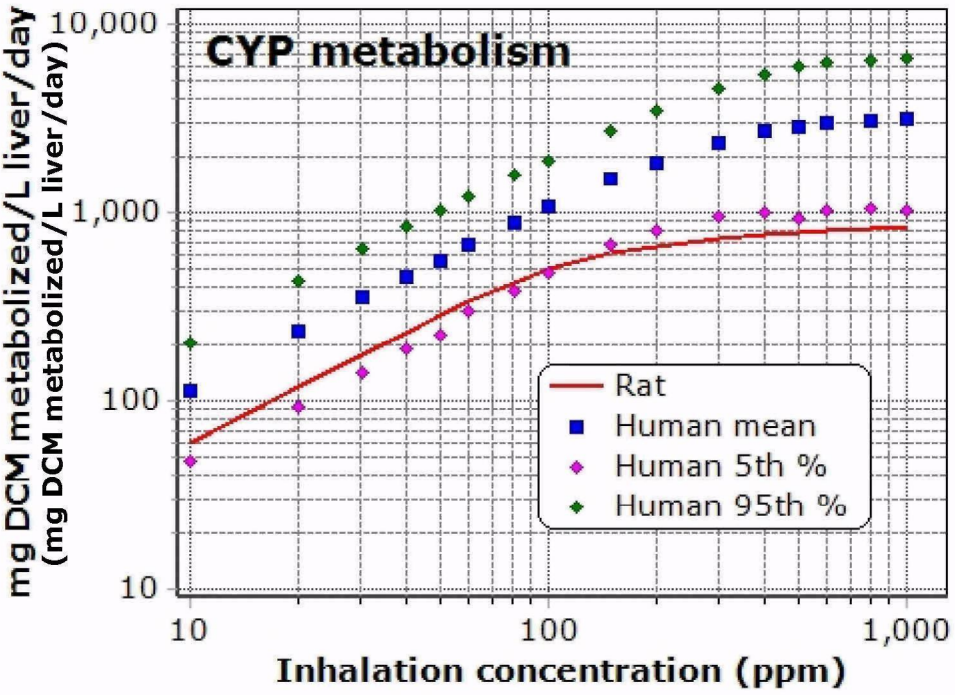
Table 5-5. Incidence data for liver lesions (hepatic vacuolation) and internal liver doses based on various metrics in female Sprague-Dawley rats exposed to dichloromethane via inhalation for 2 years

Sex	Exposure (ppm)	Liver lesion incidence[a]	Rat internal liver dose[b]			
			CYP	GST	GST and CYP	Parent AUC
Male	0	22/70 (31)	Not modeled because results from male rats were not provided for the 50 and 200 ppm groups			
	50	Not reported				
	200	Not reported				
	500	28/70 (40)				
Female (BW = 229 g)	0	41/70 (59%)	0	0	0	0
	50	42/70 (60%)	285.3	6.17	291.4	1.18
	200	41/70 (58%)	665.3	93.2	758.5	17.8
	500	53/70 (76%)[c]	782.1	360.0	1,142.1	68.6

[a]Number affected divided by total sample size.
[b]Internal doses were estimated using a rat PBPK model using exposures reported by study authors (50 ppm = 174 mg/m^3, 200 ppm = 695 mg/m^3, and 500 ppm = 1,737 mg/m^3) and are weighted-average daily values for 1 week of exposure at 6 hours/day, 5 days/week. CYP dose is in units of mg dichloromethane metabolized via CYP pathway/L tissue/day; GST dose is in units of mg dichloromethane metabolized via GST pathway/L tissue/day; GST and CYP dose is in units of mg dichloromethane metabolized via CYP and GST pathways/L tissue/day; and Parent AUC dose is in units of mg dichloromethane × hours/L tissue.
[c]Significantly ($p < 0.05$) different from control with Fisher's exact test.

Source: Nitschke et al. (1988a).

As described in Section 5.1.2, the internal dose metric used was based on total hepatic metabolism through the CYP2E1 pathway (mg dichloromethane metabolized via CYP pathway/L liver/day). Figure 5-7 shows the comparison between inhalation external and internal doses, using this dose metric for the rat and the human.



Average daily doses were calculated from simulated rat exposures of 6 hours/day, 5 days/week, while simulated human exposures were continuous. The human metabolism rates were estimated using a computational sample of 1,000 individuals per dose, including random samples of the three GST-T1 polymorphisms (+/+, +/-, -/-) in the current U.S. population based on data from Haber et al. (2002). Since a different set of samples was used for each dose, some stochasticity is evident as the human points (values) do not fall on smooth curves.

Figure 5-7. PBPK model-derived internal doses (mg dichloromethane metabolized via the CYP pathway/L liver/day) in rats and humans versus external exposures (ppm).

The seven dichotomous dose-response models available in EPA BMDS version 2.0 were fit to the female rat liver lesion incidence of Nitschke et al. (1988a) and PBPK model-derived internal dose data to derive rat internal BMD_{10} and the associated $BMDL_{10}$ values (Table 5-6). A BMR of 10% was selected because, in the absence of information regarding the magnitude of change in a response that is thought to be minimally biologically significant, a BMR of 10% is generally recommended, as it provides a consistent basis of comparison across assessments. There are no additional data to suggest that the severity of the critical effect or the power of the study would warrant a lower BMR. The log-probit model was the best fitting model for the female incidence data based on AIC value among models with adequate fit. Modeling results are shown in detail in Appendix F, Section F.2.

Table 5-6. BMD modeling results for incidence of noncancer liver lesions in female Sprague-Dawley rats exposed to dichloromethane by inhalation for 2 years, based on liver specific CYP metabolism metric (mg dichloromethane metabolized via CYP pathway/L liver tissue/day)

Model[a]	BMD_{10}	$BMDL_{10}$	χ^2 goodness of fit *p*-value	AIC
Gamma[a]	622.10	227.29	0.48	367.24
Logistic	278.31	152.41	0.14	369.77
Log-logistic[a]	706.50	506.84	0.94	365.90
Multistage (3)[a]	513.50	155.06	0.25	368.54
Probit	279.23	154.52	0.14	369.76
Log-probit[a,b]	**737.93**	**531.82**	**0.98**	**365.82**
Weibull[a]	715.15	494.87	0.95	365.88

[a]These models in EPA BMDS version 2.0 were fit to the rat dose-response data shown in Table 5-5 by using internal dose metrics calculated with the rat PBPK model. Gamma and Weibull models restrict power ≥ 1; Log-logistic and log-probit models restrict to slope >1, multistage model restrict betas ≥ 0; lowest degree polynomial with an adequate fit reported (degree of polynomial in parentheses).
[b]Bolded model is the best-fitting model in the most sensitive sex (females), which is used in the RfC derivation.

Source: Nitschke et al. (1988a).

As with the RfD derivation, the human-equivalent internal $BMDL_{10}$ was obtained by dividing this rat internal dose metric by a pharmacokinetic scaling factor based on a ratio of BWs (scaling factor = 4.09) (Table 5-7). This scaling factor was used because the metric is a rate of metabolism rather than the concentration of putative toxic metabolites, and the clearance of these metabolites may be slower per volume tissue in the human compared with the rat. (See Section 5.1.2. for a more complete discussion of this issue.) A probabilistic PBPK model for dichloromethane in humans, adapted from the model of David et al. (2006) as described in Appendix B, was then used with Monte Carlo sampling to calculate distributions of chronic HECs (in units of mg/m^3) associated with the internal $BMDL_{10}$ based on the responses in female Sprague-Dawley rats. Estimated mean, first, and fifth percentiles of this distribution are shown in Table 5-7.

Table 5-7. Inhalation RfC for dichloromethane based on PBPK model-derived probability distributions of human inhalation exposure extrapolated from liver lesion data for female rats exposed via inhalation for 2 years, based on liver-specific CYP metabolism dose metric (mg dichloromethane metabolized via CYP pathway/L liver tissue/day)

Model[a]	Rat internal $BMDL_{10}$[b]	Human internal $BMDL_{10}$[c]	HEC (mg/m^3)[d]			Human RfC (mg/m^3)[e]
			First percentile	Fifth percentile	Mean	
Log-probit	531.82	130.03	17.2	21.3	48.5	0.6

[a]Based on the best-fitting model from Table 5-6.
[b]Rat dichloromethane PBPK model-derived internal liver dose associated with lower bound on 10% extra risk for developing hepatocyte vacuolation. Units of $BMDL_{10}$: mg dichloromethane metabolized via CYP pathway/L liver tissue/day.
[c]Human dichloromethane internal liver dose, derived by dividing the rat internal $BMDL_{10}$ by a scaling factor of 4.09 ($[BW_{human}/BW_{rat}]^{0.25}$) to account for potential interspecies pharmacokinetic differences in the clearance of metabolites. Units of $BMDL_{10}$: mg dichloromethane metabolized via CYP pathway/L liver tissue/day.
[d]PBPK model-derived distributions of long-term, daily average airborne dichloromethane concentrations predicted by the PBPK model to yield an internal dose in humans equal to the dichloromethane internal $BMDL_{10}$.
[e]Human candidate RfC, based on female rat data, derived by dividing the first percentile of HEC values by a total UF of 30: 3 ($10^{0.5}$) for possible toxicodynamic differences between species, 3 ($10^{0.5}$) for variability in human toxicodynamic response, and 3 ($10^{0.5}$) for database deficiencies. The first percentile POD is a stable estimate of the lower end of the distribution. Use of this value in the lower tail of the distribution instead of the mean HEC replaces use of a default UF = 3 for human toxicokinetic variability with a lower, data-derived UF equal to the ratio of the mean:1st percentile HEC (48.54/17.21 = 2.82). Section 5.2.4 for discussion of UFs.

5.2.4. RfC Derivation—Including Application of Uncertainty Factors (UFs)

The first percentile POD is a numerically stable estimate of the lower end of the distribution. Use of this value associated with a sensitive human population addresses the uncertainty associated with human toxicokinetic variability. The RfC was calculated by dividing the first percentile of the HEC distribution in Table 5-7 by a composite UF of 30 (3 [$10^{0.5}$] to account for uncertainty about interspecies toxicodynamic equivalence, 3 [$10^{0.5}$] to account for uncertainty about toxicodynamic variability in humans, and 3 [$10^{0.5}$] for database deficiencies). The resulting RfC was 0.6 mg/m^3 based on liver lesions in female Sprague-Dawley rats in Nitschke et al. (1988a).

The uncertainty factors, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), address five areas of uncertainty:

- Uncertainty in extrapolating from laboratory animals to humans (UF_A): The use of PBPK models to extrapolate internal doses from rats to humans reduces toxicokinetic uncertainty in extrapolating from the rat liver lesion data but does not account for the possibility that humans may be more sensitive than rats to dichloromethane due to toxicodynamic differences. An UF of 3 ($10^{0.5}$) to account for this toxicodynamic uncertainty was applied, as shown previously in Table 5-7.

- Uncertainty about variation from average humans to sensitive humans (UF_H): The probabilistic human PBPK model used in this assessment incorporates the best available information about variability in toxicokinetic disposition of dichloromethane in humans but does not account for humans who may be sensitive due to toxicodynamic factors. Thus, an UF of 3 ($10^{0.5}$) was applied to account for possible toxicodynamic differences in sensitive humans.

- Uncertainty in extrapolating from LOAELs to NOAELs (UF_L): An UF for extrapolation from a LOAEL to a NOAEL was not applied because BMD modeling was used to determine the POD, and this factor was addressed as one of the considerations in selecting the BMR. The BMR was selected based on the assumption that it represents a minimum biologically significant change.

- Uncertainty in extrapolating from subchronic to chronic durations (UF_S): The derived RfC is based on results from a chronic-duration inhalation toxicity study such that no UF is necessary.

- Uncertainty reflecting database deficiencies (UF_D): An UF of 3 was selected to address the deficiencies in the dichloromethane toxicity database. The inhalation database for dichloromethane includes several well-conducted chronic inhalation studies. In these chronic exposure studies, the liver was identified as the most sensitive noncancer target organ in rats (Nitschke et al., 1988a; NTP, 1986; Burek et al., 1984). The critical effect of hepatocyte vacuolation was corroborated in the principal study (Nitschke et al., 1988a; Burek et al., 1984), both of which identified 500 ppm as the lowest inhalation LOAEL for noncancer liver lesions. Gross signs of neurologic impairment were not seen in lifetime rodent inhalation bioassays for dichloromethane at exposure levels up to 4,000 ppm (see Section 4.2.2.2 for references), and no exposure-related effects were observed 65 hours postexposure in an observational battery, a test of hind-limb grip strength, a battery of evoked potentials, or histologic examinations of nervous tissues in F344 rats exposed to dichloromethane concentrations as high as 2,000 ppm (Mattsson et al., 1990).

  A two-generation reproductive study in F344 rats reported no effect on fertility index, litter size, neonatal survival, growth rates, or histopathologic lesions at exposures ≥100 ppm dichloromethane (Nitschke et al., 1988b). Since exposure was not continuous throughout the gestation and nursing periods, however, it may not be representative of a typical human exposure and would not completely characterize reproductive and developmental toxicity associated with dichloromethane. Fertility index (measured by number of unexposed females impregnated by exposed males per total number of unexposed females mated) was reduced following inhalation exposure of male mice to 150 and 200 ppm dichloromethane 2 hours/day for 6 weeks (Raje et al., 1988), but the statistical significance of this effect varied considerably depending on the statistical test used in this analysis.

  The available developmental studies are all single-dose studies that use relatively high exposure concentrations [1,250 ppm in Schwetz et al. (1975); 4,500 ppm in Hardin and Manson (1980); and 4,500 ppm in Bornschein et al. (1980)]. In one of the single-dose studies, decreased offspring weight at birth and changed behavioral habituation of the offspring to novel environments were seen following exposure of adult Long-Evans rats to 4,500 ppm for 14 days prior to mating and during gestation (or during gestation alone) (Bornschein et al., 1980; Hardin and Manson, 1980). The

results from these single-dose developmental toxicity studies, the placental transfer of dichloromethane (Withey and Karpinski, 1985; Anders and Sunram, 1982), and the relatively high activity of CYP2E1 in the brain compared to the liver of the developing human fetus (Hines, 2007; Johnsrud et al., 2003; Brzezinski et al., 1999) raise concerns regarding possible neurodevelopmental toxicity from gestational exposure to inhaled dichloromethane. Dichloromethane, as well as the metabolite CO, has been implicated in neurological effects (reviewed in Section 4.6.3.3, Mode of Action for Neurological Effects); however, at exposures around the RfC, PBPK models have predicted that CO levels would not be high enough to result in neurodevelopmental toxicity.

In addition, Aranyi et al. (1986) demonstrated evidence of immunosuppression following a single 100 ppm dichloromethane exposure for 3 hours in CD-1 mice. This exposure is lower than the POD for the liver effects that serve as the critical effect for the RfC. This study used a functional immune assay that is directly relevant to humans (i.e., increased risk of Streptococcal pneumonia-related mortality and decreased clearance of Klebsiella bacteria). A recent study used a similar approach for the evaluation of immunosuppression from acute exposures to trichloroethylene and chloroform (Selgrade and Gilmour, 2010). Although dichloromethane was not included in this study, Selgrade and Gilmour (2010) provide support for the methodological approach used by Aranyi et al. (1986). Increases of some viral and bacterial diseases, particularly bronchitis-related mortality, is also suggested by some of the cohort studies of exposed workers (Radican et al., 2008; Gibbs et al., 1996; Lanes et al., 1993; Gibbs, 1992; Lanes et al., 1990). Systemic immunosuppression was not seen in a 4-week, 5,000-ppm inhalation exposure study measuring the antibody response to sheep red blood cells in Sprague-Dawley rats (Warbrick et al., 2003). These studies suggest a localized, portal-of-entry effect within the lung rather than a systemic immunosuppression. Because the Aranyi et al. (1986) study involved a single acute inhalation exposure, interpretation of the findings from this study in the context of chronic inhalation exposure is unclear.

In consideration of the entire database for dichloromethane, a database UF of 3 was selected. This UF accounts for limitations in the two-generation reproductive toxicity study (i.e., discontinuous exposure throughout the lifecycle) and limitations in the design of the available developmental studies (including a lack of neurodevelopmental endpoints). There is an additional potential concern for immunological effects as suggested by a single acute inhalation study, specifically immunosuppressive effects that may be relevant for infectious diseases spread through inhalation.

5.2.5. Previous RfC Assessment

No RfC was derived in the previous IRIS assessment.

5.2.6. RfC Comparison Information

Use of the mean value on the HEC distribution (48.5 mg/m^3) with an additional UF of 3 ($10^{0.5}$) to account for human toxicokinetic variability would yield an RfC of 0.5 mg/m^3.

Additional comparison RfCs were derived based on neurological endpoints from human occupational exposures. Cherry et al. (1983) compared 56 exposed and 36 unexposed workers at

an acetate film manufacturing plant for dichloromethane inhalation exposure, blood levels of dichloromethane, subjective self-reporting of general health, and two objective, quantitative measurements of neurological function (digit symbol substitution and simple reaction time). The exposed and unexposed individuals were matched to within 3 years of age. The measured dichloromethane concentrations from personal breathing zone sampling of the exposed workers ranged from 28 to 173 ppm. No information on exposure duration was given, and Cherry et al. (1983) did not indicate if the exposure measurements were indicative of historical exposure levels. There were no significant differences between exposed and unexposed workers based on visual analog scales of sleepiness, physical and mental tiredness, and general health or on tests of reaction time or digit substitution conducted at the beginning of a workshift. Exposed workers showed a slightly slower (but not statistically significant) score than the control workers on a reaction time test, but the scores did not deteriorate during the shift. During a workshift, the exposed workers deteriorated more on each of the scales than did the controls, and a significant correlation was shown between change in mood over the course of the shift and level of dichloromethane in the blood (correlation coefficients –0.37, –0.31, –0.43, and –0.50 for sleepiness, general health, mental exhaustion, and physical exhaustion, respectively; $p < 0.05$). Deterioration in the digit substitution tests at the end of the shift was also significantly related to blood dichloromethane levels (correlation coefficients = –0.37, $p < 0.01$) among the exposed workers. In the absence of data for the mean exposure levels, the exposure range midpoint of 101 ppm serves as a LOAEL for chronic neurological effects from dichloromethane exposure based on this study. A candidate RfC of 1.2 mg/m^3 was derived by dividing the NOAEL of 351 mg/m^3 (101 ppm) by a composite UF of 300 to account for potentially susceptible individuals in the human population (10), extrapolation from a LOAEL to a NOAEL (10), and database uncertainties (3). Limitations of this study include lack of information on duration of exposures and evaluation of a limited number of endpoints.

Another candidate RfC was developed by using the neurological data of potential long-term CNS effects in a study of retired aircraft maintenance workers (Lash et al., 1991). Retired aircraft maintenance workers, ages 55–75 years, employed in at least 1 of 14 targeted jobs (e.g., paint strippers) with dichloromethane exposure for ≥6 years between 1970 and 1984 (n = 25) were compared to a like group of workers without dichloromethane exposure (n = 21); the mean duration of retirement was 5 years in both groups. From 1974 to 1986, when 155 measurements of dichloromethane exposure were made, mean breathing zone TWAs ranged from 82 to 236 ppm and averaged 225 ppm for painters and 100 ppm for mechanics; information on exposure levels prior to this time was not provided, although the work histories of exposed workers goes back to the 1940s. The evaluation included several standard neurological tests, including physiological measurement of odor and color vision senses, auditory response potential, hand grip strength, measures of reaction time (simple, choice, and complex), short-term visual memory and visual retention, attention, and spatial ability. The exposed group had a

higher score on verbal memory tasks (effect size approximately 0.45, $p = 0.11$) and lower score on attention tasks (effect size approximately –0.55, $p = 0.08$) and complex reaction time (effect size approximately –0.40, $p = 0.18$) compared with the control group. None of these differences were statistically significant. Given the sample size, however, the power to detect a statistically significant difference between the groups was low (i.e., approximately 0.30 for an effect size of 0.40 using a two-tailed alpha of 0.05), and these results cannot be taken as evidence of no effect. An estimated exposure level from the study can be generated from the midpoint value from the exposure range (82–236 ppm; midpoint = 159 ppm), converted to 552 mg/m^3. If these results are viewed as a LOAEL, application of a composite UF of 300 (10 to account for potentially susceptible individuals in the human population, 10 for extrapolation from a LOAEL to a NOAEL, and 3 for database uncertainties to this estimated mean exposure level of 552 mg/m^3 would result in an RfC of 1.8 mg/m^3.

The value of the candidate RfC based on the data from Cherry et al. (1983), 1.2 mg/m^3, and the value of the candidate RfC based on the data from Lash et al. (1991), 1.8 mg/m^3, are approximately twofold and threefold higher, respectively, than the derived RfC of 0.6 mg/m^3 based on liver lesions in rats. The animal-derived RfC is preferable to the human-derived RfC because of the uncertainties about the characterization of the exposure, influence of time since exposure, effect sizes, and statistical power in the epidemiologic studies.

Additional comparisons among the RfC and candidate values developed from other endpoints or data sets using NOAEL/LOAEL methods are shown in Table 5-8 and Figure 5-8.

Table 5-8. Potential PODs with applied UFs and resulting candidate RfCs

Endpoint	POD (mg/m^3)[a]	POD Type and Description[b]	UFs[c]						RfC (mg/m^3)	Reference
			Total UF	UF_A	UF_H	UF_L	UF_S	UF_D		
Hepatocyte vacuolation, female rat[d]	**17.2**	**First percentile human equivalent concentration based on $BMDL_{10}$ for increase in incidence of liver lesion**	**30**	**3**	**3**	**1**	**1**	**3**	**0.6**	**Nitschke et al. (1988a)**
Renal tubular degeneration; NOAEL, male rat	620	NOAEL	100	3	10	1	1	3	6.2	Mennear et al. (1988); NTP (1986)
Reproductive - fertility index; NOAEL, male mouse	20.7	No effect at POD, 16% decrease in fertility index seen at LOAEL dose	100	3	10	1	1	3	0.21	Raje et al. (1988)
Increased infection susceptibility (mortality risk), female mouse	15.5	NOAEL	1,000	3	10	1	10	3	0.016	Aranyi et al. (1986)
Increased IgM production, male and female rat	17,366	NOAEL	1,000	3	10	1	10	3	17.4	Warbrick et al. (2003)
Chronic CNS effects, human male	351	NOAEL	300	1	10	10	1	3	1.2	Cherry et al. (1983)
CNS changes, human male	552	LOAEL	300	1	10	10	1	3	1.8	Lash et al. (1991)

[a]For Nitschke et al. (1988a), this is based on BMD modeling of a 10% increase in liver lesions using internal liver dose metric (mg dichloromethane metabolism via CYP pathway/L liver tissue/day) derived from a rat PBPK model. After an allometric scaling factor of 4.09 was applied, the human internal $BMDL_{10}$ was 130 mg dichloromethane metabolism via CYP pathway/L liver tissue/day. A probabilistic human PBPK model adapted from David et al. (2006) was used to generate a distribution of HECs (in units of mg/m^3) from the human internal $BMDL_{10}$, and the first percentile of this distribution was used as the POD. For other rodent studies, the NOAEL or LOAEL concentration, in mg/m^3, was adjusted to a continuous exposure taking into account hours/day and days/week of exposure. This adjusted exposure was then converted to an HEC by multiplying the value by a dosimetric adjustment factor (DAF). PBs were 8.24 for humans, 19.8 for rats, and 23 for mice. Since the PBs for both the mice and rats were greater than for humans, a DAF of 1 is recommended and was used. NOAELs or LOAELs were used as PODs in human studies since the concentrations were already human exposures.

[b]Extra risk defined for incidence data as $(Incidence_1 - Incidence_0)/(1-Incidence_0)$, where 1 = dose at observed increased and 0 = background incidence.

[c]UF_A = uncertainty in extrapolating from laboratory animals to humans, UF_H = uncertainty about variation from average humans to sensitive humans, UF_L = uncertainty about extrapolating from LOAEL to NOAEL, and UF_D = uncertainty reflecting incompleteness of the overall database. An UF extrapolating from subchronic to chronic durations (UF_S) was not used for any of these studies.

[d]Bolded value is the basis of the RfC of 0.6 mg/m^3.

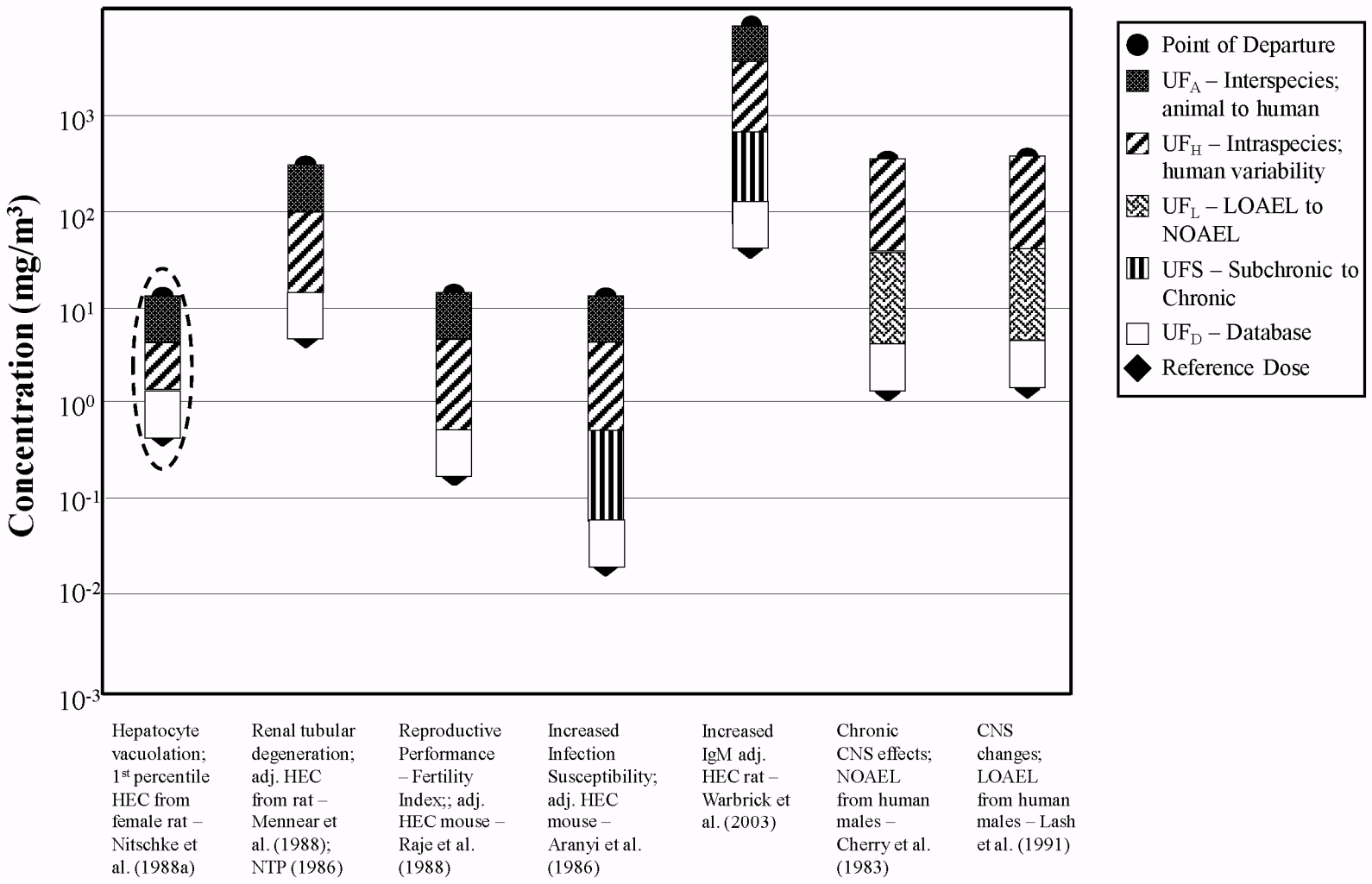


Figure 5-8. Comparison of candidate RfCs derived from selected PODs for endpoints presented in Table 5-8.

5.3. UNCERTAINTIES IN THE ORAL REFERENCE DOSE AND INHALATION REFERENCE CONCENTRATION

Risk assessments need to include a discussion of uncertainties associated with the derived toxicity values. For dichloromethane, uncertainties related to inter- and intraspecies differences in toxicodynamics and database deficiencies are treated quantitatively via the UF approach (U.S. EPA, 1994). Uncertainties in the toxicokinetic differences of dichloromethane between species and within humans are reduced by application of the PBPK models for rats and humans. These issues are discussed in Sections 5.1.5 (RfD) and 5.2.4 (RfC). Other areas of uncertainty in the derived RfD and RfC are discussed below.

Dose-response modeling. The selection of the BMD model(s) for the quantitation of the RfD and RfC does not lead to significant uncertainty in estimating the POD. It should be noted, however, that a level of uncertainty is inherent given the lack of data in the region of the BMR.

Interspecies extrapolation of dosimetry and risk. The extrapolation of internal dichloromethane dosimetry from liver lesions in rats to human risk was accomplished using PBPK models for dichloromethane in rats and humans. Uncertainties in rat and human dosimetry used for RfD and RfC derivation can arise from uncertainties in the PBPK models with regard to accurately simulating the toxicokinetics of dichloromethane for animals under bioassay conditions and humans experiencing relatively low, chronic environmental exposures. Specific uncertainties regarding the model structure are described in detail in Section 3.5.5. A structural uncertainty previously discussed arises from the indication by various data that the standard Michaelis-Menten equation used in the existing model may not accurately describe the CYP2E1-catalyzed oxidation of dichloromethane. An alternate equation described by Korzekwa et al. (1998) may better represent CYP2E1-induced oxidation of dichloromethane, which would lead to a higher fraction of total dichloromethane predicted to be metabolized by CYP2E1 at higher dichloromethane doses (or exposures). Since this shift in predicted metabolism would occur for both the human and rodent PBPK models, if the alternate equation was applied, it is difficult to estimate the net impact of using this equation on risk predictions. As described in Section 3.5.5 and Appendix C, the error in the ratio of GST:CYP metabolism at low concentrations appears to be less than 13% based on comparison of model predictions to CO metabolism data. Further, analysis of the GST-mediated metabolism of dichloromethane measured by Reitz et al. (1989a) shows that those results are within a factor of three of the GST kinetic parameters used in the current PBPK model, indicating that any error in the GST:CYP balance is no greater than that.

Also as discussed in detail in Section 3.5.2, there appears to be inconsistency in the numerical results of David et al. (2006) for the liver GST activity (coefficient), k_{fC}, between that obtained for each published data set when analyzed separately and that obtained for the

combined data set. Since the numerical average of the mean k_{fC} values for the four data sets included in the combined data set was 12.4 and the upper bound was 12, the impact of using an intermediate value of k_{fC}, specifically the DiVincenzo and Kaplan (1981) value of 5.87 $kg^{0.3}$/hour was explored. Changing only the k_{fC} is not realistic since the dichloromethane data effectively define total metabolism (sum of CYP and GST pathways), and there is naturally a negative correlation between the predicted CYP metabolic rate and the GST metabolic rate required to describe this total. It would be inconsistent with the dichloromethane data to increase k_{fC} without adjusting the CYP metabolic rate downward and likewise all other parameters. Therefore, for consistency, the distributions for all of the fitted parameters were rescaled by the ratio of the mean for DiVincenzo and Kaplan (1981) to the mean for the combined data set (e.g., the distribution for V_{maxC} was multiplied by 10.2/9.42 and the distribution for k_{fC} was multiplied by 5.87/0.852, the respective ratios of the two posterior means for each parameter). The HEC and HED calculations (to which the RfC and RfD are proportional) increased by only 25 and 12%, respectively, for the mixed GST-T1 population. Thus the impact of this model uncertainty appears to be modest for the noncancer assessment.

The dose metric used in the models is the rate of metabolism to a putative toxic metabolite rather than an average or AUC of the metabolite concentration, so the model specifically fails to account for rodent-human differences in clearance or removal of the toxic metabolite. Therefore, a scaling factor based on BW ratios was used to account for this difference. The rationale for use of this scaling factor is discussed in Section 5.1.2.

Sensitivity analysis of rat model parameters. The rat model was modified and utilized in a deterministic manner. Data were not available to perform a hierarchical Bayesian calibration in the rat. Thus, uncertainties in the rat model predictions had to be assessed qualitatively. To address these uncertainties, a sensitivity analysis was conducted to determine which model parameters most influence the predictions for a given dose metric and exposure scenario.

Sensitivity is a measure of the degree to which a given model output variable (i.e., dose metric) is influenced by perturbation in the value of model parameters. The approach implemented was a univariate analysis in which the value of an individual model parameter was perturbed by an amount (Δ) in the forward and reverse direction (i.e., an increase and decrease from the nominal value), and the change in the output variable was determined. Sensitivity coefficients were calculated as follows:

$$f'(x) \approx \frac{f(x+\Delta x) - f(x)}{\Delta x} \cdot \frac{x}{f(x)} \qquad \text{(Eq. 5-1)}$$

where x is the model parameter, $f(x)$ is the output variable, Δx is the perturbation of the parameter from the nominal value, and $f'(x)$ is the sensitivity coefficient. In equation 5-1, the sensitivity coefficients are scaled to the nominal value of x and $f(x)$ to eliminate the potential effect of units of expression. Therefore, the sensitivity coefficient is a measure of the proportional (unitless) change in the output variable produced by proportional change in the parameter value. Parameters that have higher sensitivity coefficients have greater influence on the output variable. They are considered more sensitive than parameters with lower values. The results of the sensitivity analysis are useful for assessing uncertainty in model predictions, based on the level of confidence or uncertainty in the model parameter(s) to which the dose metric is most sensitive.

Sensitivity coefficients for the noncancer dose metric (mg dichloromethane metabolized via CYP-mediated pathway/L liver/day) were determined for each of the model parameters. Sensitivity analyses for both oral and inhalation exposures were performed. The exposure conditions were set to be near or just below the lowest bioassay exposure resulting in significant increases in the critical effect.

For the CYP-mediated metabolism from oral exposure, the liver volume (VLC) and slowly perfused tissue volume (VSC) parameters exert the largest influence (Figure 5-9). The high influence of these two parameters was due to the fact that the dose metric is a tissue-specific rate of metabolism, the majority of CYP metabolism is attributed to the liver, and changes in liver volume have a greater impact on the total CYP metabolism than the individual V_{maxC} value. For inhalation exposures, V_{maxC}, VLC, and VSC have the highest sensitivity coefficients (Figure 5-10). The physiological parameters (VLC and VSC) are known with a high degree of confidence (Brown et al., 1997). V_{maxC} for the rat was estimated by fitting to the pharmacokinetic data as described in Section 3 and Appendix C, subject to model structure/equation uncertainties as detailed above, and hence is known with less certainty than the physiological parameters. That total exhaled CO, which is specific to the CYP pathway, is within 50% of measured levels (Figure C-9, panel C), and provides a similar level of confidence in the balance between CYP and GST pathways predicted by the rat PBPK model.

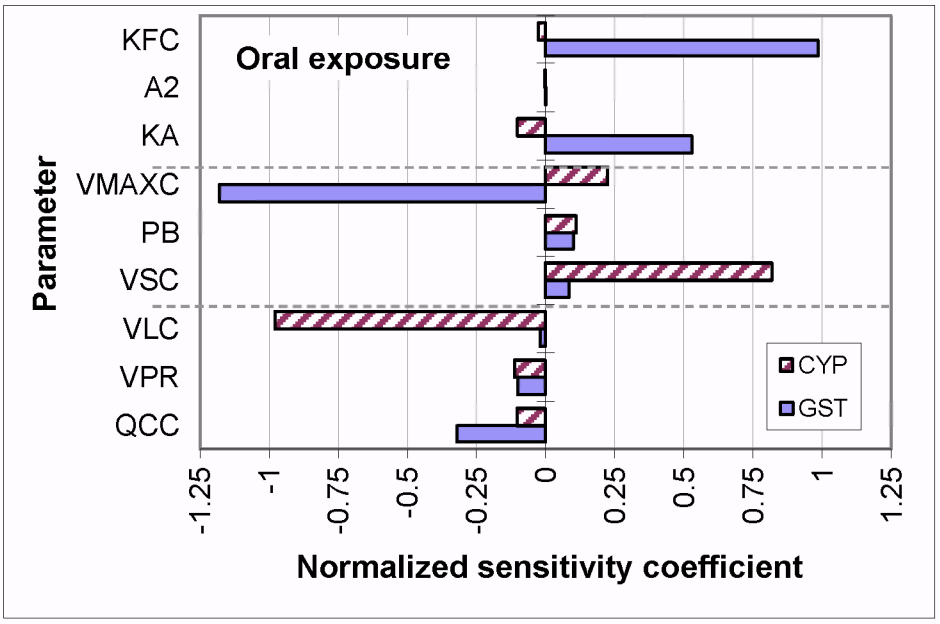
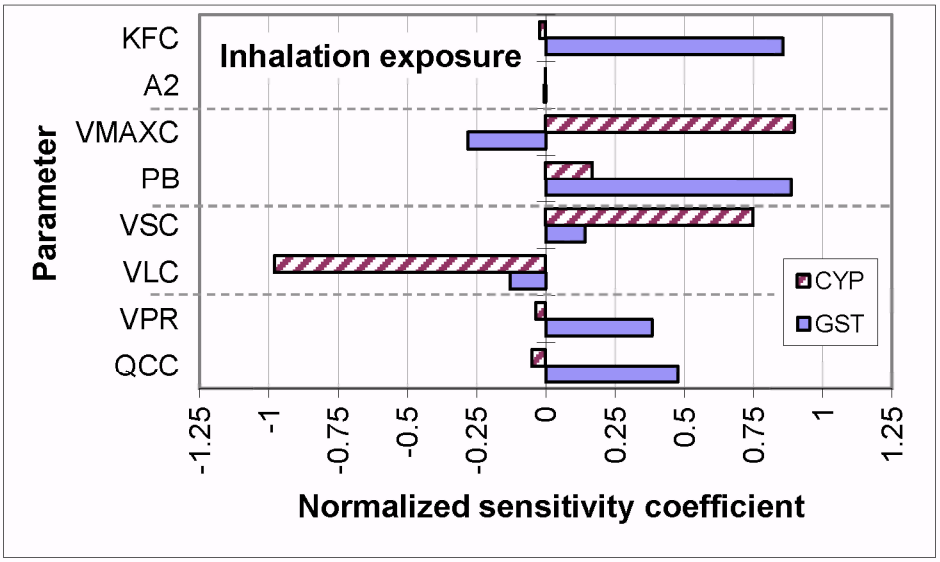


Figure 5-9. Sensitivity coefficients for long-term mass CYP- and GST-mediated metabolites per liver volume from a daily drinking water concentration of 10 mg/L in rats.



(KA is not included since it has no impact on inhalation dosimetry.)

Figure 5-10. Sensitivity coefficients for long-term mass CYP- and GST-mediated metabolites per liver volume from a long-term average daily inhalation concentration of 500 ppm in rats.

In summary, the uncertainties associated with use of the rat PBPK model should not markedly affect the values (i.e., an effect of no more than 30%) of the RfD and RfC based on the metrics considered. An additional uncertainty results from the lack of knowledge concerning the most relevant dose metric (e.g., a specific metabolite) for the noncancer endpoints considered.

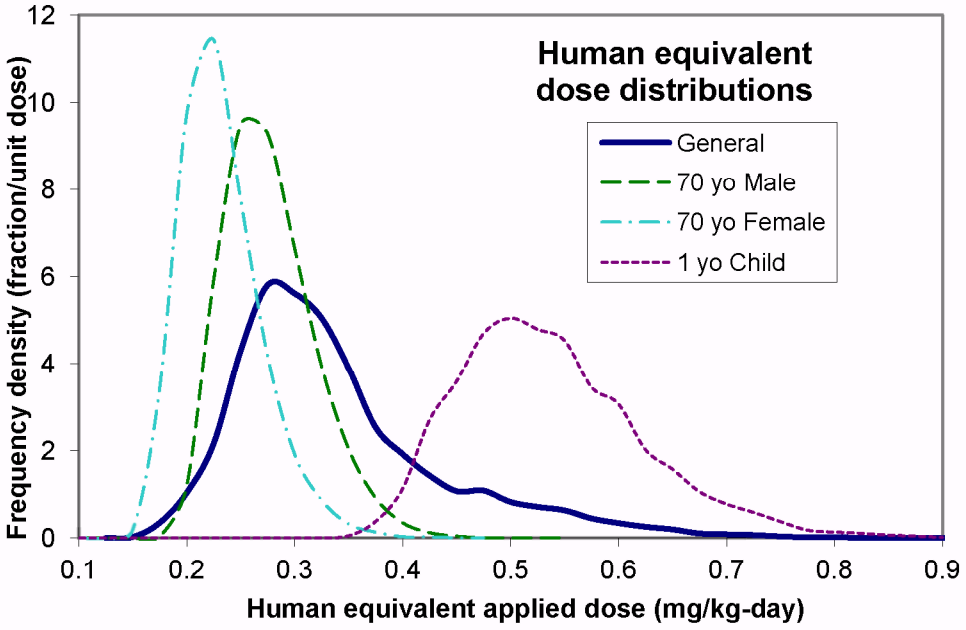
This basic research question represents a data gap. This uncertainty was addressed by considering different dose metrics (CYP metabolism alone, GST metabolism alone, sum of GST and CYP, and the AUC of the parent compound). The GST metabolism and the AUC dose metrics did not present reasonable choices based on model fit and consistency of response across studies at comparable dose levels. Given these results, the combination of hepatic metabolism through the GST and the CYP pathways would not be expected to result in an improvement to a metric based only on CYP metabolism. The CYP-metabolism dose metric seems to be most consistent with the data, and so is the metric chosen for the RfD and RfC derivations.

Sensitive human populations. The potential for sensitivity to dichloromethane in a portion of the human population due to pharmacokinetic differences was addressed quantitatively by using a human probabilistic PBPK model, as modified by EPA, to generate distributions of human exposures likely to result in a specified internal $BMDL_{10}$. The model and resulting distributions take into account the known nonchemical-specific variability in human physiology as well as total variability and uncertainty in dichloromethane-specific metabolic capability. The first percentile values of the distributions of HEDs (Table 5-3) and HECs (Tables 5-7) served as PODs for candidate RfDs and RfCs, respectively, to protect toxicokinetically sensitive individuals. Selection of the first percentile allows generation of a numerically stable estimate for the lower end of the distribution. The mean value of the human equivalent oral dose in Table 5-3 was about twofold higher than the corresponding first percentile values, and the mean value of human equivalent inhalation concentration in Table 5-7 was approximately threefold higher than the first percentile value. The internal dose metric in the analyses described in these tables was the mg dichloromethane metabolized via the CYP pathway/L liver/day, and thus the comparisons of the first percentile and mean values give estimates of the amount of variability in the population to metabolize dichloromethane by the CYP metabolic pathways on a liver-specific basis. The mean:first percentile ratios for these distributions are attributed to the dependence of the dose metric on hepatic blood flow rate (metabolism being flow-limited). This blood flow is expected to be highly and tightly correlated with liver volume, resulting in very similar delivery of dichloromethane per volume liver across the population. The mean:first percentile ratio for the oral distribution is 1.85, which is less than the default intra-human toxicokinetic UF of 3. The population-structured distributions for physiological parameters and broadened distributions for metabolic parameters used here provide a good degree of confidence that the population variability has not been underestimated.

The internal dose metric used in the RfD and RfC derivations was based on the rate of CYP metabolism. GST-T1 polymorphisms could affect this rate, as the GST-T1 null genotype would be expected to result in an increase in the metabolism through the CYP pathway, resulting in a greater sensitivity to a CYP-related effect. The effect of GST variability on the RfD and RfC values was examined by comparing results obtained specifically for the GST-T1 null

genotype to those obtained for the population of mixed genotypes. The values for HEDs and HECs were very similar for these two groups (e.g., mean HEC 47.36 and 47.49 for the mixed and the GST-T1$^{-/-}$ null genotypes, respectively; first percentile HEC 16.63 and 16.69 for the mixed and the GST-T1$^{-/-}$ null genotypes, respectively), and use of this population would not result in a change in the recommended RfD or RfC.

As a further level of sensitivity analysis, model predictions of the HED for the general population, as listed in Table 5-3 (estimates covered 0.5–80-year-old male and female individuals), were compared to three subpopulations: 1-year-old children (males and females), 70-year-old men, and 70-year-old women. For the general population and each subpopulation, a Monte Carlo simulation representing 10,000 individuals was conducted, and histograms of the resulting distribution of HEDs are shown in Figure 5-11, with corresponding statistics in Table 5-9. All groups used in these comparisons were limited to the GST-T1$^{-/-}$.



All groups were restricted to the GST-T1$^{-/-}$ population.

Figure 5-11. Frequency density of HEDs in specific populations in comparison to a general population (0.5- to 80-year-old males and females) estimate for an internal dose of 12.57 mg dichloromethane metabolized by CYP/L liver/day.

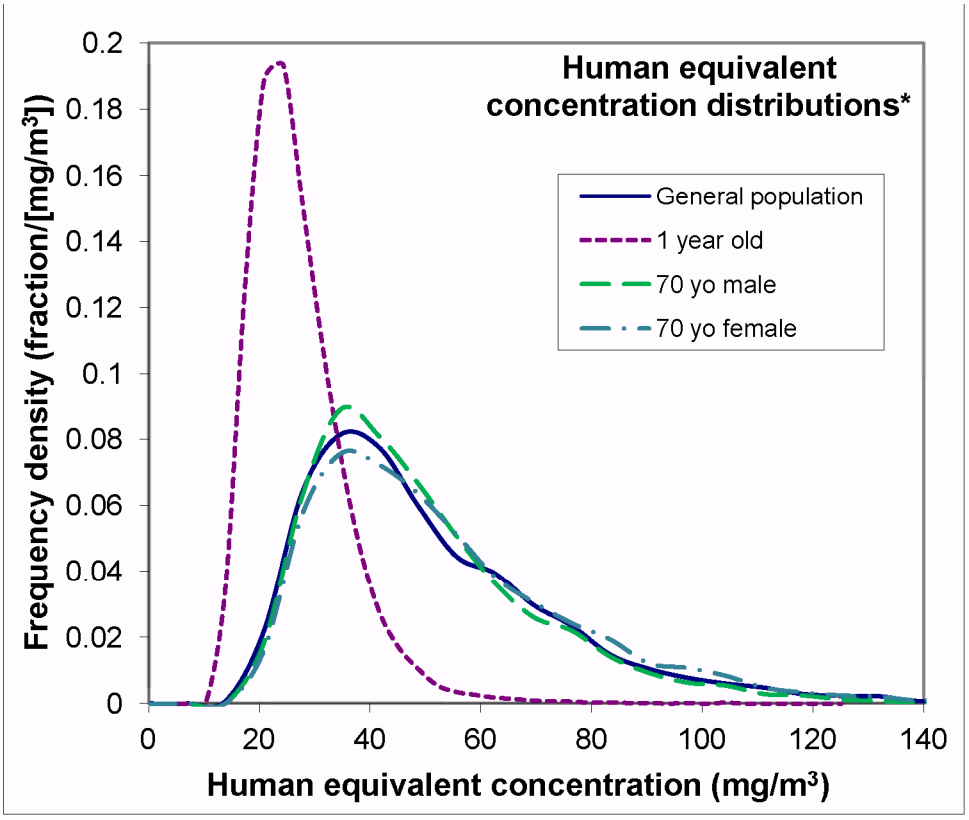
Table 5-9. Statistical characteristics of HEDs in specific populations of the GST-T1$^{-/-}$ group

Population	HED (mg/kg-d)[a]		
	Mean	Fifth percentile	First percentile
All ages[b]	3.31×10^{-1}	2.11×10^{-1}	1.79×10^{-1}
1-yr-old children	5.25×10^{-1}	4.06×10^{-1}	3.74×10^{-1}
70-yr-old men	2.64×10^{-1}	2.59×10^{-1}	2.06×10^{-1}
70-yr-old women	2.20×10^{-1}	1.68×10^{-1}	1.55×10^{-1}

[a]Exposure levels predicted to result in 15.1 mg dichloromethane metabolized via CYP pathway/L liver/day (based on $BMDL_{10}$ from the best-fitting model from Table 5-2; human dichloromethane internal liver dose, derived by dividing the rat internal $BMDL_{10}$ by a scaling factor of 4.09 $[(BW_{human}/BW_{rat})^{0.25}]$ to account for potential interspecies pharmacokinetic differences in the clearance of metabolites).
[b]0.5–80-yr-old males and females.

The results shown above for differences in HED values in different populations are qualitatively what would be expected: a relatively broad distribution for the general population with specific populations representing narrower components of that distribution. There are some differences between men and women at 70 years of age, but neither of these would be greatly misrepresented by the general population estimate. While 1-year-old children represent more of a distinct tail in the general population, in this case, the distribution of HECs in the general estimate is lower than that seen in what would otherwise be considered a more sensitive population. This difference most likely results from the higher specific respiration rate in children versus adults, which allows them to eliminate more of orally ingested dichloromethane by exhalation, leading to lower internal metabolized doses.

A similar comparison was made for inhalation HEC values, as shown in Figure 5-12 and Table 5-10. For HEC values, the distributions for 70-year-old men and women are both virtually indistinguishable from the general population, and while 1-year-old children are clearly distinct, they are less different than in the HED comparison and, in this case, are more sensitive than the population in general. As described in detail in Appendix B, the allometric alveolar ventilation constant (QAlvC) is about 28 L/hour-kg$^{0.75}$ in a 1-year-old child but averages around 14 L/hour-kg$^{0.75}$ in an adult. Combining this with the difference between a BW of 10 kg in the child and 70 kg in an "average" adult, the respiration rate per kg BW is about threefold higher in the child versus adult. As noted above, for oral exposures, this leads to faster elimination by respiration in children, while for inhalation exposures it leads to higher uptake for a given air concentration.



*All groups restricted to the GST-T1$^{-/-}$ population.

Figure 5-12. Frequency density of HECs in specific populations in comparison to a general population (0.5- to 80-year-old males and females) estimate for an internal dose of 130.0 mg dichloromethane metabolized by CYP/L liver/day.

Table 5-10. Statistical characteristics of HECs in specific populations of the GST-T1$^{-/-}$ group

Population	HEC (mg/m^3)[a]		
	Mean	Fifth percentile	First percentile
All ages[b]	48.7	21.2	16.7
1-yr-old children	25.2	14.5	12.2
70-yr-old men	46.9	21.8	17.9
70-yr-old women	51.0	22.3	18.2

[a]Exposure levels predicted to result in 128.1 mg dichloromethane metabolized via CYP pathway/L liver/day (based on $BMDL_{10}$ from the best-fitting model from Table 5-6; human dichloromethane internal liver dose, derived by dividing the rat internal $BMDL_{10}$ by a scaling factor of 4.09 ($[BW_{human}/BW_{rat}]^{0.25}$) to account for potential interspecies pharmacokinetic differences in the clearance of metabolites).
[b]0.5–80-year-old males and females.

The lack of difference in elderly adults versus the general population in HEC values is likely due to the fact that the rate of exposure and rates of metabolism (the latter being the key dose metric) both scale as $BW^{0.75}$, with the scaling coefficients being either similar (respiration) or identical (metabolism) among adults who comprise the majority of the population. Hepatic CYP-mediated metabolism is relatively stable with increasing age (Bebia et al., 2004; Schmucker, 2001). For oral exposures, the exposure rate is normalized to total BW and scales as BW^1, while elimination routes increase as $BW^{0.75}$. Moreover, oral exposures are simulated as occurring in a series of bolus exposures (drinking episodes) during the day, and the higher body-fat content occurring in the elderly (see Appendix B) means that such a dose that might saturate metabolism and, therefore, have a higher fraction exhaled in a leaner individual will tend to be more sequestered in fat and slowly released, resulting in a higher fraction metabolized (less saturation of metabolism) in a more obese individual. The difference among adults of different ages for dosimetry from oral ingestion (bolus exposure) will be greater than the difference for inhalation exposures. More careful examination of Figure 5-12 shows that the distribution for 70-year-old women, for whom the fat fraction is estimated to be greatest, has a lower peak and higher upper tail than for the general population. Thus, the physiological differences have some impact that is qualitatively consistent with what is seen from oral exposure, given the mechanistic considerations described here, but the impact of those differences is less for inhalation exposure.

No data are available regarding toxicodynamic differences within a human population. Therefore, an UF of 3 for possible differences in human toxicodynamic responses is intended to be protective for sensitive individuals.

5.4. CANCER ASSESSMENT

5.4.1. Cancer Oral Slope Factor

5.4.1.1. *Choice of Study/Data—with Rationale and Justification*

No human data are available for the quantification of potential neoplastic effects from oral exposures to dichloromethane. In the only chronic (2-year) oral exposure cancer bioassay, significant increases in the incidence of liver adenomas and carcinomas were observed in male B6C3F$_1$ mice exposed by drinking water, with incidence rates of 19, 26, 30, 31, and 28% in groups with estimated mean intakes of 0, 61, 124, 177, and 234 mg/kg-day, respectively (trend p-value = 0.058) (Table 4-29) (Serota et al., 1986b; Hazleton Laboratories, 1983). Incidences of liver tumors in female mice were not presented in the summary reports, but it was reported that exposed female mice did not show increased incidences of proliferative hepatocellular lesions (Serota et al., 1986b; Hazleton Laboratories, 1983). Evidence of a trend for increased risk of liver tumors (described as neoplastic nodule or hepatocellular carcinoma) was seen in female F344 rats but not males exposed via drinking water ($p < 0.01$) (Serota et al., 1986a). However, the potential malignant characterization of the nodules was not described, and no trend was seen in the data limited to hepatocellular carcinomas.

The derivation of the cancer oral slope factor is based on the male mouse data (Serota et al., 1986b; Hazleton Laboratories, 1983) because of their greater sensitivity compared to female mice and to male and female rats. The study authors concluded that there was no dose-related trend, that there were no significant differences comparing the individual dose groups with the combined control group, and that the observed incidences were "within the normal fluctuation of this type of tumor incidence." Although Serota et al. (1986b) state that a two-tailed significance level of $p = 0.05$ was used for all tests, this does not appear to correctly represent the statistic used by Serota et al. (1986b). Each of the p-values for the comparison of the 125, 185, and 250 mg/kg-day dose groups with the controls was $p < 0.05$. (These p-values were found in the full report of this study, see Hazleton Laboratories (1983), but were not included in the Serota et al. (1986b) publication). Hazleton Laboratories (1983) indicated that a correction factor for multiple comparisons was used specifically for the liver cancer data, reducing the nominal p-value from 0.05 to 0.0125; none of these individual group comparisons are statistically significant when a p-value of 0.0125 is used. EPA did not consider the use of a multiple comparisons correction factor for the evaluation of the liver tumor data (a primary a priori hypothesis) to be warranted. Thus, based on the Hazleton Laboratories (1983) statistical analysis, EPA concluded that dichloromethane induced a carcinogenic response in male B6C3F$_1$ mice as evidenced by a marginally increased trend test ($p = 0.058$) for combined hepatocellular adenomas and carcinomas and by small but statistically significant ($p < 0.05$) increases in hepatocellular adenomas and carcinomas at dose levels of 125 ($p = 0.023$), 185 ($p = 0.019$), and 250 mg/kg-day ($p = 0.036$).

With respect to comparisons with historical controls, the incidence in the control groups (19%) was almost identical to the mean seen in the historical controls from this laboratory (17.8% based on 354 male $B6C3F_1$ mice), so there is no indication that the observed trend is being driven by an artificially low rate in controls and no indication that the experimental conditions resulted in a systematic increase in the incidence of hepatocellular adenomas and carcinomas. Although the occurrence of one elevated rate in an exposed group may reflect normal fluctuations in the incidence of these tumors (described for this laboratory as 5–40%, with a mean of 17.8%, based on 354 male controls), the pattern of incidence rates (increased incidence in all four dose groups, with three of these increases significant at a p-value of < 0.05) suggest a treatment-related increase.

The development of liver tumors in $B6C3F_1$ mice is associated with metabolite production in this tissue via the GST metabolic pathway (Section 4.7.3), a pathway that also exists in humans. Modeling intake, metabolism, and elimination of dichloromethane in mice and humans is feasible. Thus, it is reasonable to apply the best available PBPK models to estimate equivalent internal doses in mice and humans.

5.4.1.2. *Derivation of Oral Slope Factor*

In a manner similar to the derivation of the noncancer toxicity values, PBPK models for dichloromethane in mice and humans were used in the derivation of toxicity values (cancer oral slope factor and inhalation unit risk) for cancer endpoints based on lung (for inhalation) and liver (for oral and inhalation) tumor data in the mouse (Figure 5-13). A deterministic PBPK model for dichloromethane in mice was first used to convert mouse drinking water or inhalation exposures to long-term daily average values of internal lung-specific GST metabolism (GST metabolism in lung/lung volume) or liver-specific GST metabolism (GST metabolism in liver/liver volume). The choice of these dose metrics was made based on data pertaining to the mechanism(s) involved in the carcinogenic response, specifically data supporting the involvement of a GST metabolite(s). The evidence pertaining to the GST pathway is discussed in Section 4.7 and includes the enhanced genotoxicity seen in bacterial and mammalian in vitro assays with the introduction of GST metabolic capacity (Graves et al., 1994b) and the suppression of the production of DNA SSBs by pretreatment with a GSH depletory seen in acute inhalation exposure to dichloromethane in mice (Graves et al., 1995). Although the GST metabolic pathway takes on a greater role as the CYP pathway is saturated, both the GST and CYP pathways are operating even at low exposures. The PBPK model incorporates the metabolic shift and expected nonlinearity (GST dose attenuation with low exposures) in the exposure-dose relationship across exposure levels.

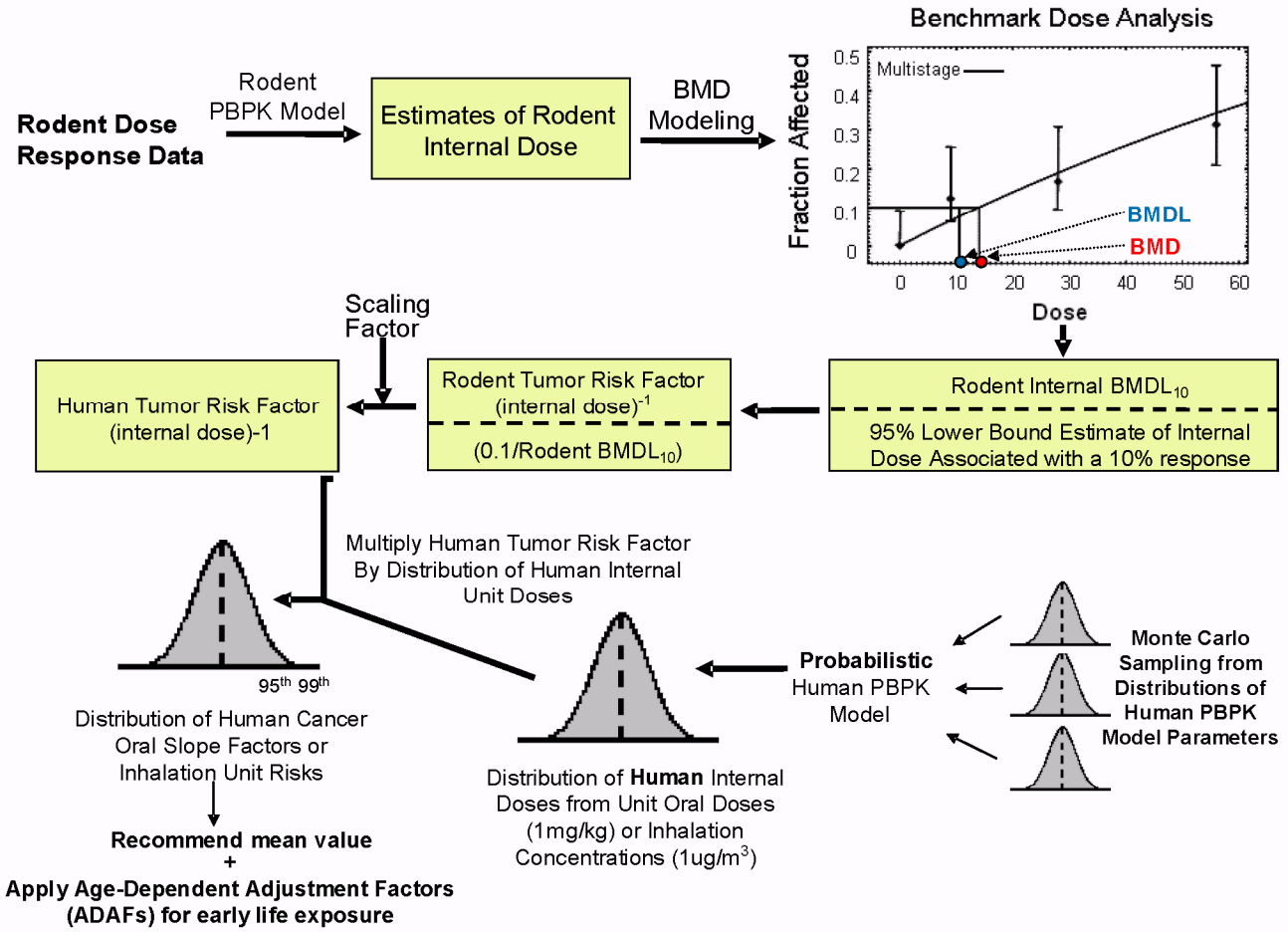


Figure 5-13. Process for deriving cancer oral slope factors and inhalation unit risks by using rodent and human PBPK models.

The multistage cancer model (using BMDS version 2.0) was fit to the tumor incidence data and internal dose data for rodents, and BMD_{10} and associated $BMDL_{10}$ values (for a BMR of 10% extra risk) were estimated. A probabilistic PBPK model for dichloromethane in humans, adapted from David et al. (2006) (see Appendix B), was used with Monte Carlo sampling to calculate distributions of internal lung or liver doses associated with chronic unit oral (1 mg/kg-day) or inhalation (1 $\mu g/m^3$) exposures. The resulting distribution of human internal doses was multiplied by a human internal dose tumor risk factor (in units of reciprocal internal dose) to generate a distribution of oral slope factors or inhalation unit risks associated with a chronic unit oral or inhalation exposure, respectively.

As discussed in Section 3.5.2, the statistics reported for the fitted metabolic parameters by David et al. (2006) (Table 4 in that publication) only represent the population mean and uncertainty in that mean for each parameter. EPA's revision of the model parameter distributions are generally described in Section 5.1.2, with details provided in Appendix B. Subsequent to this revision, the human PBPK parameter distributions are expected to appropriately account for both parametric uncertainty and interindividual variability, with sampling weighted to represent the full population from 6 months to 80 years of age. The model code also allows estimation of risk for subpopulations defined by a specific age in that range, gender, and/or GST-T1 genotype (e.g., the GST-T1 +/+ subpopulation).

5.4.1.3. *Dose-Response Data*

Data for liver tumors in male $B6C3F_1$ mice following exposure to dichloromethane in drinking water were used to develop oral cancer slope factors (Serota et al., 1986b; Hazleton Laboratories, 1983). Significant increases in incidence of liver adenomas and carcinomas were observed in male but not female $B6C3F_1$ mice exposed for 2 years (Table 5-11). No significant decreases in survival were observed in the treated groups of either sex compared with controls. The at-risk study populations (represented by the denominators in the incidence data) were determined by excluding all animals dying prior to 52 weeks.

Table 5-11. Incidence data for liver tumors and internal liver doses, based on GST metabolism dose metrics in male B6C3F$_1$ mice exposed to dichloromethane in drinking water for 2 years

Sex	Nominal (actual) daily intake (mg/kg-d)	Mouse liver tumor incidence[a]	Mouse internal liver metabolism dose[b]	Mouse whole body metabolism dose[c]
Male (BW = 37.3 g)	0 (0)	24/125 (19%)	0	0
	60 (61)	51/199 (26%)	17.5	0.73
	125 (124)	30/99 (30%)	63.3	2.65
	185 (177)	31/98 (32%)	112.0	4.68
	250 (234)	35/123 (28%)	169.5	7.1

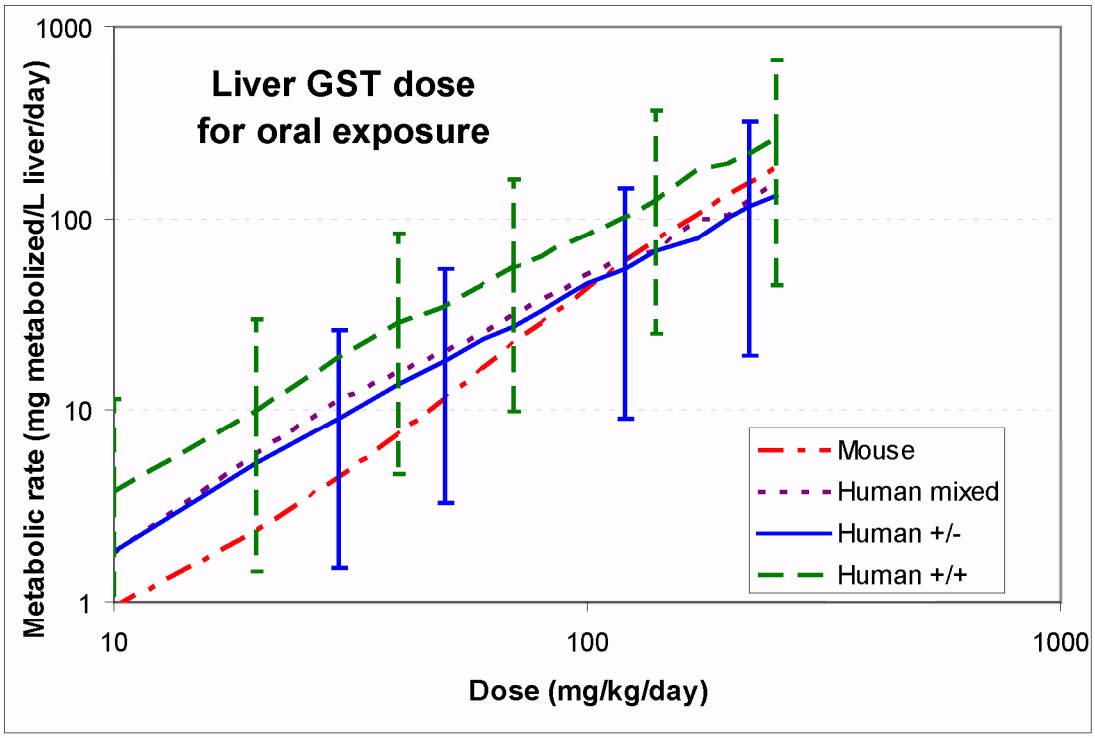
[a]Hepatocellular carcinoma or adenoma, combined. Mice dying prior to 52 weeks, as estimated from the survival data shown in Figure 1 of Hazleton Laboratories (1983), were excluded from the denominators. Cochran-Armitage trend p-value = 0.058. p-values for comparisons with the control group were 0.071, 0.023, 0.019, and 0.036 in the 60, 125, 185, and 250 mg/kg-day groups, respectively, based on statistical analyses reported by Hazleton Laboratories (1983).
[b]mg dichloromethane metabolized via GST pathway/L liver/day. Internal doses were estimated from simulations of actual daily doses reported by the study authors.
[c]Based on the sum of dichloromethane metabolized via the GST pathway in the lung plus the liver, normalized to total BW (i.e., [lung GST metabolism (mg/day) + liver GST metabolism (mg/day)]/kg BW). Units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-d.

Sources: Serota et al. (1986b); Hazleton Laboratories (1983).

5.4.1.4. *Dose Conversion and Extrapolation Methods: Cancer Oral Slope Factor*

Dose conversion. The mouse PBPK model of Marino et al. (2006) was based on the PBPK model for dichloromethane by Andersen et al. (1987), which was modified to include dichloromethane metabolism in the lung compartment and kinetics of CO and COHb (Andersen et al., 1991). For the mouse, physiological parameters and partition coefficients were adjusted to match those reported in Andersen et al. (1991; 1987) and Clewell et al. (1993), respectively, while QCC, VPR, and metabolic parameter distribution mean values were derived via MCMC model calibration reported by Marino et al. (2006). The model of Marino et al. (2006) was used to simulate daily drinking water exposures comprising six discrete drinking water episodes for specified times and percentage of total daily intake (Reitz et al., 1997), and to calculate average lifetime daily internal doses for the male mouse data shown in Table 5-11. A first-order oral absorption rate constant (k_a) of 5 hours^{-1} was taken from Reitz et al. (1997) to describe the uptake of dichloromethane from the gastrointestinal tract to the liver. Study-specific BWs were not available, so reference BWs for male B6C3F$_1$ mice in chronic studies (U.S. EPA, 1988b) were used. Based on evidence that metabolites of dichloromethane produced via the GST pathway are primarily responsible for dichloromethane carcinogenicity in mouse liver (summarized in Section 4.7.3) and the assumption that these metabolites are sufficiently reactive that they do not have substantial distribution outside the liver, the recommended selected internal dose metric for liver tumors was daily mass of dichloromethane metabolized via the GST pathway per unit volume of liver (Table 5-11). Figure 5-14 shows the comparison between

internal and external doses in the liver in mice and humans. The whole-body metabolism metric was also examined; however, this metric would be more relevant under a scenario of slowly cleared metabolites that undergo general circulation.



Six simulated drinking water episodes are described by Reitz et al. (1997). The human metabolism rates were estimated using a computational sample of 1,000 individuals per dose, including random samples of the three GST-T1 polymorphisms (+/+, +/-, -/-; "Human mixed" curve) or samples restricted to the GST +/+ or +/- populations in the current U.S. population based on data from Haber et al. (2002). Since a different set of samples was used for each dose, some stochasticity is evident as the human points (values) do not fall on smooth curves. Error bars indicate the range of 5th–95th percentile for the subpopulations sampled at select concentrations.

Figure 5-14. PBPK model-derived internal doses (mg dichloromethane metabolized via the GST pathway/L liver/day) in mice and humans and their associated external exposures (mg/kg-day) used for the derivation of cancer oral slope factors based on liver tumors in mice.

Dose-response modeling and extrapolation. The multistage dose-response model was fit to the mouse liver tumor incidence and PBPK model-derived internal dose data to derive a mouse internal BMD_{10} and $BMDL_{10}$ associated with 10% extra risk (Table 5-12). Different polynomial models were compared based on adequacy of model fit as assessed by overall χ^2 goodness of fit (p-value > 0.10) and examination of residuals at the 0 dose exposure (controls) and in the region of the BMR. Due to the lack of a monotonic increase in tumor response at the high dose, the model did not adequately fit the data. In consideration of the region of interest (i.e., low-dose risk estimation), the highest dose group was excluded. The modeling of the

remaining four dose groups exhibited an adequate fit to the data. Appendix G, Section G.1 provides details of the BMD modeling results. The mouse liver tumor risk factor (extra risk per unit internal dose) was calculated by dividing 0.1 by the mouse $BMDL_{10}$ for liver tumors.

Table 5-12. BMD modeling results and tumor risk factors for internal dose metric associated with 10% extra risk for liver tumors in male B6C3F$_1$ mice exposed to dichloromethane in drinking water for 2 years, based on liver-specific GST metabolism and whole body GST metabolism dose metrics

Internal dose metric[a]	BMDS model[b]	χ^2 goodness of fit *p*-value	Mouse BMD_{10}[c]	Mouse $BMDL_{10}$[c]	Allometric-scaled human $BMDL_{10}$[d]	Tumor risk factor[e]	
						Scaling = 1.0	Allometric-scaled
Liver-specific	Multistage (1,1)	0.56	73.0	39.6	5.66	2.53×10^{-3}	1.77×10^{-2}
Whole-body	Multistage (1,1)	0.56	3.05	1.65	0.24	–	4.24×10^{-1}

[a]Liver specific dose units = mg dichloromethane metabolized via GST pathway/L tissue/day; whole-body dose units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.
[b]The multistage model in EPA BMDS version 2.0 was fit to the mouse dose-response data shown in Table 5-11 using internal dose metrics calculated with the mouse PBPK model. Numbers in parentheses indicate (1) the number of dose groups dropped in order to obtain an adequate fit; and (2) the degree polynomial of the model.
[c]BMD_{10} and $BMDL_{10}$ refer to the BMD-model-predicted mouse internal and its 95% lower confidence limit, associated with a 10% extra risk for the incidence of tumors.
[d]Mouse $BMDL_{10}$ divided by $(BW_{human}/BW_{mouse})^{0.25} = 7$.
[e]Dichloromethane tumor risk factor (extra risk per unit internal dose) derived by dividing the BMR (0.1) by the mouse $BMDL_{10}$ and by the allometric-scaled human $BMDL_{10}$ for the scaling = 1.0 and allometric-scaled risk factors, respectively.

Linear extrapolation from the internal human $BMDL_{10}$ values ($0.1/BMDL_{10}$) was used to derive oral risk factors for liver tumors based on tumor responses in male mice. Proposed key events for dichloromethane carcinogenesis are discussed in Section 4.7.3.1. The linear low-dose extrapolation approach for agents with a mutagenic mode of action was selected consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, (2005a).

Application of allometric scaling factor. As discussed in Section 4.7, several lines of evidence point to the involvement of the GST metabolic pathway in the carcinogenic response seen in dichloromethane. The role of specific metabolites has not been firmly established, however. S-(chloromethyl)-glutathione is an intermediate to the production of formaldehyde through this pathway (Hashmi et al., 1994). Formation of the free hydrogen ion is also hypothesized, although no direct evidence supporting this has been presented. The pattern of *hprt* gene mutations seen in CHO cells incubated with GST-complete mouse liver cytosol preparations suggest that S-(chloromethyl)glutathione, rather than formaldehyde, may be responsible for the mutagenic effects associated with dichloromethane (Graves et al., 1996). DNA reaction products (e.g., DNA adducts) produced by S-(chloromethyl)glutathione have not

been quantified, possibly due to potential instability of these compounds (Watanabe et al., 2007; Hashmi et al., 1994). Other studies indicate that DNA damage resulting from formaldehyde formation, in addition to the S-(chloromethyl)glutathione intermediate, should also be considered (Hu et al., 2006; Casanova et al., 1997).

The question of the role of specific metabolites and particularly how these metabolites are transformed or removed is a key question affecting the choice of a scaling factor to be used in conjunction with the internal dose metric based on rate of GST metabolism. If the key metabolite is established and is known to be sufficiently reactive to not spread in systemic circulation, then it can be assumed that: (1) the level of reactivity and rate of clearance (i.e., disappearance due to local reactivity) for this metabolite per volume tissue is equal in rodents and humans, and (2) risk is proportional to the long-term daily average concentration of the metabolite. Under these assumptions, rodent internal $BMDL_{10}$ values based on tissue-specific dichloromethane metabolism require no allometric scaling to account for toxicodynamic differences and predict the corresponding level of human risk as a function of the metric (i.e., the scaling factor in Figure 5-13 was equal to 1.0). (A single metabolite is referenced, but the same argument holds in general for more than one metabolite.) Under this scenario and assumption, humans and rodents with the same long-term daily average metabolite formation per volume tissue (e.g., equal internal $BMDL_{10}$) should both experience the same long-term average concentration of the metabolite when the metabolite is highly reactive and, hence, experience the same extra risk.

Although the evidence points to a specific metabolic pathway and to site-specific actions resulting from a reactive metabolite that does not escape the tissue in which it is formed, some assumptions remain concerning this hypothesis. Specifically, the active metabolite(s) have not been established, and data pertaining to the reactivity or clearance rate of these metabolite(s) are lacking. Quantitative measurements of adducts of interest or of the half life of relevant compounds in humans and in mice are not available. It is not known that the rate of reaction is proportional to the liver perfusion rate, cardiac output, or body surface area, and it is not known that the rate of reaction is not proportional to these factors. To address the uncertainties in the available data, it is appropriate to use a scaling factor that addresses the possibility that the rate of clearance for the metabolite is limited by processes that are known to scale allometrically, such as blood perfusion, enzyme activity, or availability of reaction cofactors that is limited by overall metabolism. This case would result in use of a mouse:human dose-rate scaling factor of $(BW_{human}/BW_{mouse})^{0.25} = 7$ to adjust the mouse-based $BMDL_{10}$ values downward. Using this internal dose metric (liver-specific metabolism with allometric scaling), equivalent rodent and human internal $BMDL_{10}$ values result in a human liver tumor risk factor ($0.1/BMDL_{10}$) that is assumed equal to that for the mouse, given a 70-year lifetime exposure.

Another alternative that can be used is based on an allometrically-scaled whole-body metabolism metric. In this case, less weight is given to the evidence of site-specificity, as this metric allows for systemic circulation of the relevant metabolites.

The cancer toxicity values derived using each of these metrics and scaling factors (i.e., liver-specific metabolism with and without allometric-scaling and the whole-body metabolism metric) are presented in the following tables. Considering the lack of data pertaining to clearance rates or the actual AUC of the active carcinogenic metabolite(s) in mice and humans, the oral slope factor recommended by EPA is based on the allometrically-scaled tissue-specific GST metabolism rate dose metric.

Calculation of oral slope factors. The human PBPK model adapted from David et al. (2006) (see Appendix B), using Monte Carlo sampling techniques, was used to calculate distributions of human internal dose metrics of daily mass of dichloromethane metabolized via the liver-specific GST pathway per unit volume of liver resulting from a long-term average daily drinking water dose of 1 mg/kg dichloromethane. In another analysis of whole body metabolism, a dose metric based on the total metabolites formed in liver and lungs via GST metabolism per BW was used. The human model used parameter values derived from Monte Carlo sampling of probability distributions for each parameter, including MCMC-derived distributions for the metabolic parameters (David et al., 2006). The drinking water exposures comprised six discrete drinking-water episodes for specified times and percentage of total daily intake (Reitz et al., 1997) (Appendix B).

The distribution of cancer oral slope factors shown in Table 5-13 was derived by multiplying the human oral liver tumor risk factors by the respective distributions of human average daily internal doses resulting from chronic, unit oral exposures of 1 mg/kg-day dichloromethane. The mean slope factor was selected as the recommended value; other values at the upper end of the distribution are also presented.

Table 5-13. Cancer oral slope factors for dichloromethane based on PBPK model-derived internal liver doses in B6C3F$_1$ mice exposed via drinking water for 2 years, based on liver-specific GST metabolism and whole body metabolism dose metrics, by population genotype

Internal dose metric and scaling factor[a]	Population genotype[b]	Human tumor risk factor[c]	Distribution of human internal dichloromethane doses from 1 mg/kg-d exposure[d]			Resulting candidate human oral slope factor[e] (mg/kg-d)$^{-1}$		
			Mean	95th percentile	99th percentile	Mean	95th percentile	99th percentile
Liver-specific, allometric-scaled	GST-T1$^{+/+}$	1.77×10^{-2}	0.94×10^{-1}	2.98×10^{-1}	5.43×10^{-1}	1.7×10^{-3}	5.3×10^{-3}	9.6×10^{-3}
	Mixed	1.77×10^{-2}	0.53×10^{-1}	1.96×10^{-1}	3.78×10^{-1}	9.4×10^{-4}	3.5×10^{-3}	6.7×10^{-3}
Liver-specific, scaling = 1.0	GST-T1$^{+/+}$	2.53×10^{-3}	0.94×10^{-1}	2.98×10^{-1}	5.43×10^{-1}	2.4×10^{-4}	7.5×10^{-4}	1.4×10^{-3}
	Mixed	2.53×10^{-3}	0.53×10^{-1}	1.96×10^{-1}	3.78×10^{-1}	1.3×10^{-4}	5.0×10^{-4}	9.6×10^{-4}
Whole-body, allometric-scaled	GST-T1$^{+/+}$	4.24×10^{-1}	2.20×10^{-3}	7.20×10^{-3}	1.30×10^{-2}	9.3×10^{-4}	3.1×10^{-3}	5.5×10^{-3}
	Mixed	4.24×10^{-1}	1.27×10^{-3}	4.66×10^{-3}	9.41×10^{-3}	5.4×10^{-4}	2.0×10^{-3}	4.0×10^{-3}

[a]Liver specific dose units = mg dichloromethane metabolized via GST pathway/L tissue/day; Whole-body dose units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.
[b]GST-T1$^{+/+}$ = homozygous, full enzyme activity; mixed = population reflecting estimated frequency of genotypes in current U.S. population: 20% GST-T$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$ (Haber et al., 2002).
[c]Dichloromethane tumor risk factor (extra risk per unit internal dose/day) derived by dividing the BMR (0.1) by the allometric-scaled human BMDL$_{10}$ and the mouse BMDL$_{10}$ for the allometric-scaled and scaling = 1.0 risk factors, respectively (from Table 5-12).
[d]Mean, 95th, and 99th percentile of the human PBPK model-derived probability distribution of daily average internal dichloromethane dose resulting from chronic oral exposure of 1 mg/kg-day.
[e]Derived by multiplying the dichloromethane tumor risk factor by the PBPK model-derived probabilistic internal doses from daily exposure to 1 mg/kg-day.

Consideration of Sensitive Human Subpopulations. An important issue in the derivation process used by EPA, pertaining to the use of the human PBPK model, stems from the assumption regarding the population for which the derivation should be applied. The inclusion of the GST-T1 null subpopulation in effect dilutes the risk that would be experienced by those who carry a GST-T1 allele by averaging in nonresponders (i.e., the GST-$T1^{-/-}$ genotype). Thus, the cancer oral slope factor was derived specifically for carriers of the GST-T1 homozygous positive (+/+) genotype, the population that would be expected to be most sensitive to the carcinogenic effects of dichloromethane given the GST-related dose metric under consideration. In addition, cancer values derived for a population reflecting the estimated frequency of GST-T1 genotypes in the current U.S. population (20% GST-$T1^{-/-}$, 48% GST-$T1^{+/-}$, and 32% GST-$T1^{+/+}$, i.e., the "mixed" population) are also presented. All simulations also included a distribution of CYP activity based on data from Lipscomb et al. (2003).

5.4.1.5. *Cancer Oral Slope Factor*

The recommended cancer oral slope factor for dichloromethane is **2×10^{-3} (mg/kg-day)$^{-1}$** (rounded from 1.7×10^{-3}) and is based on liver tumor responses in male $B6C3F_1$ mice exposed to dichloromethane in drinking water for 2 years (Serota et al., 1986b; Hazleton Laboratories, 1983). The oral slope factor was derived by using a tissue-specific GST metabolism dose metric with allometric scaling for the population that is presumed to have the greatest sensitivity (the GST-$T1^{+/+}$ genotype). The application of ADAFs to the cancer oral slope factor is recommended and is described in Section 5.4.4.

5.4.1.6. *Alternative Derivation Based on Route-to-Route Extrapolation*

For comparison, alternative cancer oral slope factors were derived via route-to-route extrapolations from the data for liver tumors in male and female $B6C3F_1$ mice exposed by inhalation for 2 years (Mennear et al., 1988; NTP, 1986). This derivation, shown in Table 5-14, uses the cancer inhalation unit risk derived in Section 5.4.2.4 (see Table 5-19 for these inhalation unit risk values) and the distribution of human internal dichloromethane exposures from 1 mg/kg-day exposure using the tissue-specific GST metabolism dose metric (mg dichloromethane metabolized via the GST pathway/L liver/day). The cancer oral slope factors based on the route-to-route extrapolations from liver tumors in mice exposed by inhalation (Table 5-14) are about one order of magnitude lower than those based on the liver tumor responses in mice exposed via drinking water.

Table 5-14. Alternative route-to-route cancer oral slope factors for dichloromethane extrapolated from male B6C3F$_1$ mouse inhalation liver tumor incidence data using a tissue-specific GST metabolism dose metric, by population genotype

Internal dose metric and scaling factor	Population genotype[a]	Human tumor risk factor[b]	Distribution of human internal dichloromethane doses from 1 mg/kg-d exposure[c]			Resulting candidate human oral slope factor[d] $(mg/kg\text{-}d)^{-1}$		
			Mean	95th percentile	99th percentile	Mean	95th percentile	99th percentile
Liver-specific, allometric-scaled	GST-T1$^{+/+}$	1.29×10^{-3}	0.94×10^{-1}	2.98×10^{-1}	5.43×10^{-1}	1.2×10^{-4}	3.8×10^{-4}	7.0×10^{-4}
	Mixed	1.29×10^{-3}	0.53×10^{-1}	1.96×10^{-1}	3.78×10^{-1}	6.8×10^{-5}	2.5×10^{-4}	4.9×10^{-4}
Liver-specific, scaling = 1.0	GST-T1$^{+/+}$	1.84×10^{-4}	0.94×10^{-1}	2.98×10^{-1}	5.43×10^{-1}	1.7×10^{-5}	5.5×10^{-5}	1.0×10^{-4}
	Mixed	1.84×10^{-4}	0.53×10^{-1}	1.96×10^{-1}	3.78×10^{-1}	9.7×10^{-6}	3.6×10^{-5}	6.9×10^{-5}
Whole-body metabolism	GST-T1$^{+/+}$	3.03×10^{-2}	2.20×10^{-3}	7.20×10^{-3}	1.30×10^{-2}	6.7×10^{-5}	2.2×10^{-4}	3.9×10^{-4}
	Mixed	3.03×10^{-2}	1.27×10^{-3}	4.66×10^{-3}	9.41×10^{-3}	3.9×10^{-5}	1.4×10^{-4}	2.9×10^{-4}

[a]GST-T1$^{+/+}$ = homozygous, full enzyme activity; mixed = population reflecting estimated frequency of genotypes in current U.S. population: 20% GST-T$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$ (Haber et al., 2002).

[b]Dichloromethane tumor risk factor (extra risk per milligram dichloromethane metabolized via GST pathway/L tissue/day) derived by dividing the BMR (0.1) by the allometric-scaled human $BMDL_{10}$ and the mouse $BMDL_{10}$ for the allometric-scaled and scaling = 1.0 risk factors, respectively (from inhalation unit risk data, Table 5-19).

[c]Mean, 95th, and 99th percentile of the human PBPK model-derived probability distribution of daily average internal dichloromethane dose (mg dichloromethane metabolized via GST pathway/L tissue/day) resulting from chronic oral exposure of 1 mg/kg-day.

[d]Derived by multiplying the dichloromethane tumor risk factor by the PBPK model-derived probabilistic internal doses from daily exposure to 1 mg/kg-day.

5.4.1.7. *Alternative Based On Administered Dose*

One comparison that can be made is with an alternative oral slope factor based on liver tumors in mice, using the external concentrations of dichloromethane in the mouse as converted to HEDs and then applying this by using BMD modeling to obtain the $BMDL_{10}$ and resulting oral cancer risk. Mouse bioassay exposures were adjusted to HEDs as follows:

HED = (nominal daily intake/BW scaling factor) × daily exposure adjustment factor

where BW scaling factor = $(BW_{human}/BW_{mouse})^{0.25} = 7$
and
daily exposure adjustment factor = 5/7

The HEDs for the 0, 60, 125, 185, and 250 mg/kg-day dose groups used in the liver tumor analysis (Table 5-11) [from Serota et al. (1986b)] were 0, 6.12, 12.75, 18.87, and 25.51 mg/kg-day, respectively. The BMD modeling and oral slope factor derived from these values are shown in Table 5-15. The resulting oral slope factor based on the liver tumors in the mouse is approximately one order of magnitude higher than the current recommended value obtained by using the mouse and human PBPK models.

Table 5-15. Cancer oral slope factor based on a human $BMDL_{10}$ using administered dose for liver tumors in male $B6C3F_1$ mice exposed to dichloromethane in drinking water for 2 years

Sex, tumor type	BMDS model[a]	χ^2 goodness of fit *p*-value	Human BMD_{10}[b]	Human $BMDL_{10}$[b]	Cancer oral slope factor[c] $(mg/kg\text{-}d)^{-1}$
Male, liver	Multistage (0,1)	0.55	19.4	10.4	1.0×10^{-2}

[a]The multistage model in EPA BMDS version 2.0 was fit to the mouse liver tumor data shown in Table 5-11. The HEDs for the 0, 60, 125, 185, and 250 mg/kg-day dose groups used in the liver tumor analysis were 0, 6.12, 12.75, 18.87, and 25.51 mg/kg-day, respectively, based on application of BW scaling factor = $(BW_{human}/BW_{mouse})^{0.25} = 7$ and adjusting for daily exposure by multiplying by 5/7 d. Numbers in parentheses indicate: (1) the number of dose groups dropped in order to obtain an adequate fit, starting with the highest dose group; and (2) the degree polynomial of the model.
[b]BMD_{10} and $BMDL_{10}$ refer to the BMD-model-predicted HED (mg/kg-day) and its 95% lower confidence limit, associated with a 10% extra risk for the incidence of tumors.
[c]Cancer oral slope factor (risk per mg/kg-day) = 0.1/human $BMDL_{10}$.

The administered dose methodology can be considered equivalent to using a single-compartment, whole-body model of dichloromethane where the internal dose metric is the AUC of dichloromethane itself and clearance of dichloromethane scales from mice to humans as $BW^{0.75}$. The estimates based on the PBPK model, in contrast, use the rate of GST metabolism of dichloromethane as the metric. Another difference is that the administered dose methodology does not account in any way for the GST polymorphism and so might be considered as representing the general/mixed-GST-genotype population rather than the +/+ subpopulation.

5.4.1.8. *Previous IRIS Assessment: Cancer Oral Slope Factor*

The previous IRIS assessment derived a cancer oral slope factor of 7.5×10^{-3} (mg/kg-day)$^{-1}$ by the application of the multistage model to combined incidence of hepatocellular adenomas and carcinomas from two studies; a 2-year drinking water study in $B6C3F_1$ mice (Hazleton Laboratories, 1983) and a 2-year inhalation study in $B6C3F_1$ mice (NTP, 1986). The slope factor was the arithmetic mean of two candidate slope factors, 1.2×10^{-2} (mg/kg-day)$^{-1}$ (Hazleton Laboratories, 1983) and 2.6×10^{-3} (mg/kg-day)$^{-1}$ (NTP, 1986). Since the NTP (1986) animal data were from inhalation exposures, the estimated inhaled doses were calculated for mice and humans (assuming near complete uptake into lung tissues and blood) and converted to administered doses in units of mg/kg-day. Assumed inhalation rates of 0.0407 and 20 m^3/day were used for mice and humans, respectively. A default factor based on body surface area scaling was used to adjust the estimated dose-rates for species differences in metabolism, to obtain human equivalent dose rates, from which the oral cancer slope factor was then derived.

5.4.1.9. *Comparison of Cancer Oral Slope Factors Using Different Methodologies*

Cancer oral slope factors derived using different dose metrics and assumptions are summarized in Table 5-16. The recommended oral slope factor of 2×10^{-3} per mg/kg-day (rounded to one significant digit) is based on a tissue-specific GST-internal dose metric with allometric scaling (= 7) because of some uncertainty regarding the rate of clearance of the relevant metabolite(s) formed via the GST pathway. The value derived specifically for the GST-$T1^{+/+}$ population is recommended to provide protection for the population that is hypothesized to be most sensitive to the carcinogenic effect. The values based on the GST-$T1^{+/+}$ group are approximately twofold higher than those for the full population for the dose metrics used in this assessment (Table 5-16). Within a genotype population, the values of the oral slope factor among most of the various dose metrics vary by about one to two orders of magnitude. The slope of the linear extrapolation from the BMD10, the central estimate of exposure associated with 10% extra cancer risk, was also derived based on the male mouse liver tumor incidence data (Table 5-11). The slope of the linear extrapolation from the central estimate BMD_{10} is 0.1/111 mg/kg-day = 9×10^{-4} per mg/kg-day.

Table 5-16. Comparison of oral slope factors derived using various assumptions and metrics, based on tumors in male mice

Population[a]	Dose metric	Species, sex	Tumor	Scaling factor	Mean oral slope factor $(mg/kg\text{-}d)^{-1}$	Source (table)
GST-T1$^{+/+}$[b]	**Tissue-specific GST-metabolism rate**	**Mouse, male**	**Liver**	**7.0**	**1.7×10^{-3}**	**Table 5-13**
	Tissue-specific GST-metabolism rate	Mouse, male	Liver	1.0	2.4×10^{-4}	Table 5-13
	Whole-body GST metabolism rate	Mouse, male	Liver	7.0	9.3×10^{-4}	Table 5-13
	Route-to-route extrapolation, tissue-specific metabolism	Mouse, male	Liver	7.0	1.2×10^{-4}	Table 5-14
	Route-to-route extrapolation, tissue-specific metabolism	Mouse, male	Liver	1.0	1.7×10^{-5}	Table 5-14
	Route-to-route extrapolation, whole-body metabolism	Mouse, male	Liver	7.0	6.7×10^{-5}	Table 5-14
Mixed	Tissue-specific GST-metabolism rate[b]	Mouse, male	Liver	7.0	9.4×10^{-4}	Table 5-13
	Tissue-specific GST-metabolism rate	Mouse, male	Liver	1.0	1.3×10^{-4}	Table 5-13
	Whole-body GST metabolism rate	Mouse, male	Liver	7.0	5.4×10^{-4}	Table 5-13
	Route-to-route extrapolation, tissue-specific metabolism	Mouse, male	Liver	7.0	6.8×10^{-5}	Table 5-14
	Route-to-route extrapolation, tissue-specific metabolism	Mouse, male	Liver	1.0	9.7×10^{-6}	Table 5-14
	Route-to-route extrapolation, whole-body metabolism	Mouse, male	Liver	7.0	3.9×10^{-5}	Table 5-14
	Applied dose (HED)	Mouse, male	Liver		1.0×10^{-2}	Table 5-15
	1995 IRIS assessment	Mouse, male	Liver		7.5×10^{-3}	

[a]GST-T1$^{+/+}$ = homozygous, full enzyme activity; Mixed = genotypes based on a population reflecting the estimated frequency of genotypes in the current U.S. population: 20% GST-T1$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$ (Haber et al., 2002).
[b]Bolded value is the basis for the recommended oral slope factor of 2×10^{-3} per mg/kg-day.

5.4.2. Cancer Inhalation Unit Risk

5.4.2.1. *Choice of Study/Data—with Rationale and Justification*

As discussed in Section 4.7, results from several cohort mortality studies of workers repeatedly exposed to dichloromethane and several case-control studies provide supporting evidence of carcinogenicity in humans, specifically with respect to liver and brain cancer, and some hematopoietic cancers. However, the epidemiologic studies do not provide adequate data to estimate exposure-response relationships for dichloromethane exposure and these cancers.

Results from several bioassays provide sufficient evidence of the carcinogenicity of dichloromethane in mice and rats exposed by inhalation, as well as adequate data to describe dose-response relationships. As discussed in Section 4.7.2, repeated inhalation exposure to concentrations of 2,000 or 4,000 ppm dichloromethane produced increased incidences of lung and liver tumors in male and female $B6C3F_1$ mice (Maronpot et al., 1995; Foley et al., 1993; Kari et al., 1993; Mennear et al., 1988; NTP, 1986). A weaker trend ($p = 0.08$) was seen with respect to liver tumor incidence (described as neoplastic nodules or hepatic carcinomas) in female rats, but this trend was not seen when limited to hepatic carcinomas (NTP, 1986). A statistically significant increased incidence of brain tumors has not been observed in any of the animal cancer bioassays, but a 2-year study using relatively low exposure levels (0, 50, 200, and 500 ppm) in Sprague-Dawley rats observed a total of six astrocytoma or glioma (mixed glial cell) tumors (combining males and females) in the exposed groups (Nitschke et al., 1988a). These tumors are exceedingly rare in rats, and there are few examples of statistically significant trends in animal bioassays (Sills et al., 1999). Male and female F344 rats exposed to 2,000 or 4,000 ppm showed significantly increased incidences of benign mammary tumors (adenomas or fibroadenomas), and the male rats also exhibited a low rate of sarcoma or fibrosarcoma in mammary gland or subcutaneous tissue around the mammary gland (NTP, 1986).

The NTP inhalation study in $B6C3F_1$ mice (NTP, 1986) was used to derive an inhalation unit risk for dichloromethane because of the completeness of the data, adequate sample size, and clear dose response with respect to liver and lung tumors. The liver tumor incidence in male mice increased from 44% in controls to 66% in the highest dose group; in females, the incidence of this tumor rose from 6 to 83%. For lung tumors, the incidence rose from 10 to 80% in males and from 6 to 85% in females. Compelling evidence exists for the role of GST-mediated metabolism of dichloromethane in carcinogenicity in mice (Section 4.7.3), and both mice and humans possess this metabolic pathway. Modeling intake, metabolism, and elimination of dichloromethane in mice and humans is feasible. Thus, it is reasonable to apply the best available PBPK models to estimate equivalent internal doses in mice and humans.

The mammary tumor data from the NTP (1986) study was also used to derive a comparative inhalation unit risk. However, the toxicokinetic or mechanistic events that might lead to mammary gland tumor development in rats are unknown, and so a clear choice of the optimal internal dose metric could not be made. Thus, this derivation is based on the average

daily AUC for dichloromethane in blood. The role of CYP- or GST-mediated metabolism in the mammary gland is uncertain, although both GST-T1 (Lehmann and Wagner, 2008) and CYP2E1 (El-Rayes et al., 2003; Hellmold et al., 1998) expressions have been detected in human mammary tissue. It is also possible that some metabolites enter systemic circulation from the liver and lung where they are formed.

The female rat liver cancer data from the NTP (1986) inhalation study were not used to derive an inhalation unit risk because the trend was weaker than that seen in the mouse (incidence increased from 4% in controls to 10% in the highest dose group, trend $p = 0.08$), and because the category included neoplastic nodule or hepatocellular carcinoma. The brain tumor data of Nitschke et al. (1988a) in Sprague-Dawley rats were not used to develop an inhalation unit risk because of the low incidence of this rare tumor (a total of four astrocytoma or glioma tumors in exposed males and two in exposed females). The mechanistic issues with respect to mammary tumors and the interpretation of the brain tumor data represent data gaps in the understanding of the health effects of dichloromethane and relevance of the rat data to humans.

5.4.2.2. *Derivation of the Cancer Inhalation Unit Risk*

The derivation of the inhalation unit risk parallels the process described in Section 5.4.1.2 for the cancer oral slope factor. Since modeling metabolism and elimination kinetics of dichloromethane in mice and humans is feasible, it is reasonable to apply the best available PBPK models to determine equivalent target organ doses in mice and humans. Although the GST metabolic pathway takes on a greater role as the CYP pathway is saturated, both the GST and CYP pathways are operating even at low exposures. The PBPK model incorporates the metabolic shift and expected nonlinearity (GST dose attenuation with low exposures) in the exposure-dose relationship across exposure levels.

5.4.2.3. *Dose-Response Data*

Data for liver and lung tumors in male and female $B6C3F_1$ mice following exposure to airborne dichloromethane were used to develop inhalation unit risks for dichloromethane (Mennear et al., 1988; NTP, 1986). As discussed in Section 5.4.1.6, the liver tumor dose-response data were also the basis of an oral slope factor derived by route-to-route extrapolation using the PBPK models to compare with an oral slope factor based on liver tumor data in mice exposed to dichloromethane in drinking water (Serota et al., 1986b). In the NTP (1986) study, significant increases in incidence of liver and lung adenomas and carcinomas were observed in both sexes of $B6C3F_1$ mice exposed 6 hours/day, 5 days/week for 2 years (Table 5-17). Since significant decreases in survival were observed in the treated groups of both sexes, the at-risk study populations (represented by the denominators in the incidence data) were determined by excluding all animals dying prior 52 weeks.

Table 5-17. Incidence data for liver and lung tumors and internal doses based on GST metabolism dose metrics in male and female B6C3F$_1$ mice exposed to dichloromethane via inhalation for 2 years

Sex, tumor type	BW (g)	External dichloromethane concentration (ppm)	Mouse tumor incidence	Mouse internal tissue dose[a]	Mouse whole body metabolism dose[b]
Male, liver[c]	–	0	22/50 (44%)[d]	0	0
	34.0	2,000	24/47 (51%)	2,363.7	100.2
	32.0	4,000	33/47 (70%)	4,972.2	210.7
Male, lung[e]	–	0	5/50 (10%)[d]	0	0
	34.0	2,000	27/47 (55%)	475.0	100.2
	32.0	4,000	40/47 (85%)	992.2	210.7
Female, liver[c]	–	0	3/47 (6%)[d]	0	0
	30.0	2,000	16/46 (35%)	2,453.2	104.0
	29.0	4,000	40/46 (87%)	5,120.0	217.0
Female, lung[e]	–	0	3/45 (6%)[d]	0	0
	30.0	2,000	30/46 (65%)	493.0	104.0
	29.0	4,000	41/46 (89%)	1,021.8	217.0

[a]For liver tumors: mg dichloromethane metabolized via GST pathway/L liver tissue/day from 6 hours/day, 5 days/week exposure; for lung tumors: mg dichloromethane metabolized via GST pathway/L lung tissue/day from 6 hours/day, 5 days/week exposure.
[b]Based on the sum of dichloromethane metabolized via the GST pathway in the lung plus the liver, normalized to total BW (i.e., [lung GST metabolism (mg/day) + liver GST metabolism (mg/day)]/kg BW). Units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.
[c]Hepatocellular carcinoma or adenoma. Mice dying prior to 52 weeks were excluded from the denominators.
[d]Statistically significant increasing trend (by incidental and life-table tests; $p \leq 0.01$).
[e]Bronchoalveolar carcinoma or adenoma. Mice dying prior to 52 weeks were excluded from the denominators.

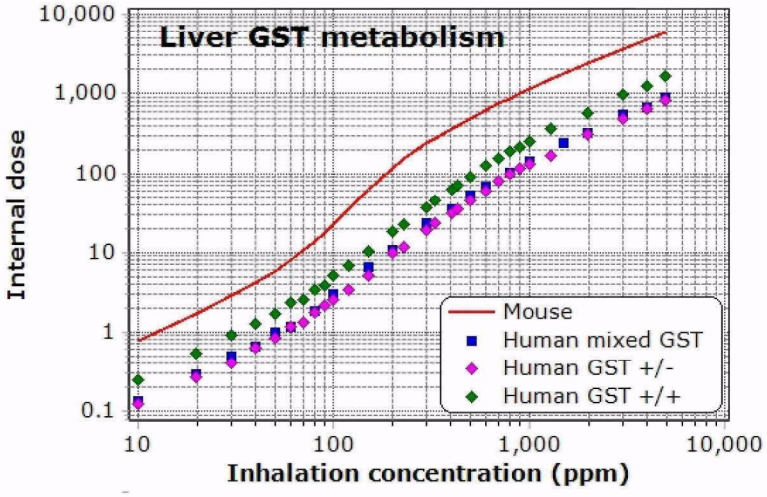
Sources: Mennear et al. (1988); NTP (1986).

5.4.2.4. *Dose Conversion and Extrapolation Methods: Cancer Inhalation Unit Risk*

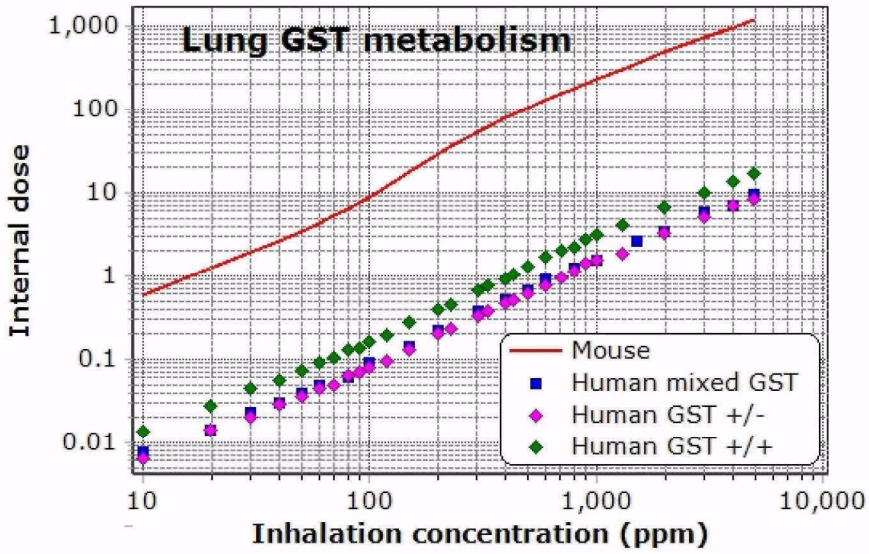
Dose conversion. The PBPK model of Marino et al. (2006) for dichloromethane in the mouse was used to simulate inhalation exposures of 6 hours/day, 5 days/week (Mennear et al., 1988; NTP, 1986) and to calculate long-term daily average internal doses. Study-, group-, and sex-specific mean BWs were used. Based on evidence that metabolites of dichloromethane produced via the GST pathway are primarily responsible for dichloromethane carcinogenicity in mouse liver and lung (summarized in Section 4.7.3) and the assumption that these metabolites are sufficiently reactive that they do not have substantial distribution outside these tissues, the recommended selected internal dose metrics for liver tumors and lung tumors were long-term average daily mass of dichloromethane metabolized via the GST pathway per unit volume of liver and lung, respectively (Table 5-17). Figure 5-15 shows the comparison between inhalation external and internal doses in the liver and lung using this dose metric for the mouse and for the

human. A whole-body metabolism metric was also examined; however, this metric would be more relevant under a scenario of slowly cleared metabolites that undergo general circulation.

A.



B.



Average daily doses were calculated from simulated mouse exposures of 6 hours/day, 5 days/week, while simulated human exposures were continuous. The GST metabolism rate in each simulated human population was obtained by generating 1,000 random samples from each population (ages 0.5–80 years, males and females) for each exposure level and calculating the average GST metabolic rate for each sample.

Figure 5-15. PBPK model-derived internal doses (mg dichloromethane metabolized via the GST pathways/L tissue/day) for liver (A) and lung (B) in mice and humans and their associated external exposures (ppm) used for the derivation of cancer inhalation unit risks.

Dose-response modeling and extrapolation. The multistage dose-response model was fit to the mouse tumor incidence and PBPK model-derived internal dose data to derive mouse internal BMD_{10} and $BMDL_{10}$ values associated with 10% extra risk (Table 5-18). Different polynomial models were compared based on adequacy of model fit as assessed by overall χ^2 goodness of fit (p-value > 0.10) and examination of residuals at the 0 dose exposure (controls) and in the region of the BMR (U.S. EPA, 2000c). Appendix G, Section G.2 provides details of the BMD modeling results for the male mouse. The mouse liver and lung tumor risk factors (extra risk per unit internal dose) were calculated by dividing 0.1 by the mouse $BMDL_{10}$ for liver and lung tumors, respectively.

Linear extrapolation from the internal $BMDL_{10}$ ($0.1/BMDL_{10}$) was used to derive inhalation risk factors for lung and liver tumors in male and female mice (Table 5-18). Consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, (2005a), a linear low-dose extrapolation approach is applied for agents that are DNA reactive and have direct mutagenic activity (see discussion of the cancer mode of action for dichloromethane in Section 4.7.3).

Application of allometric scaling factor. As discussed in Section 5.4.1.4, the choice of a scaling factor is based on the question of the role of specific metabolites and particularly how these metabolites are transformed or removed. If the key metabolite is established and is known to be sufficiently reactive to not spread in systemic circulation, then it can be assumed that: (1) the level of reactivity and rate of clearance (i.e., disappearance due to local reactivity) for this metabolite per volume tissue is equal in rodents and humans; and (2) risk is proportional to the long-term daily average concentration of the metabolite. Under these assumptions, rodent internal $BMDL_{10}$ values based on tissue-specific dichloromethane metabolism require no allometric scaling to account for toxicodynamic differences and predict the corresponding level of human risk as a function of the metric (i.e., the scaling factor in Figure 5-13 was equal to 1.0). (A single metabolite is referenced, but the same argument holds in general for more than one metabolite.) Under this scenario and assumption, humans and rodents with the same long-term daily average metabolite formation per volume tissue (e.g., equal internal $BMDL_{10}$) should both experience the same long-term average concentration of the metabolite when the metabolite is highly reactive and, hence, experience the same extra risk. However, some uncertainties remain concerning the hypothesized role of a highly reactive metabolite in the carcinogenic effects seen with dichloromethane. The active metabolite(s) have not been established, and data pertaining to the reactivity or removal (clearance) rate of these metabolite(s) are lacking. For example, quantitative measurements of adducts of interest or of the half-life of relevant compounds in humans and mice are not available. It is not known that the rate of reaction is proportional to the liver perfusion rate, cardiac output, or body surface area, and it is not known that the rate of reaction is not proportional to these factors. To address these uncertainties, use of a scaling

factor that addresses the possibility that the rate of clearance for the metabolite is limited by processes that scale allometrically, such as blood perfusion, reaction cofactor supply (e.g., antioxidant supply), or enzyme activity, may be appropriate. This case would result in use of a mouse:human dose-rate scaling factor of $(BW_{human}/BW_{mouse})^{0.25} = 7$ to adjust the mouse-based $BMDL_{10}$ values downward. Using this internal dose metric (liver-specific metabolism with allometric scaling), equivalent rodent and human internal $BMDL_{10}$ values result in a human liver tumor risk factor ($0.1/BMDL_{10}$) that is assumed equal to that for the mouse, given a 70-year lifetime exposure. Another alternative that can be used is based on an allometrically-scaled, whole-body metabolism metric. In this case, less weight is given to the evidence of site-specificity of the effects. As with the oral slope factor derivations, the cancer toxicity values derived using each of these metrics and scaling factors (i.e., liver-specific metabolism with and without allometric-scaling and the whole-body metabolism metric) are presented in the following tables. Considering the lack of data pertaining to clearance rates or the actual AUC of the active carcinogenic metabolite(s) in mice and humans, the inhalation unit risks recommended by EPA are based on the allometrically-scaled tissue-specific GST metabolism rate dose metric.

Calculation of Inhalation Unit Risks. A probabilistic PBPK model for dichloromethane in humans, adapted from David et al. (2006) (see Appendix B), was used with Monte Carlo sampling to calculate distributions of internal lung, liver, or blood doses associated with chronic unit inhalation (1 $\mu g/m^3$) exposures. The data on which the model is based indicate that the relationship between exposure and internal dose is linear at low doses. Parameters in the human PBPK model developed by David et al. (2006) are distributions that incorporate information about dichloromethane toxicokinetic variability and uncertainty among humans. Monte Carlo sampling was performed in which each human model parameter was defined by a value randomly drawn from each respective parameter distribution. The model was then executed by using the external unit exposure as input, and the resulting human equivalent internal dose was recorded. This process was repeated for 10,000 iterations to generate a distribution of human internal doses.

The resulting distribution of inhalation unit risks shown in Table 5-19 was derived by multiplying the human internal dose tumor risk factor (in units of reciprocal internal dose) by the respective distributions of human average daily internal dose resulting from a chronic unit inhalation exposure of 1 $\mu g/m^3$ dichloromethane. Table 5-19 presents the analysis using the male mouse data. Analyses based on the female mouse data produced very similar results and are summarized in Appendix H. The mean slope factor was selected as the recommended value; other values at the upper end of the distribution are also presented. As with the cancer oral slope factor derivation, the cancer inhalation unit risk is derived for a population composed entirely of carriers of the GST-T1 homozygous positive genotype (the group that would be expected to be most sensitive to the carcinogenic effects of dichloromethane), and a population reflecting the

estimated frequency of GST-T1 genotypes in the current U.S. population (20% GST-T1$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$, the "mixed" population). All simulations also included a distribution of CYP activity, based on data from Lipscomb et al. (2003).

Table 5-18. BMD modeling results and tumor risk factors associated with 10% extra risk for liver and lung tumors in male and female B6C3F$_1$ mice exposed by inhalation to dichloromethane for 2 years, based on liver-specific GST metabolism and whole body GST metabolism dose metrics

Internal dose metric[a]		BMDS model[b]	χ^2 goodness of fit *p*-value	Mouse BMD_{10}[c]	Mouse $BMDL_{10}$[c]	Allometric-scaled human $BMDL_{10}$[d]	Tumor risk factor[e]	
							Scaling = 1.0	Allometric-scaled
Tissue-specific	Male, liver	Multistage (1)	0.40	913.9	544.4	77.8	1.84×10^{-4}	1.29×10^{-3}
	Male, lung	Multistage (1)	0.64	61.7	48.6	7.0	2.06×10^{-3}	1.44×10^{-2}
	Female, liver	Multistage (2)	0.53	1,224.1	659.7	94.2	1.52×10^{-4}	1.06×10^{-3}
	Female, lung	Multistage (1)	0.87	51.2	40.7	5.8	2.46×10^{-3}	1.72×10^{-2}
Whole body	Male, liver	Multistage (1)	0.40	38.7	23.1	3.3	–	3.03×10^{-2}
	Male, lung	Multistage (1)	0.66	13.1	10.3	1.5	–	6.80×10^{-2}
	Female, liver	Multistage (2)	0.53	51.9	28.0	4.0	–	2.50×10^{-2}
	Female, lung	Multistage (1)	0.88	10.8	8.6	1.2	–	8.14×10^{-2}

[a]Tissue-specific dose units = mg dichloromethane metabolized via GST pathway/L tissue (liver or lung)/day; whole-body dose units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.
[b]The multistage model in EPA BMDS version 2.0 was fit to the mouse dose-response data shown in Table 5-17 using internal dose metrics calculated with the mouse PBPK model. Numbers in parentheses indicate the degree polynomial of the model.
[c]BMD_{10} and $BMDL_{10}$ refer to the BMD-model-predicted mouse internal dose and its 95% lower confidence limit, associated with a 10% extra risk for the incidence of tumors.
[d]Mouse $BMDL_{10}$ divided by $(BW_{human}/BW_{mouse})^{0.25} = 7$.
[e]Dichloromethane tumor risk factor (extra risk per unit internal dose) derived by dividing the BMR (0.1) by the mouse $BMDL_{10}$ and by the allometric-scaled human $BMDL_{10}$, for the scaling = 1.0 and allometric-scaled risk factors, respectively.

Table 5-19. Inhalation unit risks for dichloromethane based on PBPK model-derived internal liver and lung doses in B6C3F$_1$ male mice exposed via inhalation for 2 years, based on liver-specific GST metabolism and whole body metabolism dose metrics, by population genotype

Internal dose metric and scaling factor[a]	Population genotype[b]	Tumor type	Human tumor risk factor[c]	Distribution of human internal dichloromethane doses from 1 µg/m^3 exposure[d]			Resulting candidate human inhalation unit risk[e] (µg/m^3)$^{-1}$		
				Mean	95th percentile	99th percentile	Mean	95th percentile	99th percentile
Tissue-specific, allometric-scaled	GST-T1$^{+/+}$	Liver	1.29×10^{-3}	6.61×10^{-6}	2.21×10^{-5}	4.47×10^{-5}	8.5×10^{-9}	2.8×10^{-8}	5.8×10^{-8}
	GST-T1$^{+/+}$	Lung	1.44×10^{-2}	3.89×10^{-7}	1.24×10^{-6}	2.42×10^{-6}	5.6×10^{-9}	1.8×10^{-8}	3.5×10^{-8}
	Mixed	Liver	1.29×10^{-3}	3.71×10^{-6}	1.43×10^{-5}	3.03×10^{-5}	4.8×10^{-9}	1.8×10^{-8}	3.9×10^{-8}
	Mixed	Lung	1.44×10^{-2}	2.20×10^{-7}	8.06×10^{-7}	1.69×10^{-6}	3.2×10^{-9}	1.2×10^{-8}	2.4×10^{-8}
Tissue-specific, scaling = 1.0	GST-T1$^{+/+}$	Liver	1.84×10^{-4}	6.61×10^{-6}	2.21×10^{-5}	4.47×10^{-5}	1.2×10^{-9}	4.1×10^{-9}	8.2×10^{-9}
	GST-T1$^{+/+}$	Lung	2.06×10^{-3}	3.89×10^{-7}	1.24×10^{-6}	2.42×10^{-6}	8.0×10^{-10}	2.6×10^{-9}	5.0×10^{-9}
	Mixed	Liver	1.84×10^{-4}	3.71×10^{-6}	1.43×10^{-5}	3.03×10^{-5}	6.8×10^{-10}	2.6×10^{-9}	5.6×10^{-9}
	Mixed	Lung	2.06×10^{-3}	2.20×10^{-7}	8.06×10^{-7}	1.69×10^{-6}	4.5×10^{-10}	1.7×10^{-9}	3.5×10^{-9}
Whole-body, allometric-scaled	GST-T1$^{+/+}$	Liver	3.03×10^{-2}	1.80×10^{-7}	6.38×10^{-7}	1.41×10^{-6}	5.5×10^{-9}	1.9×10^{-8}	4.3×10^{-8}
	GST-T1$^{+/+}$	Lung	6.80×10^{-2}	1.80×10^{-7}	6.38×10^{-7}	1.41×10^{-6}	1.2×10^{-8}	4.3×10^{-8}	9.6×10^{-8}
	Mixed	Liver	3.03×10^{-2}	1.01×10^{-7}	4.00×10^{-7}	9.43×10^{-7}	3.1×10^{-9}	1.2×10^{-8}	2.9×10^{-8}
	Mixed	Lung	6.80×10^{-2}	1.01×10^{-7}	4.00×10^{-7}	9.43×10^{-7}	6.9×10^{-9}	2.7×10^{-8}	6.4×10^{-8}

[a]Tissue specific dose units = mg dichloromethane metabolized via GST pathway/L tissue (liver or lung, respectively, for liver and lung tumors)/day; whole-body dose units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.
[b]GST-T1$^{+/+}$ = homozygous, full enzyme activity;); mixed = population reflecting estimated frequency of genotypes in current U.S. population: 20% GST-T$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$ (Haber et al., 2002).
[c]Dichloromethane tumor risk factor (extra risk per unit internal dose) derived by dividing the BMR (0.1) by the allometric-scaled human $BMDL_{10}$ or by the mouse $BMDL_{10}$ (from Table 5-18) for the allometric-scaled and scaling = 1.0 risk factors, respectively.
[d]Mean, 95th, and 99th percentile of the human PBPK model-derived probability distribution of daily average internal dichloromethane dose resulting from chronic exposure to 1 µg/m^3 (0.00029 ppm).
[e]Derived by multiplying the dichloromethane tumor risk factor by the PBPK model-derived probabilistic internal doses from daily exposure to 1 µg/m^3.

5.4.2.5. *Cancer Inhalation Unit Risk*

The recommended cancer inhalation unit risks for the development of liver cancer and lung cancer, respectively, are 9×10^{-9} $(\mu g/m^3)^{-1}$ and 6×10^{-9} $(\mu g/m^3)^{-1}$, based on the mean for the GST-T1$^{+/+}$ population (the group with the greatest presumed sensitivity). These values are based on male B6C3F$_1$ mice, using a tissue-specific GST metabolism dose metric with allometric scaling (Table 5-19). Risk estimates were similar to the values based on female mice in the NTP (1986) inhalation study: 7×10^{-9} $(\mu g/m^3)^{-1}$ and 7×10^{-9} $(\mu g/m^3)^{-1}$ for the development of liver and lung cancer, respectively, in the GST-T1$^{+/+}$ population (see Appendix H).

Consideration of combined risk (summing risk across tumors). With two significant tumor sites, focusing on the more sensitive response may underestimate the overall cancer risk associated with exposure to this chemical. Following the recommendations of the NRC (1994) and the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), an upper bound on total risk was estimated in order to gain some understanding of the total risk from multiple tumor sites in the selected data set. Note that this estimate of overall risk describes the risk of developing either tumor type, not just the risk of developing both simultaneously.

NRC (1994) stated that an approach based on counts of animals with one or more tumors (or tumor-bearing animals) would tend to underestimate overall risk when tumor types occur independently and that an approach based on combining the risk estimates from each separate tumor type should be used. For dichloromethane, there is no reason to expect that the occurrence of one tumor type depends on the incidence of the other, given the association of different dose metrics (i.e., different tissue-specific metabolism) with each tumor response. Therefore, it appears reasonable to assume that the two tumor types occur independently. However, simply summing upper limit risks may result in an overestimation of overall combined risk because of the statistical issues with respect to summing variances of distributions. An additional challenge results from the use of different internal dose metrics for different tumors. Statistical methods based on a common metric cannot be used with the tissue-specific metabolism metric used in these derivations.

An alternative approach is to derive an upper bound on the combined risk estimates by summing central tendency risks and calculating a pooled SD by using BMD_{10} and $BMDL_{10}$ values for liver and lung tumors. The SD associated with the inhalation unit risk for each tumor site is calculated as the difference between 95th percentiles of the distribution for upper bound and maximum likelihood estimate inhalation unit risks (based on either female or male mouse tumor risk factors), divided by 1.645 (the relevant *t* statistic, assuming normal distributions of summed quantities). Variances for each tumor site are the squares of the SDs. Pooled variance and SD are defined as the sum of variances for lung and liver tumors and the square root of that sum, respectively. Finally, the upper bound on the combined lung and liver cancer risk is determined by multiplying the cumulative SD by 1.645 and adding it to the summed central

tendency inhalation unit risks. The calculations of these upper bound estimates for combined liver and lung tumor risks are shown in Table 5-20.

Using this approach and the male mouse-derived risk factors, the combined human equivalent inhalation unit risk values for both tumor types is **1×10^{-8} $(\mu g/m^3)^{-1}$** (rounded from 1.3×10^{-8}) in the most sensitive (GST-$T1^{+/+}$) population. This is the recommended inhalation cancer unit risk value to be used for chronic exposure to dichloromethane. The application of ADAFs to the cancer inhalation unit risk is recommended and is described in Section 5.4.4.

5.4.2.6. *Comparative Derivation Based on Rat Mammary Tumor Data*

Mammary gland tumor data from male and female F344 rats following an inhalation exposure to dichloromethane were considered in development of a comparative inhalation unit risk for dichloromethane (Mennear et al., 1988; NTP, 1986). In both the male and female rats, there were significant increases in the incidence of adenomas, fibroadenomas, or fibromas in or near the mammary gland. These were characterized as benign tumors in the NTP report (NTP, 1986). Increased numbers of benign mammary tumors per animal in exposed groups were also seen in two studies of Sprague-Dawley rats (Nitschke et al., 1988a; Burek et al., 1984). A gavage study in Sprague-Dawley rats reported an increased incidence of malignant mammary tumors, mainly adenocarcinomas (8, 6, and 18% in the control, 100, and 500 mg/kg dose groups, respectively), but the increase was not statistically significant. Data were not provided to allow an analysis that accounts for differing mortality rates (Maltoni et al., 1988). There are considerably more uncertainties regarding the interpretation of these data with respect to carcinogenic risk compared with the data pertaining to liver and lung tumors. The trends were driven in large part by benign tumors; adenocarcinomas and carcinomas were seen only in the females with incidences of 1, 2, 2, and 0 in the 0, 1,000, 2,000, and 4,000 ppm exposure groups, respectively. There are little data to guide the choice of relevant dose metric, and the genotoxicity and mechanistic studies have not included mammary tissue. For these reasons, the analysis and the calculation of the comparative inhalation unit risk based on rat mammary tumor data are presented in Appendix I. The alternative inhalation unit risk based on the female rat data was 1×10^{-7} $(\mu g/m^3)^{-1}$.

Table 5-20. Upper bound estimates of combined human inhalation unit risks for liver and lung tumors resulting from lifetime exposure to 1 µg/m^3 dichloromethane based on liver-specific GST metabolism and whole body metabolism dose metrics, by population genotype

Internal dose metric and scaling factor[a]	Population genotype[b]	Tumor site	Upper bound inhalation unit risk[c]	Central tendency inhalation unit risk[d]	Variance of tissue-specific tumor risk[e]	Combined tumor risk SD[f]	Upper bound on combined tumor risk[g] $(\mu g/m^3)^{-1}$
Tissue-specific, allometric-scaled	GST-T1$^{+/+}$	Liver	8.5×10^{-9}	5.1×10^{-9}	4.36×10^{-18}	–	–
		Lung	5.6×10^{-9}	4.4×10^{-9}	5.18×10^{-19}	–	–
		Liver or lung		9.5×10^{-9}	–	2.2×10^{-9}	1.3×10^{-8}
	Mixed	Liver	4.8×10^{-9}	2.8×10^{-9}	1.37×10^{-18}	–	–
		Lung	3.2×10^{-9}	2.5×10^{-9}	1.66×10^{-19}	–	–
		Liver or lung		5.3×10^{-9}	–	1.2×10^{-9}	7.4×10^{-9}
Tissue-specific, scaling = 1.0	GST-T1$^{+/+}$	Liver	1.2×10^{-9}	7.2×10^{-10}	8.91×10^{-20}	–	–
		Lung	8.0×10^{-10}	6.3×10^{-10}	1.07×10^{-20}	–	–
		Liver or lung		1.4×10^{-9}	–	3.2×10^{-10}	1.9×10^{-9}
	Mixed	Liver	6.8×10^{-10}	4.1×10^{-10}	2.81×10^{-20}	–	–
		Lung	4.5×10^{-10}	3.6×10^{-10}	3.41×10^{-21}	–	–
		Liver or lung		7.6×10^{-10}	–	1.7×10^{-10}	1.1×10^{-9}
Whole-body, allometric-scaled	GST-T1$^{+/+}$	Liver	5.5×10^{-9}	3.3×10^{-9}	1.79×10^{-18}	–	–
		Lung	1.2×10^{-8}	9.6×10^{-9}	2.55×10^{-18}	–	–
		Liver or lung		1.3×10^{-8}	–	2.1×10^{-9}	1.6×10^{-8}
	Mixed	Liver	3.1×10^{-9}	1.8×10^{-9}	5.62×10^{-19}	–	–
		Lung	6.9×10^{-9}	5.4×10^{-9}	8.03×10^{-19}	–	–
		Liver or lung		7.2×10^{-9}	–	1.2×10^{-9}	9.2×10^{-9}

[a]Tissue specific dose units = mg dichloromethane metabolized via GST pathway/L tissue (liver or lung, respectively, for liver and lung tumors)/day; whole-body dose units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.
[b]GST-T1$^{+/+}$ = homozygous, full enzyme activity; mixed = population reflecting estimated frequency of genotypes in current U.S. population: 20% GST-T$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$ (Haber et al., 2002).
[c]Estimated at the human equivalent $BMDL_{10}$ ($0.1/BMDL_{10}$) (see Table 5-18).
[d]Estimated at the human equivalent BMD_{10} (0.1/BMD) (see Table 5-18).
[e]Calculated as the square of the difference of the upper bound and central tendency inhalation unit risks divided by the *t* statistic, 1.645.
[f]Calculated as the square root of the sum of the variances for liver and lung tumors.
[g]Calculated as the product of the cumulative tumor risk SD and the *t* statistic, 1.645, added to the sum of central tendency inhalation unit risks.

5.4.2.7. *Alternative Based on Administered Concentration*

Another comparison that can be made is with an alternative inhalation unit risk based on liver and lung tumors in mice using the external concentrations of dichloromethane in the mouse studies as converted to HECs, and then applying this using BMD modeling to obtain the $BMDL_{10}$ and resulting inhalation unit risk. Mouse bioassay exposures were adjusted to HECs as follows:

- Adjusting to continuous exposure: External $concentration_{ADJ}$ = External concentration × (6 hours/24 hours) × (5 days/7 days);
- Concentrations in mg/m^3 = concentrations in ppm × 84.93/24.45; and
- $[H_{b/g}]_A/[H_{b/g}]_H$ = the ratio of blood:gas (air) partition coefficients in animals and humans. Because the partition coefficient for mice (23.0) is higher than for humans (9.7), a value of 1.0 was used, as per U.S. EPA (1994) guidance.

Thus, HECs = External $concentration_{ADJ} \times [H_{b/g}]_A/[H_{b/g}]_H$ = External $concentration_{ADJ} \times 1$.

The HECs (mg/m^3) for the 0, 2,000, and 4,000 ppm exposure groups were 0, 1,241, and 2,481 mg/m^3, respectively. The BMD modeling and inhalation unit risks derived from these values, in conjunction with the liver and lung tumor data from Table 5-17 (NTP, 1986), are shown in Table 5-21. The resulting inhalation unit risks based on the liver or lung tumors in the mouse are approximately one order of magnitude higher than the currently recommended value obtained by using the mouse and human PBPK models.

Table 5-21. Inhalation units risks based on human $BMDL_{10}$ values using administered concentration for liver and lung tumors in $B6C3F_1$ mice exposed by inhalation to dichloromethane for 2 years

Sex, tumor type	BMDS model[a]	χ^2 goodness of fit *p*-value	BMD_{10}[b]	$BMDL_{10}$[b]	Inhalation unit risk[c] $(\mu g/m^3)^{-1}$
Male, liver	Multistage (1)	0.37	463.89	276.15	3.6×10^{-7}
Male, lung	Multistage (1)	0.54	157.23	124.10	8.1×10^{-7}
Female, liver	Multistage (2)	0.38	601.84	342.83	2.9×10^{-7}
Female, lung	Multistage (1)	0.77	126.40	100.61	9.9×10^{-7}

[a]The multistage model in EPA BMDS version 2.0 was fit to each of the four sets of mouse dose-response data shown in Table 5-17. The HEC used in these models for the 0, 2,000, and 4,000 ppm exposure groups were 0, 1,241, and 2,481 mg/m^3, respectively. Numbers in parentheses indicate the lowest degree polynomial of the model showing an adequate fit.
[b]BMD_{10} and $BMDL_{10}$ refer to the BMD-model-predicted HECs (mg dichloromethane/m^3), and its 95% lower confidence limit associated with a 10% extra risk for the incidence of tumors.
[c]Inhalation unit risk (risk/μg-m^3) = 0.1/human $BMDL_{10}$.

Sources: Mennear et al. (1988); NTP (1986).

The difference between the administered concentration methodology and PBPK-based approaches depends on two key differences: the use of a dichloromethane-metabolite dose-metric rather than dichloromethane AUC, and the fact that the rate of dichloromethane conversion to that metabolite is estimated in humans by using human data rather than default allometric scaling ($BW^{0.75}$). In addition, the administered concentration methodology does not account in any way for the GST polymorphism and so might be considered as representing the general/mixed-GST-genotype population rather than the +/+ subpopulation.

5.4.2.8. *Previous IRIS Assessment: Cancer Inhalation Unit Risk*

The inhalation unit risk in the previous IRIS assessment was determined from the combined incidence of liver and lung adenomas and carcinomas in $B6C3F_1$ mice exposed to dichloromethane for 2 years by NTP (1986). A value of 4.7×10^{-7} $(\mu g/m^3)^{-1}$ was derived by the application of a modified version of the PBPK model of Andersen et al. (1987), which incorporated the pharmacokinetics and metabolism of dichloromethane. Internal dose estimates based on dichloromethane metabolism via the GST pathway were used and corrected for differences in interspecies sensitivity by applying to the human risks an interspecies scaling factor of 12.7, which was based on dose equivalence adjusted to BW to the 2/3 power (Rhomberg, 1995; U.S. EPA, 1987b).

5.4.2.9. *Comparison of Cancer Inhalation Unit Risk Using Different Methodologies*

In this assessment, cancer inhalation unit risks derived by using different dose metrics and assumptions were examined, as summarized in Table 5-22. The recommended inhalation unit risk value of 1×10^{-8} $(\mu g/m^3)^{-1}$ is based on a tissue-specific, GST-internal dose metric with allometric scaling because of the evidence for the involvement of highly reactive metabolites formed via the GST pathway. The value derived specifically for the GST-$T1^{+/+}$ population is recommended to provide protection for the population that is hypothesized to be most sensitive to the carcinogenic effect. The values based on the GST-$T1^{+/+}$ group are approximately two- to fivefold higher than those for the full population for all dose metrics used in this assessment. Within a genotype population, the values of the inhalation unit risk among the various dose metrics vary by about one to two orders of magnitude. The slope of the linear extrapolation from the BMD_{10}, the central estimate of exposure associated with 10% extra cancer risk, was also derived based on the male mouse liver and lung tumor incidence data (Table 5-17). The slope of the linear extrapolation from the central estimate BMD_{10} is 0.1/10,500 mg/m^3 = 9.5×10^{-9} per $\mu g/m^3$.

Table 5-22. Comparison of inhalation unit risks derived by using various assumptions and metrics

Population[a]	Dose metric	Species, sex	Tumor type	Scaling factor	Inhalation unit risk[b] $(\mu g/m^3)^{-1}$	Source (Table)
GST-T1$^{+/+}$	**Tissue-specific GST-metabolism rate[c]**	**Mouse, male**	**Liver and lung**	**7.0**	**1.3×10^{-8}**	**Table 5-20**
GST-T1$^{+/+}$	Tissue-specific GST-metabolism rate	Mouse, male	Liver	7.0	8.5×10^{-9}	Table 5-19
GST-T1$^{+/+}$	Tissue-specific GST-metabolism rate	Mouse, male	Lung	7.0	5.6×10^{-9}	Table 5-19
GST-T1$^{+/+}$	Tissue-specific GST-metabolism rate	Mouse, male	Liver and lung	1.0	1.9×10^{-9}	Table 5-20
GST-T1$^{+/+}$	Tissue-specific GST-metabolism rate	Mouse, male	Liver	1.0	1.2×10^{-9}	Table 5-19
GST-T1$^{+/+}$	Tissue-specific GST-metabolism rate	Mouse, male	Lung	1.0	8.0×10^{-10}	Table 5-19
GST-T1$^{+/+}$	Whole-body GST metabolism rate	Mouse, male	Liver and lung	7.0	1.6×10^{-8}	Table 5-20
GST-T1$^{+/+}$	Whole-body GST metabolism rate	Mouse, male	Liver	7.0	5.5×10^{-9}	Table 5-19
GST-T1$^{+/+}$	Whole-body GST metabolism rate	Mouse, male	Lung	7.0	1.2×10^{-8}	Table 5-19
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Liver and lung	7.0	7.4×10^{-9}	Table 5-20
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Liver	7.0	4.8×10^{-9}	Table 5-19
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Lung	7.0	3.2×10^{-9}	Table 5-19
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Liver and lung	1.0	1.1×10^{-9}	Table 5-20
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Liver	1.0	6.8×10^{-10}	Table 5-19
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Lung	1.0	4.5×10^{-10}	Table 5-19
Mixed	Whole-body GST metabolism rate	Mouse, male	Liver and lung	7.0	9.2×10^{-9}	Table 5-20
Mixed	Whole-body GST metabolism rate	Mouse, male	Liver	7.0	3.1×10^{-9}	Table 5-19
Mixed	Whole-body GST metabolism rate	Mouse, male	Lung	7.0	6.9×10^{-9}	Table 5-19
	Administered concentration (HEC)	Mouse, male	Liver	–	3.6×10^{-7}	Table 5-21
	Administered concentration (HEC)	Mouse, male	Lung	–	8.1×10^{-7}	Table 5-21
	1995 IRIS assessment	Mouse, male	Liver and lung	12.7	4.7×10^{-7}	

[a]GST-T1$^{+/+}$ = homozygous, full enzyme activity; mixed = genotypes based on a population reflecting the estimated frequency of genotypes in the current U.S. population: 20% GST-T1$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$ (Haber et al., 2002).
[b]Based on mean value of the derived distributions.
[c]Bolded value is the basis for the recommended inhalation unit risk of 1×10^{-8} $(\mu g/m^3)^{-1}$.

5.4.3. Differences Between Current Assessment and Previous IRIS PBPK-based Assessment

To better understand the changes in assessment risk predictions between previous EPA evaluations and the current assessment, the differences in PBPK model parameters for the $B6C3F_1$ mouse were evaluated. Values that differed significantly between the model version used previously and that of Marino et al. (2006), along with derived group parameters that lend further insight, are shown in Table 5-23.

Table 5-23. Comparison of key $B6C3F_1$ mouse parameters differing between prior and current PBPK model application

Parameter[a]	Marino et al. (2006); mean values as applied (posterior)	U.S. EPA (1988a, 1987a, b)
Partition coefficients		
PB blood/air	23	8.29
PF fat/blood	5.1	14.5
PF·PB (fat/blood)·(blood/air) = fat/air	117.3	120.2
PL liver/blood	1.6	1.71
PL·PB (liver/blood)·(blood/air) = liver/air	36.8	14.2
PLu lung (tissue)/blood	0.46	1.71
PLu·PB (lung/blood)·(blood/air) = lung/air	10.6	14.2
PR rapidly perfused/blood	0.52	1.71
PR·PB rapidly perfused/air	12.0	14.2
PS slowly perfused/blood	0.44	0.96
PS·PB slowly perfused/air	10.1	7.96
Flow rates		
QCC cardiac output (L/hr/kg$^{0.74}$)	24.2	14.3
VPR ventilation:perfusion ratio	1.45	1.0
Metabolism parameters		
V_{maxC} maximum CYP metabolic rate (mg/hr/kg$^{0.7}$)	9.27	11.1
K_m CYP affinity (mg/L)	0.574	0.396
V_{maxC}/K_m (L/hr/kg$^{0.7}$)	16.1	28
A1 ratio of lung V_{maxC} to liver V_{maxC}	0.207	0.416
Total lung + liver V_{maxC}/K_m	19.5	39.7
k_{fC} first-order GST metabolic rate constant (kg$^{0.3}$/hr)	1.41	1.46
A2 ratio of lung k_{fC} to liver k_{fC}	0.196	0.137
Total lung + liver k_{fC}	1.69	1.66

[a]Parameters not listed differed by <10% between versions. See Table 3-5 and associated text for details.

While a number of the tissue:blood partition coefficients in Table 5-23 differ significantly between the two models (e.g., PF, PLu, and PR), the corresponding tissue:air coefficients (e.g., the products PF·PB, Plu·PB, and PR·PB) generally do not. Since the latter tend to determine the long-term equilibration between the tissue (tissue group) and air, the differences in the tissue:blood coefficients are not expected to significantly impact long-term risk predictions. Thus, the partition coefficients that most significantly differ (the blood:air and liver:air partition coefficients) are, respectively, 2.8- and 2.6-fold higher in the current version. The increased PB results in a tendency for simulated blood concentrations to rise more quickly and reach higher

values with other parameters being equal. The significantly increased QCC and VPR contribute even more to this difference, resulting in an even faster rise to steady state during inhalation exposure simulations, but also more rapid delivery to the liver (decreasing blood-flow limitation of hepatic metabolism) and more rapid exhalation. The increased liver:air partition coefficient leads to higher predicted liver concentrations (again, other parameters being equal) and, hence, higher rates of metabolism.

For metabolism, a much reduced oxidative metabolism is seen, which at low doses depends on V_{maxC}/K_m. The revised hepatic metabolism is over 40% lower, and the total of lung plus liver metabolism is 50% lower than previously used. This lower rate of metabolism means that far less of parent dichloromethane will be removed through metabolism and, hence, predicted blood concentrations will be higher still relative to the impact of changes in partition coefficient, QCC, and VPR, as noted above.

The result of having higher predicted blood and liver dichloromethane concentrations is that, while the GSH-pathway metabolic constant, k_{fC}, is virtually the same for the mouse liver in both cases, the much higher concentration of dichloromethane available will lead to a much higher predicted rate of metabolism via this pathway. For the lung, since the A2 is 43% higher in the model of Marino et al. (2006), the relative increase will be even greater.

Because the revised rate of GST metabolism in mice was estimated by using data with CYP2E1 inhibited by a suicide inhibitor, there is considerable confidence in the relative rate of metabolism by these two pathways and the GST pathway in particular. The partition coefficients used by Marino et al. (2006) are as measured by Clewell et al. (1993) and expected to be at least as reliable as those used in the 1995 assessment. Considering that the revised PBPK model does an excellent job of reproducing closed-chamber gas uptake data that were not available for calibration of the 1987 model as well as blood concentrations after intravenous injection, there is fairly high confidence in its predictions.

The net result of these model changes is that, under mouse bioassay conditions, the predicted dose metrics for liver and lung cancer (i.e., GST-mediated metabolism) are higher than those obtained with the previous model, resulting in a lower risk estimated per unit of dose.

The other piece of the PBPK-based risk estimation is the human model. In updating the parameter estimates for the human model (see Appendix B for details), the oxidative metabolism V_{maxC}/K_m approximately doubled, which leads to lower predicted blood concentrations of dichloromethane available for metabolism by GST. In addition, the liver GST was reduced by almost 60%, and the lung:liver GST ratio decreased by almost fivefold for a net change in lung GST of over 90%. Given the larger human data set available to David et al. (2006) and the sophisticated Bayesian analysis used to recalibrate the model, the expectation is that these values are more reliable than the values used in the 1995 IRIS assessment.

Since actual rates of metabolism at a given exposure level also depend on respiration rate and blood flows, these changes in metabolic parameters do not completely determine the relative

(predicted) dosimetry. But the difference in cancer risk predictions between the current and previous assessments is primarily explained by the overall prediction of higher GST-mediated dosimetry in the mouse (at bioassay conditions) and lower human GST metabolism (due in part to greater predicted clearance of dichloromethane by oxidative metabolism). In addition to these changes in PBPK parameters, the reduction of scaling factor from 12.7 to 7 is a significant factor in the overall change from the previous assessments.

5.4.4. Application of Age-Dependent Adjustment Factors (ADAFs)

The available dichloromethane studies do not include an evaluation of early-life susceptibility to dichloromethane cancer risk. In the absence of this type of data, and if a chemical follows a mutagenic mode of action for carcinogenicity like dichloromethane, the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) recommends that ADAFs be applied to the cancer values. Since the oral slope factor of 2×10^{-3} (mg/kg-day)$^{-1}$ and the inhalation unit risk of 1×10^{-8} ($\mu g/m^3$)$^{-1}$ were calculated from chronic (2-year) dichloromethane exposure beginning after early development (e.g., beginning at 7-9 weeks of age), early-life cancer susceptibility has not been accounted for in these values, and ADAFs need to be applied in combination with exposure information when estimating cancer risks that include early-life exposures. Sample calculations that incorporate ADAFs into the cancer risks are presented in subsequent sections. Additional examples of evaluations of cancer risks incorporating early-life exposure are provided in Section 6 of the *Supplemental Guidance* (U.S. EPA, 2005b).

In the *Supplemental Guidance* (U.S. EPA, 2005b), ADAFs are established for three age groups. An ADAF of 10 is applied for age groups <2 years, 3 is applied for ages 2–<16 years, and 1 is applied for ≥16 years (U.S. EPA, 2005b). The 10- and 3-fold adjustments in cancer values are combined with age-specific exposure estimates when early-life exposure considerations need to be included in cancer risk estimates. The most current information on usage of ADAFs can be found at http://www.epa.gov/cancerguidelines. For estimation of risk, the *Supplemental Guidance* (U.S. EPA, 2005b) recommends obtaining and using age-specific values for exposure and cancer potency. In the absence of age-specific cancer potency values, as is true for dichloromethane, age-specific cancer values are estimated by using the appropriate ADAFs. Using this process, a cancer risk is derived for each age group. The risks are summed across the age groups to get the total cancer risk for the age-exposure period of interest.

5.4.4.1. *Application of ADAFs in Oral Exposure Scenarios*

Sample calculations incorporating the use of ADAFs are presented for three exposure duration scenarios. These scenarios include full lifetime exposure (assuming a 70-year lifespan), and two 30-year exposures at ages 0–30 and ages 20–50. A constant dichloromethane exposure of 1 mg/kg-day was assumed for each scenario.

Table 5-24 lists the four factors (ADAFs, oral slope factor, assumed exposure, and duration adjustment) that are needed to calculate the partial cancer risk based on the early age-specific group. The partial cancer risk for each age group is the product of the four factors in columns 2–4. Therefore, the partial cancer risk following daily dichloromethane oral exposure in the age group 0 to <2 years is the product of the values in columns 2–4 or $10 \times (2 \times 10^{-3}) \times 1 \times 2/70 = 5.7 \times 10^{-4}$. The partial risks that are listed in the last column of Table 5-24 are added together to get the total risk. Thus, a 70-year (lifetime) risk estimate for continuous exposure to 1 mg/kg-day dichloromethane is 3.3×10^{-3}, which is adjusted for early-life susceptibility and assumes a 70-year lifetime and constant exposure across age groups.

Table 5-24. Application of ADAFs to dichloromethane cancer risk following a lifetime (70-year) oral exposure

Age group (yrs)	ADAF	Oral slope factor (per mg/kg-d)	Exposure concentration (mg/kg-d)	Duration adjustment	Partial risk
0–<2	10	2×10^{-3}	1	2 yrs/70 yrs	5.7×10^{-4}
2–<16	3	2×10^{-3}	1	14 yrs/70 yrs	1.2×10^{-3}
≥16	1	2×10^{-3}	1	54 yrs/70 yrs	1.5×10^{-3}
				Total risk	**3.3×10^{-3}**

In calculating the cancer risk for a 30-year constant exposure to dichloromethane at an exposure level of 1 mg/kg-day from ages 0–30, the duration adjustments would be 2/70, 14/70, and 14/70, and the partial risks for the three age groups would be 5.7×10^{-4}, 1.2×10^{-3}, and 4.0×10^{-4}, which would result in a total risk estimate of 2.2×10^{-3}.

In calculating the cancer risk for a 30-year constant exposure to dichloromethane at an exposure level of 1 mg/kg-day from ages 20–50, the duration adjustments would be 0/70, 0/70, and 30/70. The partial risks for the three groups are 0, 0, and 8.6×10^{-4}, which would result in a total risk estimate of 8.6×10^{-4}.

5.4.4.2. *Application of ADAFs in Inhalation Exposure Scenarios*

Sample calculations incorporating the use of ADAFs are presented for three exposure duration scenarios involving inhalation exposure. These scenarios include full lifetime exposure (assuming a 70-year lifespan) and two 30-year exposures from ages 0–30 and ages 20–50. A constant dichloromethane inhalation exposure of 1 $\mu g/m^3$ was assumed for each scenario.

Similar to the oral exposure scenarios presented in Section 5.4.4.1, Table 5-25 lists the four factors (ADAFs, unit risk, assumed exposure, and duration adjustment) that are needed to calculate the partial cancer risk based on the early age-specific group. The partial cancer risk for each age group is the product of the four factors in columns 2–4. Therefore, the partial cancer risk following daily dichloromethane inhalation exposure in the age group 0 to <2 years is the product of the values in columns 2–4 or $10 \times (1 \times 10^{-8}) \times 1 \times 2/70 = 2.9 \times 10^{-9}$. The partial risks

that are listed in the last column of Table 5-25 are added together to get the total risk. Thus, a 70-year (lifetime) risk estimate for continuous exposure to 1 μg/m^3 dichloromethane is 1.7×10^{-8}, which is adjusted for early-life susceptibility and assumes a 70-year lifetime and constant exposure across age groups.

Table 5-25. Application of ADAFs to dichloromethane cancer risk following a lifetime (70-year) inhalation exposure

Age group (yrs)	ADAF	Unit risk (μg/m^3)	Exposure concentration (μg/m^3)	Duration adjustment	Partial risk
0–<2	10	1×10^{-8}	1	2 yrs/ 70 yrs	2.9×10^{-9}
2–<16	3	1×10^{-8}	1	14 yrs/ 70 yrs	6.0×10^{-9}
≥16	1	1×10^{-8}	1	54 yrs/ 70 yrs	7.7×10^{-9}
				Total risk	**1.7×10^{-8}**

In calculating the cancer risk for a 30-year constant exposure to dichloromethane at a concentration of 1 μg/m^3 from ages 0–30, the duration adjustments would be 2/70, 14/70, and 14/70, and the partial risks for the three age groups are 2.9×10^{-9}, 6.0×10^{-9}, and 2.0×10^{-9}. These partial risks result in a total risk estimate of 1.1×10^{-8}.

In calculating the cancer risk for a 30-year constant exposure to dichloromethane at a concentration of 1 μg/m^3 from ages 20–50, the duration adjustments would be 0/70, 0/70, and 30/70, and the partial risks for the three age groups are 0, 0, and 4.3×10^{-9}, resulting in a total risk estimate of 4.3×10^{-9}.

5.4.5. Uncertainties in Cancer Risk Values

The derivation of cancer risk values often involves a number of uncertainties in the extrapolation of dose-response data in animals to cancer risks in human populations. Several types of uncertainty have been quantitatively integrated into the derivation of the recommended oral slope factors and inhalation unit risks for dichloromethane, while others are qualitatively considered. Table 5-26 summarizes principal uncertainties identified in previous sections, their possible effects on the cancer risk values, and decisions made in the derivations. Additional issues and considerations are discussed below.

Table 5-26. Summary of uncertainty in the derivation of cancer risk values for dichloromethane

Consideration and impact on cancer risk value	Decision	Justification and discussion
Selection of data set (Selection of an alternative data set could change the recommended cancer risk values.)	NTP (1986) selected as principal inhalation study and Serota et al. (1986b) as principal oral (drinking water) study to derive cancer risks for humans	NTP (1986) inhalation mouse bioassay provides the strongest cancer responses (liver and lung tumors) and the best dose-response data in the animal database. The oral mouse study (Serota et al., 1986b; Hazleton Laboratories, 1983) provides an adequate basis for dose-response modeling.
Selection of target organ (Selection of a target organ could change the recommended cancer risk values.)	Liver (oral and inhalation) and lung (inhalation) selected as target organs. Cancer risk values based on mammary gland tumors in rats also examined; potential brain cancer risk and hematopoietic cancer risk were identified as data gaps.	The evidence for mammary gland tumors from dichloromethane exposure is less consistent than evidence for liver and lung tumors. Inhalation cancer risk values based on mammary tumors in rats are about one order of magnitude higher than risk values based on liver or lung tumors in mice. No data are available to allow derivation of unit risks based on brain or hematopoietic cancers.
Selection of extrapolation approach (Selection of extrapolation approach could change the recommended cancer risk values.)	Oral data used for oral slope factor and inhalation data used for inhalation unit risk. Oral cancer risk values based on route-to-route extrapolation from inhalation study also examined.	Uncertainty associated with an oral slope factor derived from oral exposure data was considered lower than with an oral slope factor derived by route-to-route extrapolation when oral data from a well-conducted study were available (in this case, a 2-year drinking water study with complete histopathology). Oral cancer risk values based on route-to-route extrapolation from the NTP (1986) inhalation mouse study were about one order of magnitude lower than values based on oral exposure study.
Selection of dose metric (Selection of dose metric could change the recommended cancer risk values.)	Tissue-specific GST-metabolism used as dose metric. Cancer risk estimates based on alternative (whole-body) metrics also examined.	Contribution of CYP pathway to cancer risk unknown, but strong evidence of GST role in carcinogenesis supports focus on this pathway. Values based on tissue-specific GST metabolism recommended based on evidence of site locality of effects.
Dose-response modeling (Human risk values could increase or decrease, depending on fits of alternative models)	Use multistage dose-response model to derive a BMD and BMDL	The multistage model has biological support and is the model most consistently used in EPA cancer assessments.
Low-dose extrapolation (Human risk values would be expected to decrease with the application of nonlinear tumor responses in low-dose regions of dose-response curves.)	Linear extrapolation of risk in low-dose region used	PBPK model incorporates the metabolic shift and expected nonlinearity (GST dose attenuation) in the exposure-dose relationship across exposure levels. Linear low-dose extrapolation for agents with a mutagenic mode of action is supported.

(Table 5-26; page 1 of 2)

Table 5-26. Summary of uncertainty in the derivation of cancer risk values for dichloromethane

Consideration and impact on cancer risk value	Decision	Justification and discussion
Interspecies extrapolation of dosimetry and risk (Alternative values for PBPK model parameters and cross-species scaling factor could increase or decrease human cancer risk values.)	PBPK model and allometric scaling factor used for the primary dose metric	Use of rodent and human PBPK models reduced uncertainty due to interspecies differences in toxicokinetics. Examination of impact of different values for key parameters in human model, and sensitivity analysis of rodent PBPK model parameters identified influential metabolic parameters for which limited experimental data exist.
Sensitive subpopulations (Differences in CYP and GST metabolic rates could change cancer risk values.)	Risk estimates generated for presumed most sensitive (GST-T1$^{+/+}$) genotype; CYP variability incorporated into PBPK model	No data are available to determine the range of human toxicodynamic variability or sensitivity, including whether children are more sensitive than adults.

(Table 5-26; page 2 of 2)

Extrapolation approach. A route-to-route extrapolation from the NTP (1986) inhalation mouse bioassay was used to develop an oral slope factor for purposes of comparison. In this case, the uncertainty associated with an oral slope factor derived using oral exposure data from a well-conducted study (i.e., a 2-year drinking water study with complete histopathology) was considered lower than with an oral slope factor derived by route-to-route extrapolation. Therefore, although the exposure-response effect seen in the oral exposure study (Serota et al., 1986b) is not strong, direct derivation from oral exposure is recommended over route-to-route extrapolation as the basis for the oral slope factor.

The comparison of the oral slope factor derived from the oral exposure data and from the route-to-route extrapolation from the inhalation data provides a crude measure of the uncertainty in recommending a human oral slope factor based on the available rodent bioassay data. The cancer oral slope factors based on route-to-route extrapolation from liver tumors in mice exposed by inhalation are about an order of magnitude lower than those based on the liver tumor responses in mice exposed via drinking water. This difference may be explained at least partially by the heterogeneity of hepatic cell types within the sinusoid, resulting in region-specific toxicity. Oral exposure may result in a higher internal exposure of hepatocytes in the periportal region (particularly those lining the portal vein through which all gastrointestinal-absorbed dichloromethane passes) than in the centrilobular region (SRC, 1989). Further, the metabolic capacity of hepatic cells is also region-specific, with higher CYP activity found in the centrilobular region compared to the periportal region. Thus, liver perfusion via the systemic arterial circulation or portal drainage of the gastrointestinal tract, through which inhaled dichloromethane would be introduced, may influence region-specific hepatotoxicity, resulting in the route-of-exposure effects on toxicity. The available PBPK models do not have the capability to predict region-specific disposition of dichloromethane in the liver.

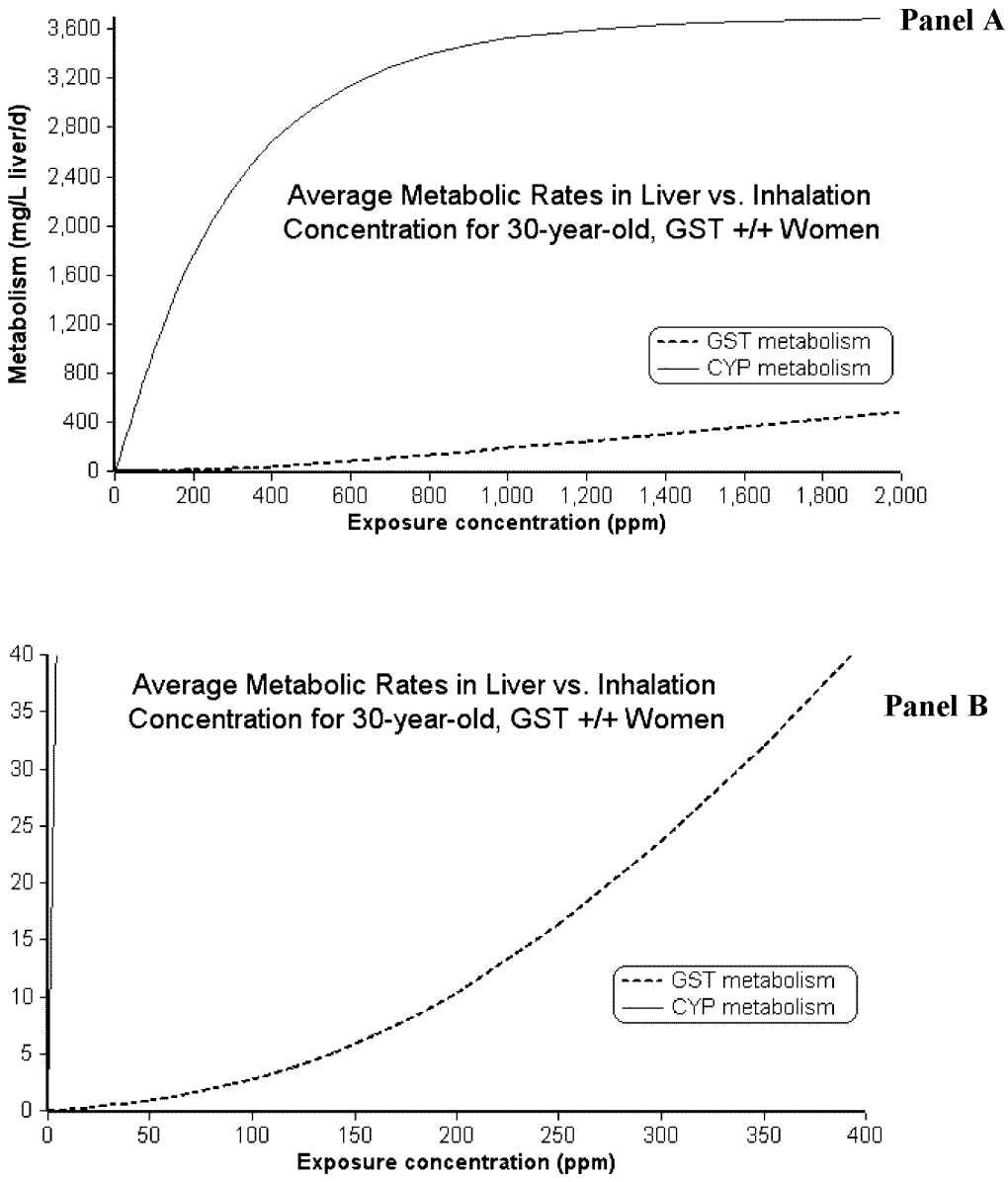
Dose-response modeling. For human oral exposure, ingestion is assumed to occur as six discrete boluses during the course of the day: 25% of the daily dose consumed at 7 am, noon, and 6 pm; 10% at 10 am and 3 pm; and 5% at 10 pm. When exposure occurs as a bolus, the short-term (peak) concentration of dichloromethane will be higher, leading to a higher degree of CYP saturation and, hence, a higher fraction metabolized by GST as compared to more continuous exposure, such as occurs by inhalation. Thus, if actual ingestion is in fewer/larger boluses than those assumed, the cancer risk will be somewhat underpredicted. If ingestion is in more/smaller boluses, the opposite will occur. However, when ingestion is fairly small, such that the peak concentration is well below the saturation constant (Km) for CYP, the difference in metabolic outcome will be negligible. The pattern used here assumes, in effect, that the amount of food and liquid ingested is divided neatly into meals and snacks or breaks as indicated *and* that the concentration of dichloromethane in the food and beverages ingested is constant. Thus, if one meal or drink happens to include the bulk of that ingested for a day, total ingestion will be more like a single daily bolus. But to the extent that people sip beverages or ingest foods over longer periods of time, actual ingestion will be more continual. Given that both of these are likely to occur to some extent, the population ingestion pattern is expected to be a distribution that includes the one used for simulation purposes. While it cannot be said that this pattern is an exact average, given that the differences in saturation at low total exposure levels will be small, it is considered sufficiently representative of the population, and the uncertainty resulting from inexact knowledge of actual ingestion is unlikely to be significant.

Low-dose extrapolation. The mode of action is a key consideration in determining how risks should be estimated for low-dose exposure. The in vitro and in vivo genotoxicity data suggest that mutagenicity is the most plausible mode of action. Because it was concluded that dichloromethane acts through a mutagenic mode of action, a linear-low-dose extrapolation approach was used to estimate oral slope factors and inhalation unit risks.

While the rate equation for GST metabolism in the PBPK model is first-order, consistent with metabolism increasing in direct proportion to the concentration of dichloromethane, and because the GST and CYP pathways compete for dichloromethane and the CYP kinetics are nonlinear (saturable), the interaction of the two pathways within the whole-body system results in a nonlinear exposure-dose relationship for both pathways. These nonlinearities are demonstrated for a simulated group of 30-year-old women (population mean kinetics for continuous inhalation exposure) in Figure 5-16. The upper panel (A) of Figure 5-16 provides a full-scale plot of CYP liver metabolism (mg/L liver/day) up to 2000 ppm exposure while the lower panel expands the CYP metabolism curve up to 400 ppm exposure (with CYP metabolism still included, but not far off the y-axis with that scale). Because both CYP and GST metabolism are linear at very low concentrations (below 10–30 ppm), the exposure-response relationship at

low exposures is linear for both pathway metrics, initially increasing from zero dose at zero concentration in direct proportion to the exposure level until CYP saturation begins. As CYP becomes saturated, starting around 50 ppm and reaching half saturation at around 200 ppm, a lower fraction of dichloromethane is eliminated by CYP metabolism. As a lower fraction is metabolized by CYP, the blood concentration increases faster than directly proportional to exposure concentration, with the result that GST metabolism also increases faster than direct proportionality: the upward curvature seen in the lower panel (B) of Figure 5-16. While GST metabolism remains less than CYP over the entire exposure range shown here for humans, in mice, where the GST pathway has relatively higher activity compared to CYP, GST metabolism increases above CYP metabolism in the range of bioassay exposures. This transition from CYP-dominated (or vastly- dominated) clearance at low exposures to a higher fraction of GST metabolism at high exposures has at times been referred to as a "switch", but as shown in Figure 5-16, the transition is smooth and continuous, and there is some GST metabolism at all exposure levels with the exposure-response approaching linearity without a threshold at low exposures.

One other important note is that in calculating inhalation unit risk for humans, the relationship between external exposure and internal dose was determined using the PBPK model at a very low level of exposure (i.e.,1 $\mu g/m^3$ or 0.00029 ppm) where the relationship is effectively linear: the difference between the actual exposure-dose curve and a straight line is <1%. However, as one goes to higher concentrations, the relationship becomes significantly nonlinear, and application of the cancer toxicity values (inhalation unit risk) will not accurately represent the risk. Because GST metabolism increases faster than proportional to exposure level with concentration, the inhalation unit risk will underpredict risk at those higher exposure levels. Analysis of the PBPK model versus the low-exposure linear estimate shows that the extent of nonlinearity is <20% for oral exposures at low doses and for inhalation exposures at <30 ppm (100 $\mu g/m^3$). The dose used for calculating the internal dose:exposure ratio for oral exposures, 1 mg/kg-day, was above the transition to nonlinear dosimetry, but only to a small extent. For oral exposures, the linear approximation used differed from the full model by <30% for exposures <2 mg/kg-day, but at doses below 1 mg/kg-day, the error would be in the direction of an overprediction of risk (i.e., actual cancer risks may be somewhat lower, but no more than 1.3-fold lower) than indicated by the linear model.



The curves represent average results for a simulated population of 1,000 women with the GST-T1 +/+ genotype. A) Relationships scaled to show full range of CYP metabolism up to 2,000 ppm inhalation exposure. B) Relationships scaled to show low-dose linearity (<50 ppm) and curvature (transition) in GST metabolism (above 50 ppm) as CYP metabolism saturates.

Figure 5-16. PBPK-model-predicted exposure-response relationships for hepatic CYP and GST metabolism for continuous inhalation exposure to dichloromethane in 30-year-old GST +/+ women.

Interspecies extrapolation of dosimetry and risk. Target organ dosimetry for neoplastic mouse responses and estimation of equivalent internal human doses were accomplished using PBPK models for dichloromethane in mice and humans. Uncertainty in the ability of the PBPK models to estimate animal and human internal doses from lifetime bioassay low-level environmental exposures may affect the confidence in the cancer risk extrapolated from animal data. Uncertainties in the mouse and human model parameter values were integrated quantitatively into parameter estimation by utilizing hierarchical Bayesian methods to calibrate the models at the population level (David et al., 2006; Marino et al., 2006). The use of Monte Carlo sampling to define human model parameter distributions allowed for derivation of human distributions of dosimetry and cancer risk, providing for bounds on the recommended risk values.

A detailed discussion of PBPK model structure (CYP rate equation) and parameter uncertainties is provided in Sections 3.5.2 and 3.5.5, respectively. While the structure and equations used in the existing model have been described in multiple peer-reviewed publications over the past two decades, there are discrepancies between dichloromethane kinetics observed in vitro and the model parameters obtained from in vivo data, and the model poorly fits some of the in vivo data (e.g., fraction of dose exhaled as CO at higher exposure levels in mice). The discrepancies are significant enough that simply re-estimating model parameters is unlikely to resolve them, but based on a limited analysis, it appears that an alternative (dual-binding-site) CYP metabolic equation (Korzekwa et al., 1998) may provide the resolution. At present, the suggestion of this alternate equation is a hypothesis that should be tested experimentally. Further, integration of the alternate rate equation into the PBPK modeling and then quantitative risk assessment will likely require several years of further research and, hence, is beyond the scope of the current assessment. Since the GST activity in the current model is within a factor of three of that measured in vitro (when both are evaluated per gram of liver), the impact of that model uncertainty is also expected to be no more than a factor of 3.

Also, as detailed in Section 3.5.2, the results of David et al. (2006) for the GST-T1 activity parameter, k_{fC}, for the combined human data set appear to be discrepant with the range of results for each of the individual data sets. Therefore, sensitivity to the human PBPK parameter distributions was evaluated by rescaling the parameters to the mean values obtained by David et al. (2006) for a specific data set (DiVincenzo and Kaplan, 1981) for which the GST activity was intermediate among those obtained across individual data sets. Specifically, the hepatic GST internal dose for a 1 $\mu g/m^3$ inhalation exposure or a 1 mg/kg-day oral exposure was simulated in the GST-$T1^{+/+}$ population using the alternate parameters. The upper bounds on internal dose for both exposure routes increased by just over an order of magnitude, and the mean values increased by approximately 20-fold. Thus, it is possible that revision or refinement of the PBPK model could have a larger impact on the cancer risk estimates. The ultimate impact

will depend on how revisions affect model predictions for both the animal and the human. If the predicted GST metabolism per unit exposure increases in both mice and humans by a similar factor, there will be little impact on the risk estimate. But if the GST activity predicted in the mouse is decreased by a factor of 3, while that in the human is increased by a factor of 3, for example, then the net impact would be an increase of ninefold in human risk estimates.

Sensitivity analysis of the mouse PBPK parameters. The mouse and rat PBPK models were utilized deterministically (i.e., the single-value parameter estimates for the rat PBPK model were used for rat dosimetry simulations, and the mean parameter estimates from the Bayesian analysis of Marino et al. (2006) were used for the mouse dosimetry simulations). To assess the effect of using point estimates of parameter values for calculation of rodent dosimetry, a sensitivity analysis was performed to identify model parameters most influential on the predictions of dose metrics used to estimate oral and inhalation cancer risks. As was described in the RfD and RfC sensitivity analysis calculation, this procedure used a univariate analysis in which the value of an individual model parameter was perturbed by an amount (Δ) in the forward and reverse direction (i.e., an increase and decrease from the nominal value), and the change in the output variable was determined. Results are for the effects of a perturbation of ±1% from the nominal value of each parameter on the output values at the end of a minimum of 10,000 simulated hours. This time was chosen to achieve a stable daily value of the dose metric, given that the simulated bioassay exposures did not include weekend exposures. The exposure conditions represented the lowest bioassay exposure resulting in significant increases in the critical effect. For inhalation exposures in mice, the PB, followed closely by the first-order GST-mediated metabolism rate (k_{fC}), had the greatest impact on the dose metric for liver cancer (mg dichloromethane metabolized via GST pathway/L liver/day) (Figure 5-17). For drinking water exposures in mice, the k_{fC}, followed by the CYP-mediated maximum reaction velocity (V_{maxC}), affected the liver cancer dose metric to the greatest extent (Figure 5-18). For mice inhaling dichloromethane, the lung cancer dose metric (mg dichloromethane metabolized via GST pathways/L lung/day), like the liver cancer metric, was highly affected by the k_{fC} and the PB (Figure 5-19). However, since GST-mediated lung metabolism is calculated as a constant fraction of the liver metabolism rate ($A2 \times k_{fC}$), the lung cancer dose metric was most sensitive to the proportional yield of liver GST-mediated metabolic activity attributed to the lung. The PB was experimentally determined, lending high confidence to its value. Values for the three metabolic parameters were determined by computational optimization against data sets not directly measuring dichloromethane or its metabolites in the target/metabolizing tissues. It is uncertain how alternative values for these three parameters would affect the estimation of animal $BMDL_{10}$ values and, ultimately, the oral slope factors and inhalation unit risks.

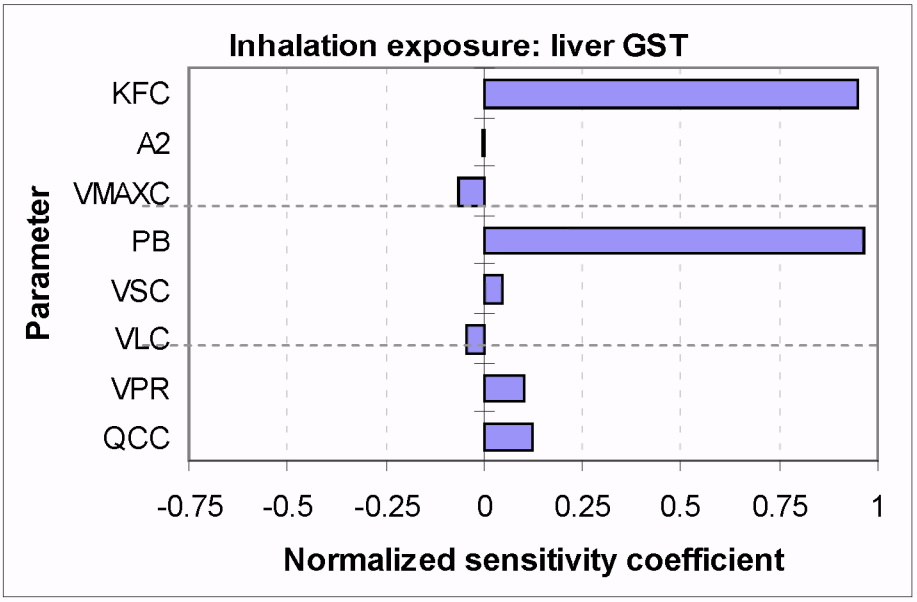


Figure 5-17. Sensitivity coefficients for long-term mass GST-mediated metabolites per liver volume from a long-term average daily inhalation concentration of 2,000 ppm in mice.

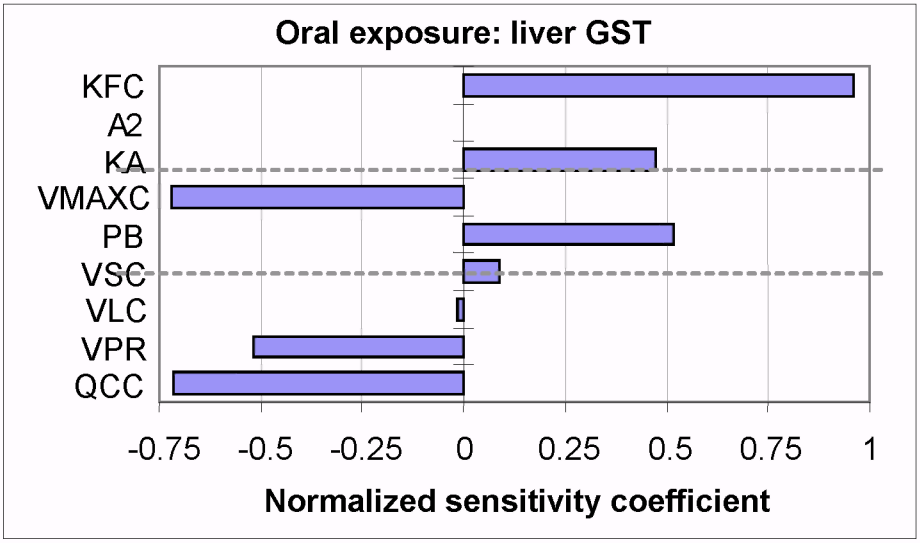


Figure 5-18. Sensitivity coefficients for long-term mass GST-mediated metabolites per liver volume from a long-term average daily drinking water concentration of 500 mg/L in mice.

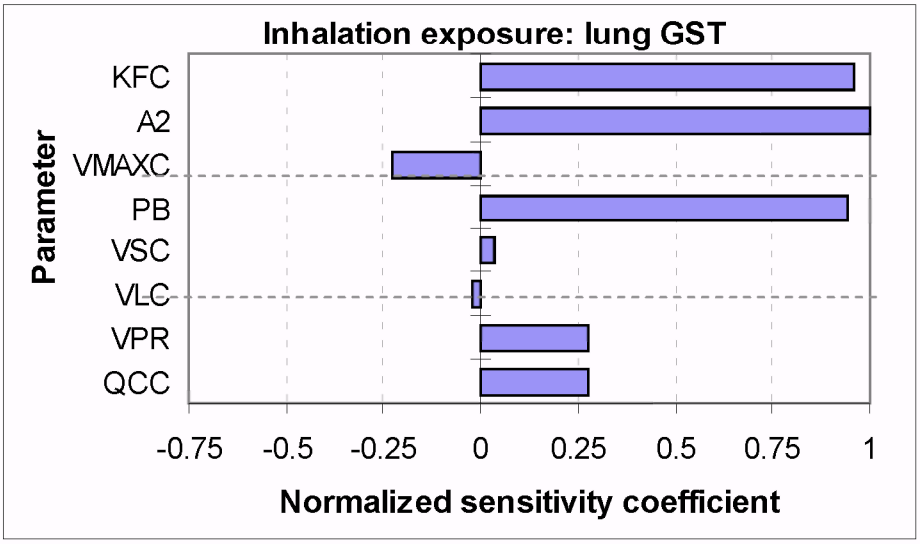


Figure 5-19. Sensitivity coefficients for long-term mass GST-mediated metabolites per lung volume from a long-term average daily inhalation concentration of 500 ppm in mice.

There is uncertainty as to whether the reactivity of the toxic dichloromethane metabolites is sufficiently high enough to preclude systemic distribution. Therefore, alternative derivations of cancer risk values were performed under the assumption that high reactivity leads to complete clearance from the tissue in which the active metabolite is formed (scaling factor = 1.0). The difference in scaling factor (7.0 for allometric scaling versus 1.0) results in a sevenfold decrease in estimated cancer toxicity values. Using a whole-body GST metabolism dose metric, the resulting oral slope factor and inhalation unit risk for liver cancer was approximately fivefold lower than when tissue-specific dose metrics were used (Tables 5-16 and 5-22); however, the inhalation unit risks for lung cancer and for the combined liver and lung cancer risk were higher with the whole-body compared with the tissue-specific metric (Table 5-22). This difference reflects the lower metabolism that occurs in human versus mouse lung (relative to total); lung-specific metabolism is lower in humans than mice, so the predicted risk in the lung is lower when based on that metabolism versus when whole-body metabolism is used. The mechanistic data support the hypothesis that reactive metabolites produced in the target tissues do not distribute significantly beyond those tissues and cause deleterious effects in the metabolizing tissues soon after generation. Thus, there is less uncertainty in the cancer risk values derived by using a tissue-specific GST metabolism dose metric compared with those derived using a whole-body GST metabolism dose metric.

Sensitive human populations. Possible sensitive populations include persons with altered CYP (e.g., obese individuals, alcoholics, diabetics, and the very young) and GST (e.g., GST-T1 homozygous conjugators) metabolic capacity. The PBPK model includes an estimate of the

variability of CYP metabolism (sixfold variation) within the general population but does not specifically address what could be greater variation in these other groups. However, the known polymorphisms for GST-T1 expression were integrated into the human model. The distributions of human inhalation unit risk values (from which the recommended [i.e., mean] values were taken) show that the 99th percentiles are approximately seven- and sixfold higher than means for liver and lung cancer, respectively. For the distribution of oral slope factors, the 99th percentile is approximately twofold higher than the mean for liver cancer.

To further characterize the potential sensitivity of specific subpopulations, internal dose distributions for oral exposure to 1 mg/kg-day or inhalation exposure to 1 mg/m^3 were estimated for 1-year-old children and 70-year-old men and women to compare with the broader population results used to estimate cancer risks above. Since the recommended cancer risk estimate is based on the GST-T1$^{+/+}$ subpopulation, this analysis was also restricted to that subpopulation so that only the factors of age and gender would differ. The impact of considering other GST-T1 groups can be seen where risk estimates for the GST-T1$^{+/-}$ and entire population mix are given above. Specification of age- and gender-specific parameters are as described in Appendix B. This sensitivity analysis is qualitatively similar to that described previously for the noncancer assessments of dichloromethane, where the variability in HED and HEC values was estimated.

For this analysis, however, consideration of exclusively GST-T1$^{+/+}$ individuals will clearly narrow any estimate of variability. This analysis will also differ from that for noncancer effects in that the inverse of the former relationship is being considered (i.e., the variation in a specific internal dose for a fixed exposure is being computed, whereas for the HED and HEC, the variability in exposure levels corresponding to a fixed internal dose are estimated). The results of this analysis are shown in Figure 5-20 and Table 5-27 for oral exposures and in Figure 5-21 and Table 5-28 for inhalation exposures.

For the oral exposure analysis, the distribution of internal doses shows little variation among the different age/gender groups (Figure 5-20, Table 5-27). The cancer analysis is based on a very low internal dose where little enzymatic saturation is expected to occur, allowing for efficient first-pass metabolism which is independent of differences in respiration; differences will be more significant at the higher doses analyzed for the noncancer human equivalent applied dose. Thus, the consideration of only GST metabolism and the narrower range of metabolic rate for that pathway in the +/+ population at low oral exposure rates results in minimal age/gender sensitivity differences (the 70-year-old female is only 5% more sensitive from pharmacokinetic factors than the general population).

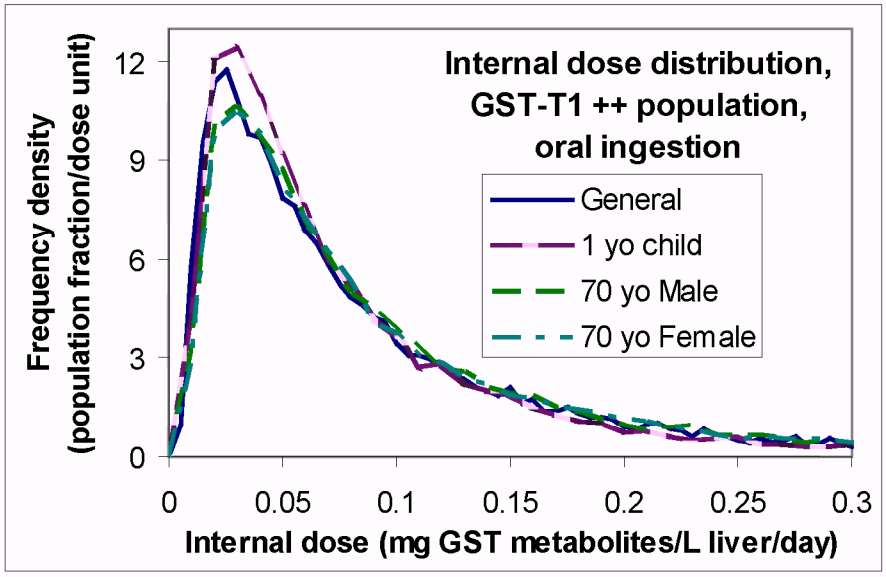


Figure 5-20. Histograms for a liver-specific dose of GST metabolism (mg GST metabolites/L liver/day) for the general population (0.5–80-year-old males and females), and specific age/gender groups within the population of GST-T1$^{+/+}$ genotypes, given a daily oral dose-rate of 1 mg/kg-day dichloromethane.

Table 5-27. Statistical characteristics of human internal doses for 1 mg/kg-day oral exposures in specific populations

Population	Internal dose (mg/L liver per d)[a]		
	Mean	95th percentile	99th percentile
All ages[b]	9.43×10^{-2}	2.98×10^{-1}	5.43×10^{-1}
1-yr-old children	7.82×10^{-2}	2.41×10^{-1}	4.00×10^{-1}
70-yr-old men	9.71×10^{-2}	2.99×10^{-1}	5.51×10^{-1}
70-yr-old women	1.01×10^{-1}	5.33×10^{-1}	9.84×10^{-1}

[a]Liver-specific GST-T1 metabolism in GST-T1$^{+/+}$ individuals exposed orally to 1 mg/kg-day dichloromethane.
[b]0.5- to 80-yr-old males and females.

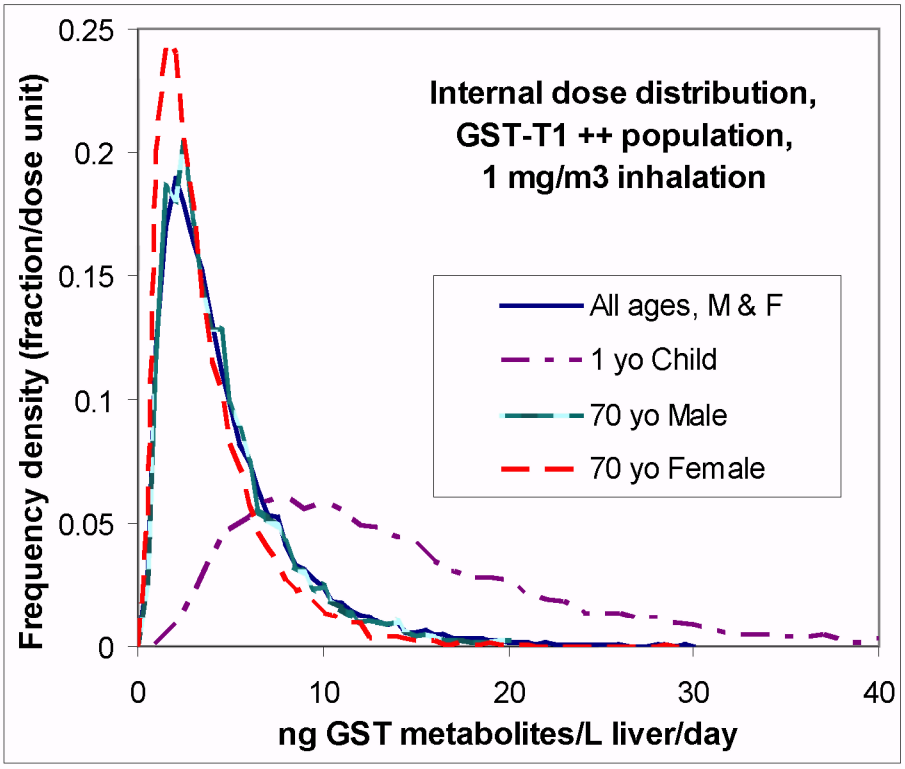


Figure 5-21. Histograms for liver-specific dose of GST metabolism (mg GST metabolites/L liver/day) for the general population (0.5–80-year-old males and females), and specific age/gender groups within the population of GST-T1$^{+/+}$ genotypes, given a continuous inhalation exposure to 1 mg/m^3 dichloromethane.

Table 5-28. Statistical characteristics of human internal doses for 1 mg/m^3 inhalation exposures in specific subpopulations

Population	Internal dose (mg/L liver per d)[a]		
	Mean	95th percentile	99th percentile
All ages[b]	6.61×10^{-6}	2.21×10^{-5}	4.47×10^{-5}
1-yr-old children	1.65×10^{-5}	5.11×10^{-5}	9.04×10^{-5}
70-yr-old men	5.09×10^{-6}	1.68×10^{-5}	3.12×10^{-5}
70-yr-old women	4.14×10^{-6}	1.37×10^{-5}	2.56×10^{-5}

[a]Liver-specific GST-T1 metabolism in GST-T1$^{+/+}$ individuals exposed continuously by inhalation to 1 mg/m^3 dichloromethane.
[b]0.5–80-year-old males and females.

For inhalation, an internal liver GST dose (mean value) about 2.5 times higher in the child than the general population is predicted due to the higher inhalation rate. The results for the liver GST dose for inhalation (Figure 5-21 and Table 5-28) indicate that the 70-year-old male and female populations are only slightly shifted from the general population, while the population for the 1-year-old child is a distinct upper tail of the general distribution.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Dichloromethane (CASRN 75-09-2), also known as methylene chloride, is a colorless liquid with a penetrating, ether-like odor. It is produced by the direct reaction of methane with chlorine at either high temperatures or low temperatures under catalytic or photolytic conditions. The principal uses for dichloromethane have been in paint strippers and removers, as a propellant in aerosols, in the manufacture of drugs, pharmaceuticals, film coatings, electronics, and polyurethane foam, and as a metal-cleaning solvent.

Dichloromethane is rapidly absorbed through both oral administration and inhalation exposure with a near steady-state saturation occurring with inhalation. Results from studies of animals show that following absorption, dichloromethane is rapidly distributed throughout the body and has been detected in all tissues that have been evaluated. Metabolism of dichloromethane involves two primary pathways. Dichloromethane is metabolized to CO in a CYP-dependent oxidative pathway (CYP2E1) that is predominant at low exposure levels. The other major pathway for dichloromethane metabolism involves the conjugation of dichloromethane to GSH, catalyzed by GST (GST-T1). This results in the formation of a GSH conjugate that is eventually metabolized to CO_2. The conjugation of dichloromethane to GSH results in the formation of two reactive intermediates that have been hypothesized to be involved in dichloromethane carcinogenicity, S-(chloromethyl)glutathione and formaldehyde. Formation of formaldehyde leads to several covalent modifications of cellular macromolecules, including DNA-protein cross-links (Casanova et al., 1996) and RNA-formaldehyde adducts (Casanova et al., 1997). Evidence is also available that S-(chloromethyl)glutathione can result in both DNA SSBs and DNA mutations, presumably through DNA alkylation (Green, 1997; Graves et al., 1996; Graves and Green, 1996; Graves et al., 1994b; Hashmi et al., 1994). However, DNA reaction products (e.g., DNA adducts) produced by S-(chloromethyl)glutathione have not been found in vivo, possibly due to potential instability of these compounds or due to the limited doses used in attempts to detect them (Watanabe et al., 2007; Hashmi et al., 1994). DNA adducts, however, have been observed in in vitro studies in which calf thymus DNA was incubated with dichloromethane and GSH or was incubated with S-(1-acetoxymethyl)glutathione, a compound structurally similar to S-(chloromethyl)glutathione (Marsch et al., 2004; Kayser and Vuilleumier, 2001).

Information on noncancer effects in humans exposed orally to dichloromethane are restricted to case reports of neurological impairment (general CNS depression), liver and kidney effects (as severe as organ failure), and gastrointestinal irritation in individuals who ingested

amounts ranging from about 25 to 300 mL (Chang et al., 1999; Hughes and Tracey, 1993). The animal toxicity database identifies hepatic effects (hepatic vacuolation, liver foci) as the critical dose-dependent noncancer endpoint associated with oral exposure to dichloromethane. The most frequently observed liver effect was hepatocyte vacuolation, seen with drinking water exposure (90 days) in F344 rats at ≥166 mg/kg-day and B6C3F$_1$ mice at 586 mg/kg-day (Kirschman et al., 1986) and with gavage exposure (14 days) in CD-1 mice at 333 mg/kg-day (Condie et al., 1983). Hepatocyte degeneration or necrosis was observed in female F344 rats exposed via drinking water for 90 days to 1,469 mg/kg-day (Kirschman et al., 1986) and in female F344 rats exposed by gavage for 14 days to 337 mg/kg-day (Berman et al., 1995). In the chronic-duration (104-week) study, liver effects (areas of foci alteration) were observed in F344 rats exposed to drinking water doses between 50 and 250 mg/kg-day (Serota et al., 1986a). In the reproductive oral administration studies, no significant effect on reproductive function or parameters was observed in rats up to 225 mg/kg-day (General Electric Company, 1976) or in mice up to 500 mg/kg-day (Raje et al., 1988). A two-generation oral exposure study is not available. The NOAEL and LOAEL for altered neurological functions in female F344 rats were 101 and 337 mg/kg-day [as reported by Moser et al. (1995)].

Acute inhalation exposure of humans to dichloromethane has been associated with decreased oxygen availability from COHb formation and neurological impairment from interaction of dichloromethane with nervous system membranes (Bos et al., 2006; ACGIH, 2001; ATSDR, 2000; Cherry et al., 1983; Putz et al., 1979; Gamberale et al., 1975; Winneke, 1974). Relatively little is known about the long-term neurological effects of chronic exposures, although there are studies that provide some evidence of an increased prevalence of neurological symptoms among workers with average exposures of 75–100 ppm (Cherry et al., 1981) and long-term effects on some neurological measures (i.e., possible detriments in attention and reaction time in complex tasks) in retired workers whose past exposures were in the 100–200 ppm range (Lash et al., 1991). These studies are limited by the relatively small sample sizes and low power for detecting statistically significant results for these endpoints.

Following repeated inhalation exposure to dichloromethane, the liver is the most sensitive target for noncancer toxicity in rats and mice. Lifetime exposure was associated with hepatocyte vacuolation and necrosis in F344 rats exposed to 1,000 ppm for 6 hours/day (Mennear et al., 1988; NTP, 1986), hepatocyte vacuolation in Sprague-Dawley rats exposed to 500 ppm for 6 hours/day (Nitschke et al., 1988a; Burek et al., 1984), and hepatocyte degeneration in B6C3F$_1$ mice exposed to 2,000 ppm for 6 hours/day (lower concentrations were not tested in mice) (Mennear et al., 1988; NTP, 1986). Other effects observed include renal tubular degeneration in F344 rats and B6C3F$_1$ mice at 2,000 ppm, testicular atrophy in B6C3F$_1$ mice at 4,000 ppm, and ovarian atrophy in B6C3F$_1$ mice at 2,000 ppm.

A two-generation inhalation exposure to dichloromethane revealed no significant effects on reproductive performance in rats (up to 1,500 ppm) (Nitschke et al., 1988b). This study is

limited in its ability to fully evaluate reproductive and developmental toxicity, however, since exposure was not continued through the gestation and nursing periods. Some evidence of a decrease in fertility index was seen in male mice exposed to 150 and 200 ppm (Raje et al., 1988), and no adverse effects on fetal development of mice or rats exposed to up to 1,250 ppm were seen by Schwetz et al. (1975). Decreases in fetal BW and changes in behavioral habituation were observed in offspring of Long-Evans rats exposed to 4,500 ppm during the gestational period (Bornschein et al., 1980; Hardin and Manson, 1980). Exposure-related noncancer effects on the lungs consisted of foreign-body pneumonia in rats exposed to 8,400 ppm, 6 hours/day for 13 weeks (NTP, 1986). Several neurological mediated parameters, including decreased activity (Kjellstrand et al., 1985; Weinstein et al., 1972; Heppel et al., 1944; Heppel and Neal, 1944), impairment of learning and memory (Alexeeff and Kilgore, 1983), and changes in responses to sensory stimuli (Rebert et al., 1989), are reported from acute and short-term dichloromethane exposure. Evidence of a localized immunosuppressive effect in the lung resulting from inhalation dichloromethane exposure was seen in an acute exposure (3 hours, 100 ppm) study in CD-1 mice (Aranyi et al., 1986).

Dichloromethane is "likely to be carcinogenic in humans" under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Results from 2-year bioassays provide adequate evidence of the carcinogenicity of dichloromethane in mice and rats exposed by inhalation, as well as adequate data to describe dose-response relationships. Oral exposure to dichloromethane produced statistically significant increases in hepatocellular adenomas and carcinomas in male $B6C3F_1$ mice (Serota et al., 1986b; Hazleton Laboratories, 1983). Inhalation exposure to concentrations of 2,000 or 4,000 ppm dichloromethane produced increased incidences of lung and liver tumors in male and female $B6C3F_1$ mice (Maronpot et al., 1995; Foley et al., 1993; Kari et al., 1993; Mennear et al., 1988; NTP, 1986). Significantly increased incidences of benign mammary tumors (adenomas or fibroadenomas) were observed in male and female F344/N rats exposed by inhalation to 2,000 or 4,000 ppm (Mennear et al., 1988; NTP, 1986). A statistically significant increased incidence of brain or CNS tumors has not been observed in any of the animal cancer bioassays, but a 2-year study using relatively low exposure levels (0, 50, 200, and 500 ppm) in Sprague-Dawley rats observed a total of six astrocytoma or glioma (mixed glial cell) tumors in the exposed groups (Nitschke et al., 1988a). These tumors are exceedingly rare in rats, and there are few examples of statistically significant trends in animal bioassays (Sills et al., 1999). Studies in humans also provide evidence for an association between occupational exposure to dichloromethane and increased risk for some specific cancers, including brain cancer (Hearne and Pifer, 1999; Heineman et al., 1994; Tomenson, In Press), liver cancer (Lanes et al., 1993; Lanes et al., 1990), non-Hodgkin lymphoma (Barry et al., 2011; Wang et al., 2009; Seidler et al., 2007; Miligi et al., 2006), and multiple myeloma (Gold et al., 2010).

The hypothesized mode of action for dichloromethane-induced lung and liver tumors is through a mutagenic mode of carcinogenic action. Key events within this hypothesis are (1) dichloromethane metabolized via GST metabolism, with increasing metabolism through this pathway with increasing exposure levels above the saturation of CPY2E1; (2) reaction of GST-pathway metabolites with DNA, leading to (3) mutations in critical genes that result in tumor initiation; and (4) tumor growth promoted by unidentified molecular or cellular events. In this proposed mode of action, metabolism by CYP2E1, which is more predominant at lower exposures (or tissues concentrations) than metabolism by GST, is considered a protective mechanism against the formation of putatively carcinogenic metabolites from the GST pathway.

The weight of evidence from a large collection of in vivo and in vitro studies support the proposed mutagenicity of dichloromethane and the key role of GST metabolism and the formation of DNA-reactive GST-pathway metabolites. The data pertaining to chromosomal damage provide greater weight to this collection of evidence than the indicator genotoxicity assays; among chromosomal damage studies, in vivo evidence provides greater weight than in vitro evidence. The database for dichloromethane provides support along each of these lines: 1) in vivo evidence of chromosomal mutations (chromosomal aberrations) in the mouse lung and peripheral red blood cells, in the absence of evidence of cytoxicity. These observations were not seen in the mouse bone marrow, a site that would be expected to be much more limited in terms of degree of dichloromethane metabolism; 2) in vitro chromosomal instability evidence in human cells, other mammalian cells (i.e., CHO), and in bacterial systems; and 3) positive DNA damage assays in numerous vivo and in vitro studies. As noted previously, unscheduled DNA synthesis is generally a relatively insensitive measure of genotoxicity, and is given little weight in this synthesis of the data. Available studies also demonstrate target tissue specificity, with DNA-protein cross-links, DNA SSBs, and sister chromatid exchanges seen in liver and lung cells of $B6C3F_1$ mice following acute inhalation exposure to concentrations producing liver and lung tumors with chronic exposure (Casanova et al., 1996; Graves et al., 1995; Casanova et al., 1992; Allen et al., 1990). Dose-response concordance and temporality have also been seen, with evidence of mutagenicity (as determined by the micronucleus test or presence of chromosome aberrations) and genotoxicity (as determined by the presence of DNA-protein cross links or DNA SSBs) at the exposure levels inducing liver tumors in $B6C3F_1$ mice (NTP, 1986). Similar results were seen in inhalation exposure studies in liver and lung cells of $B6C3F_1$ mice in studies using the dose range used in the NTP study.

6.2. DOSE RESPONSE

6.2.1. Oral RfD

The available oral toxicity data for animals identify hepatic effects (hepatic vacuolation, liver foci) as the most sensitive noncancer endpoint associated with chronic oral exposure to dichloromethane. The 104-week drinking-water study in F344 rats (Serota et al., 1986a) was

selected as the principal study for RfD derivation because the study provided a sensitive endpoint (liver foci) and used lower doses in comparison to other chronic oral administration studies. In this study, four doses (6, 52, 125, and 235 mg/kg-day in males; 6, 58, 136, and 263 mg/kg-day in females) were used. A NOAEL of 6 mg/kg-day in males and females and a LOAEL of 52 (male) and 58 mg/kg-day (female) for alterations of liver foci were identified.

An RfD of 6×10^{-3} mg/kg-day is recommended. The RfD derivation process involved first fitting all available dichotomous models in BMDS version 2.0 to the incidence data for male and female rats; the male data were used because a greater sensitivity was seen in males compared with females in this study. A dose metric of average daily mass of dichloromethane metabolized via the CYP pathway per unit volume of liver was derived from a EPA-modified rat PBPK model (see Appendix C). This metric was chosen because there are no data to support the role of a specific metabolite in the development of the noncancer liver lesions seen in oral and inhalation exposure studies, and the CYP-metabolism dose metric was determined to be most consistent with the data. Then, the $BMDL_{10}$ for liver lesions was derived based on the best fitting model (in terms of the value of the AIC and examination of model fit and residuals). Because the metric is a rate of metabolism rather than the concentration of putative toxic metabolites, and the clearance of these metabolites may be slower per volume tissue in the human compared with the rat, this rodent internal dose metric for noncancer effects was adjusted by dividing by a pharmacokinetic allometric $BW^{0.75}$ scaling factor (operationalized as $[BW_{human}/BW_{rat}]^{0.25} \approx 4.09$) to obtain a human equivalent internal $BMDL_{10}$. This $BMDL_{10}$ was then converted to the HED by using a human PBPK model (adapted from David et al. (2006); see Appendix B) that utilizes Monte Carlo sampling techniques to provide a distribution of HEDs. The first percentile of the distribution of HEDs (0.189 mg/kg-day) was chosen to include the most sensitive population while staying within bounds of what is considered computationally stable. The first percentile HED was used as a POD and was divided by a composite UF of 30 (3 [$10^{0.5}$] to account for uncertainty about interspecies toxicodynamic equivalence, 3 [$10^{0.5}$] to account for uncertainty about toxicodynamic variability in humans, and 3 [$10^{0.5}$] for database deficiencies) to arrive at an RfD of 6×10^{-3} mg/kg-day.

Use of the mean value (3.50×10^{-1} mg/kg-day) of the HED distribution instead of the first percentile, with an additional UF of 3 ($10^{0.5}$) to account for human toxicokinetic variability, would yield a candidate RfD of 4×10^{-3} mg/kg-day, which is relatively similar to the recommended RfD of 6×10^{-3} mg/kg-day.

A confidence level of high, medium, or low is assigned to the study used to derive the RfD, the overall database, and the RfD itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Confidence in the principal study, Serota et al. (1986a), for dichloromethane is high. The 2-year drinking water study in rats is a well-conducted, peer-reviewed study that used four dose groups plus a control. Confidence in the oral database is medium-high. The oral

database includes a 2-year drinking water study in rats (Serota et al., 1986a) and mice (Serota et al., 1986b) as well as a supporting subchronic exposure study (Kirschman et al., 1986) that reports similar liver effects to those observed in the chronic oral exposure studies. The toxicity of orally-administered dichloromethane has also been investigated in an oral administration immunotoxicity study (Warbrick et al., 2003), a one-generation oral reproductive toxicity study (General Electric Company, 1976), and an orally dosed developmental toxicity study (Narotsky and Kavlock, 1995). Several studies have also evaluated neurotoxicity associated with oral exposure to dichloromethane. The oral database lacks a two-generation reproductive study and a developmental neurotoxicity study; neurodevelopmental outcomes are relevant endpoints given that dichloromethane is capable of crossing the placental barrier and entering fetal circulation (Withey and Karpinski, 1985; Anders and Sunram, 1982) and has neurotoxic effects. Overall, confidence in the RfD is high.

6.2.2. Inhalation RfC

The liver is the most sensitive target for noncancer toxicity in rats and mice following repeated inhalation exposure to dichloromethane. Liver lesions (specifically, hepatic vacuolation, consistent with fatty changes) in rats are the critical noncancer effect from chronic dichloromethane inhalation exposure in animals. The evidence is consistent with hepatic vacuolation as a precursor of toxicity. Accordingly, hepatic vacuolation is considered a toxicologically relevant effect. Inhalation bioassays with Sprague-Dawley rats identified the lowest inhalation LOAEL for liver lesions in the database: 500 ppm (6 hours/day, 5 days/week for 2 years) (Nitschke et al., 1988a; Burek et al., 1984). Nitschke et al. (1988a) identified a NOAEL of 200 ppm for hepatocyte vacuolation in female rats. Because the Nitschke et al. (1988a) study more adequately covers the range spanning the BMR compared with the study by Burek et al. (1984), the former study was selected as the principal study for derivation of a chronic inhalation RfC.

An RfC of 0.6 mg/m^3 is derived based on the observed critical effect in the principal study. As was described above for the RfD, the RfC derivation process was based on a dose metric of average daily mass of dichloromethane metabolized via the CYP pathway per unit volume of liver. This metric was derived from an EPA-modified rat PBPK model (see Appendix C). Then, the $BMDL_{10}$ for liver lesions was derived based on the best fitting model in terms of the value of the AIC and examination of model fit and residuals. Because the metric is a rate of metabolism rather than the concentration of putative toxic metabolites, and the clearance of these metabolites may be slower per volume tissue in the human compared with the rat, this rodent internal dose metric for noncancer effects was adjusted by dividing by a pharmacokinetic allometric $BW^{0.75}$ scaling factor (operationalized as $[BW_{human}/BW_{rat}]^{0.25} \approx 4.09$) to obtain a human-equivalent internal $BMDL_{10}$. This $BMDL_{10}$ was then converted to the HEC by using a

human PBPK model (adapted from David et al. (2006); see Appendix B) that utilizes Monte Carlo sampling techniques to provide a distribution of HECs.

The first percentile HEC (17.2 mg/m^3) was used as a POD. This percentile was chosen because it included the most sensitive population while staying within bounds of what is considered computationally stable. This POD was divided by a composite UF of 30 (3 [$10^{0.5}$] to account for uncertainty about interspecies toxicodynamic equivalence, 3 [$10^{0.5}$] to account for uncertainty about toxicodynamic variability in humans, and 3 [$10^{0.5}$] for database deficiencies) to arrive at an RfC of 0.6 mg/m^3.

Use of the mean value (48.5 mg/m^3) of the HEC distribution instead of the first percentile with an additional UF of 3 ($10^{0.5}$) to account for human toxicokinetic variability would yield a candidate RfC of 0.5, similar to the recommended value of 0.6 mg/m^3. In addition, two comparison values derived from occupational studies produced values of 1.2 mg/m^3 (Cherry et al., 1983) and 1.8 mg/m^3 (Lash et al., 1991). The animal-derived candidate RfC is preferable to the human-derived candidate RfC because of the uncertainties about the characterization of the exposure, influence of time since exposure, effect sizes, and statistical power in the epidemiologic studies.

As noted in Section 6.2.1, a confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in EPA (U.S. EPA, 1994), Section 4.3.9.2. Confidence in the principal study, Nitschke et al. (1988a), is high. The 2-year inhalation study in mice is a well-conducted, peer-reviewed study that used three concentration groups plus a control. Confidence in the inhalation database is medium to high. The inhalation database includes several well-conducted chronic inhalation studies that consistently identified the liver as the most sensitive noncancer target organ in rats (Nitschke et al., 1988a; NTP, 1986; Burek et al., 1984). A two-generation reproductive toxicity study (Nitschke et al., 1988b), developmental studies at relatively high exposures (≥1,250 ppm), several neurotoxicity studies, and an immunotoxicity study have been conducted in animals following inhalational exposures to dichloromethane. However, the two-generation study is limited in its ability to fully evaluate reproductive and developmental toxicity, since exposure was not continued through the gestation and nursing periods. The results from the single dose developmental toxicity study in rats (Bornschein et al., 1980; Hardin and Manson, 1980), the placental transfer of dichloromethane, and the relatively high activity of CYP2E1 in the brain compared to the liver of the developing human fetus (Hines, 2007; Johnsrud et al., 2003; Brzezinski et al., 1999), raise uncertainty regarding possible neurodevelopmental toxicity from gestational exposure to inhaled dichloromethane. An acute, 3-hour exposure to 100 ppm dichloromethane demonstrated evidence of immunosuppression in CD-1 mice (Aranyi et al., 1986). This study used a functional immune assay that is relevant to humans (i.e., increased risk of Streptococcal pneumonia-related mortality and decreased clearance of Klebsiella bacteria). Chronic and/or repeated exposure studies evaluating functional immunity are not available and

represent a data gap. The inhalation database lacks adequate developmental neurotoxicity and immunotoxicity studies at chronic low exposures. Overall confidence in the RfC is medium to high.

6.2.3. Uncertainties in RfD and RfC Values

One data uncertainty identified is the potential for neurodevelopmental effects. Animal bioassays have not identified gross or microscopic effects on neural tissues from long-term exposures or single (Schwetz et al., 1975) or multigenerational (Nitschke et al., 1988b) developmental toxicity studies, albeit with the limitations regarding dosing protocol. However, behavioral changes were observed in pups born to rats exposed to high levels (4,500 ppm) of dichloromethane (Bornschein et al., 1980; Hardin and Manson, 1980); 4,500 ppm was the only dose used in this study. Thus, uncertainty exists as to the development of neurological effects from lower gestational exposures in animals or in humans. Immunotoxicity data revealed an additional area of data uncertainty specifically with respect to inhalation exposure. Data from Aranyi et al. (1986) demonstrated evidence of immunosuppression following a single 100 ppm dichloromethane exposure for 3 hours in CD-1 mice. The weight of evidence for nonneoplastic effects in humans and animals suggests that the development of liver lesions is the most sensitive effect, with an UF applied because of deficiencies in the reproductive and developmental studies for the RfD and the additional uncertainty regarding immune system toxicity (specifically, a portal-of-entry immune suppression effect) at low exposures for the RfC.

The extrapolation of internal dichloromethane dosimetry from rat liver responses to human risk was accomplished by using PBPK models for dichloromethane in rats and humans. Uncertainties in rat and human dosimetry used for RfD and RfC derivation can arise from uncertainties in the PBPK models to accurately simulate the toxicokinetics of dichloromethane for animals under bioassay conditions and humans experiencing relatively low, chronic environmental exposures. Specific uncertainties identified with the PBPK models used here are described in detail in Sections 3.5.2 and 3.5.5. In brief, there is a structural uncertainty in the equation used to describe the CYP2E1-mediated metabolism that could be the source of discrepancies between the model and some of the data (both in vitro and in vivo), although the impact of that uncertainty does not appear to be large. There is also a parametric uncertainty in the GST-T1 parameter, k_{fC}, evident from an inconsistency in the values obtained by David et al. (2006). The impact of this uncertainty was evaluated by re-estimating human dosimetry with the mean values for the fitted metabolic parameter reset to match those obtained by David et al. (2006) from analysis of one data set (DiVincenzo and Kaplan, 1981) rather than the combined data. The impact of this change on the RfC and RfD was modest (HEC and HED values increased by 25 and 12%, respectively), but cancer risk estimates were increased by 10–20-fold.

Given the level of uncertainty identified by this sensitivity analysis, EPA obtained the MCSim model code and data sets from the authors and re-ran the MCMC analysis in order to

validate the results of David et al. (2006). As was done by David et al. (2006), EPA ran the chains for 50,000 iterations, saved the output from every 5th iteration (i.e., a total of 10,000 entries), then analyzed the final 4,000 of these entries. The resulting population-parameter mean values and CVs were virtually identical to those reported for the combined data set by David et al. (2006), providing assurance that the computational result was reproducible. When the output was analyzed by current methods for convergence of the Markov Chain, however, not all of those measures were satisfied. Visual inspection of plots of the chains did not reveal any observable trend towards higher or lower values for any of the parameters (i.e., it appeared that continuing the chains would not significantly alter the estimated population mean values). There was a high degree of auto-correlation in the chains, however, indicating that the statistical procedure had not yet obtained a good measure of the covariance among the parameters.

Autocorrelation in the Markov Chains used to estimate the population parameters indicates that the assumed degree of independence among the parameters is overpredicted (i.e., some sets of population means for all parameters predicted as "likely" given the current results are probably not likely). If some combinations of parameters are less likely than other combinations (because the combination does not reflect the true correlation), and the current estimate treats those combinations as equally likely, then the level of uncertainty that is reflected in the width of the predicted confidence bounds (distribution percentiles) will be overestimated. If the chains are run longer to reach convergence, the correlation among parameters should be better identified and the resulting prediction uncertainty (e.g., difference between the predicted mean and 95th percentile for a dose metric) should be decreased. In short, these results indicate a lack of convergence in the chains that leads to an overestimation of parameter uncertainty, such that the first percentiles of HECs or HEDs are lower than would be predicted after full convergence was obtained. Hence, these results likely lead to values of the RfC and RfD that are more sensitive than would be obtained if the chains are continued to convergence. Since population mean dose estimates (for the GST-T1$^{+/+}$ subpopulation) were used to determine cancer risks, the direction in which risk estimates would change with convergence cannot be predicted for that endpoint. As indicated by the sensitivity analysis, estimated risks are sensitive to possible changes in the population mean values. But given the variance in the current estimates of those means, the estimate is not expected to change by more than a factor of 3 after full convergence. Accordingly, EPA chose to continue using those values while providing an analysis of the uncertainty.

The dose metric used in the models is the rate of metabolism to a putative toxic metabolite rather than the concentration (average or area under the concentration curve of the metabolite), so the model specifically fails to account for rodent-human differences in clearance or removal of the toxic metabolite. A scaling factor based on BW ratios was used to account for this difference.

Uncertainties in the human population model parameters and variability in that population were quantitatively accounted for by utilizing hierarchical Bayesian calibration methods during model development (David et al., 2006), as modified here by EPA. The rat model was modified, recalibrated, and utilized in a deterministic manner (Appendix C). Data were not available to perform a hierarchical Bayesian calibration in the rat, but uncertainties in the rat model predictions were assessed qualitatively. For both oral and inhalation exposures, the liver volume, followed closely by the volume of slowly perfused tissues, had the greatest impact on the internal dose of mg dichloromethane metabolized via CYP pathway/L tissue/day. This was due to the fact that the dose metric is a tissue-specific measure, the majority of CYP metabolism is attributed to the liver, and changes in liver volume have a greater impact on the total CYP metabolism than either of the individual V_{max} values. There is high confidence in the values used for volume of liver and slowly perfused tissues in the rat, as these are well studied (Brown et al., 1997). Therefore, except as described in the preceding paragraph, the uncertainties associated with use of the rat PBPK model should not markedly affect the values of the RfD and RfC.

An additional uncertainty inherent in this process, however, is the lack of knowledge concerning the most relevant dose metric (e.g., a specific metabolite) within the context of the development of the noncancer liver effects. This basic research question represents a data gap, and this uncertainty is not addressed quantitatively or qualitatively in the assessment.

The effect of dichloromethane on human populations that are sensitive due to pharmacokinetic differences was addressed quantitatively by using a human probabilistic PBPK model to generate distributions of human exposures likely to occur given a specified internal $BMDL_{10}$. The model and resulting distributions take into account the known differences in human physiology and metabolic capability with regard to dichloromethane dosimetry. The first percentile values of the distributions of HEDs (Table 5-3) and HECs (Table 5-7) served as PODs for candidate RfDs and RfCs, respectively, to protect toxicokinetically sensitive individuals. No data are available regarding toxicodynamic differences within a human population. Therefore, an UF of 3 for possible differences in human toxicodynamic responses is intended to be protective for sensitive individuals.

6.2.4. Oral Cancer Slope Factor

The recommended cancer oral slope factor for dichloromethane is 2×10^{-3} $(mg/kg\text{-}day)^{-1}$, which is based on liver tumor responses in male $B6C3F_1$ mice exposed to dichloromethane in drinking water for 2 years (Serota et al., 1986b; Hazleton Laboratories, 1983). This value was derived by using a tissue-specific GST metabolism dose metric with allometric scaling to account for uncertainty regarding the reactivity and clearance of the metabolite(s) involved in the carcinogenic response.

There was only one adequate oral exposure cancer bioassay evaluating the carcinogenic potential of orally administered dichloromethane in F344 rats and $B6C3F_1$ mice (Serota et al., 1986a, b; Hazleton Laboratories, 1983). Significant increases in incidence of liver adenomas and carcinomas were observed in male but not female $B6C3F_1$ mice (female data were not presented in the summary reports) (Serota et al., 1986b; Hazleton Laboratories, 1983). The study authors concluded that in the male bioassay that there was no dose-related trend, there were no significant differences comparing the individual dose groups with the combined control group, and the observed incidences were "within the normal fluctuation of this type of tumor incidence." Although Serota et al. (1986b) state that a two-tailed significance level of $p = 0.05$ was used for all tests, this does not appear to correctly represent the statistic used by Serota et al. (1986b). Each of the p-values for the comparison of the 125, 185, and 250 mg/kg-day dose groups with the controls was $p < 0.05$. (These p-values were found in the full report of this study, see Hazleton Laboratories (1983) but were not included in the Serota et al. (1986b) publication). Hazleton Laboratories (1983) indicated that a correction factor for multiple comparisons was used specifically for the liver cancer data, reducing the nominal p-value from 0.05 to 0.0125; none of these individual group comparisons are statistically significant when a p-value of 0.0125 is used. EPA did not consider the use of a multiple comparisons correction factor for the evaluation of the liver tumor data (a primary a priori hypothesis) to be warranted. Thus, based on the Hazleton Laboratories (1983) statistical analysis, EPA concluded that dichloromethane induced a carcinogenic response in male $B6C3F_1$ mice as evidenced by a marginally increased trend test ($p = 0.058$) for combined hepatocellular adenomas and carcinomas, and by small but statistically significant ($p < 0.05$) increases in hepatocellular adenomas and carcinomas at dose levels of 125 ($p = 0.023$), 185 ($p = 0.019$), and 250 mg/kg-day ($p = 0.036$).

With respect to the issue of the comparison to historical controls, the incidence in the control groups (19%) was almost identical to the mean seen in the historical controls from this laboratory (17.8% based on 354 male $B6C3F_1$ mice), so there is no indication that the observed trend is being driven by an artificially low rate in controls, no indication that the experimental conditions resulted in a systematic increase in the incidence of hepatocellular adenomas and carcinomas, and no indication that the pattern of incidence rates observed in this study (increased incidence in all four dose groups, with three of these increases significant at a p-value < 0.05) reflect normal fluctuations in the incidence of these tumors. In F344 rats (Serota et al., 1986a), no increased incidence of liver tumors was seen in male rats, and the pattern in female rats was characterized by a jagged stepped pattern of increasing incidence of hepatocellular carcinoma or neoplastic nodules. However, the potential malignant characterization of the nodules was not described, and the data for hepatocellular carcinomas are much more limited. The derivation of the oral cancer slope factor is based on the male mice data because of their greater sensitivity to liver cancer compared with female mice and with male and female rats.

A modified mouse PBPK model of Marino et al. (2006) was used to approximate the internal dose of daily dichloromethane (mg) metabolized via the GST pathway per unit volume of liver from the daily oral administered doses. This approach was taken based on evidence that GST-pathway metabolites produced from dichloromethane are primarily responsible for dichloromethane carcinogenicity in mouse liver. The multistage dose-response model (BMDS version 2.0) was used to fit the mouse liver tumor incidence and PBPK model-derived internal dose data and to derive a mouse internal BMD and $BMDL_{10}$. Because the metric is a rate of metabolism rather than the concentration of putative toxic metabolites, and data pertaining to the reactivity or clearance rate of the relevant metabolite(s) are lacking, the human $BMDL_{10}$ was derived by multiplying the mouse $BMDL_{10}$ by a $BW^{0.75}$ allometric scaling factor (operationalized as $[BW_{human}/BW_{mouse}]^{0.25} \approx 7$) to account for the potential slower clearance per volume tissue in the human compared with the mouse. Linear extrapolation from the internal human $BMDL_{10}$ ($0.1/BMDL_{10}$) was used to derive oral risk factors for liver tumors based on tumor responses in male mice. The linear low-dose extrapolation approach for agents with a mutagenic mode of action was selected because GST-metabolism of dichloromethane is expected to occur at and below exposures producing the mouse $BMDL_{10}$, even though CYP2E1 metabolism is expected to be unsaturated and to represent the predominant metabolic pathway in the liver. Currently, there are no data from chronic oral cancer bioassays in mice providing support for a nonlinear dose-response relationship.

Probability distributions of human oral cancer slope factors were derived by using a human PBPK model (adapted from David et al. (2006); see Appendix B). The cancer reference values (oral slope factor and inhalation unit risk) were derived for a sensitive population: a population composed entirely of carriers of the GST-$T1^{+/+}$ homozygous genotype (that is, the group that would be expected to be most sensitive to the carcinogenic effects of dichloromethane). In addition, cancer values derived for a population reflecting the estimated frequency of GST-T1 genotypes in the current U.S. population (20% GST-$T1^{-/-}$, 48% GST-$T1^{+/-}$, and 32% GST-$T1^{+/+}$) were presented. All simulations also included a distribution of CYP activity based on data from Lipscomb et al. (2003). The mean oral slope factor based on liver tumors in mice exposed to dichloromethane in drinking water, 2×10^{-3} $(mg/kg\text{-}day)^{-1}$, based on what is assumed to be the most sensitive of the populations (the GST-$T1^{+/+}$ group), is the recommended oral slope factor for chronic oral exposures to dichloromethane.

An oral slope factor derived from the liver tumor data in the Serota et al. (1986b) study using administered dose dosimetry, 1×10^{-2} $(mg/kg\text{-}day)^{-1}$, rather than PBPK modeling is approximately one order of magnitude higher than the current recommended value of 2×10^{-3} $(mg/kg\text{-}day)^{-1}$. There is approximately one to two orders of magnitude difference among the values based on different dose metrics, scaling factors, and populations (Table 6-1).

The recommended oral slope factor of 2×10^{-3} $(mg/kg\text{-}day)^{-1}$ is based on a tissue-specific GST internal dose metric with allometric scaling. Although involvement of the GST pathway in

carcinogenic response has been established, some uncertainty remains as to the metabolite(s) involved and the rate at which those metabolites are cleared. The value derived specifically for the GST-T1$^{+/+}$ population is recommended to provide protection for the population that is hypothesized to be most sensitive to the carcinogenic effect. Application of ADAFs to the cancer oral slope factor is recommended in combination with appropriate exposure data when assessing risks associated with early-life exposure (see Section 5.4.4 for more details).

Table 6-1. Comparison of oral slope factors derived by using various assumptions and metrics, based on liver tumors in male mice

Population[a]	Dose metric	Species, sex	Tumor	Scaling factor	Mean oral slope factor $(mg/kg\text{-}d)^{-1}$	Source (table)
GST-T1$^{+/+}$[b]	**Tissue-specific GST-metabolism rate[b]**	**Mouse, male**	**Liver**	**7.0**	**1.7×10^{-3}**	**Table 5-13**
	Tissue-specific GST-metabolism rate	Mouse, male	Liver	1.0	2.4×10^{-4}	Table 5-13
	Whole-body GST metabolism rate	Mouse, male	Liver	7.0	9.3×10^{-4}	Table 5-13
	Route-to-route extrapolation, tissue-specific metabolism	Mouse, male	Liver	7.0	1.2×10^{-4}	Table 5-14
	Route-to-route extrapolation, tissue-specific metabolism	Mouse, male	Liver	1.0	1.7×10^{-5}	Table 5-14
	Route-to-route extrapolation, whole-body metabolism	Mouse, male	Liver	7.0	6.7×10^{-5}	Table 5-14
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Liver	7.0	9.4×10^{-4}	Table 5-13
	Tissue-specific GST-metabolism rate	Mouse, male	Liver	1.0	1.3×10^{-4}	Table 5-13
	Whole-body GST metabolism rate	Mouse, male	Liver	7.0	5.4×10^{-4}	Table 5-13
	Route-to-route extrapolation, tissue-specific metabolism	Mouse, male	Liver	7.0	6.8×10^{-5}	Table 5-14
	Route-to-route extrapolation, tissue-specific metabolism	Mouse, male	Liver	1.0	9.7×10^{-6}	Table 5-14
	Route-to-route extrapolation, whole-body metabolism	Mouse, male	Liver	7.0	3.9×10^{-5}	Table 5-14
	Applied dose (HED)	Mouse, male	Liver		1.0×10^{-2}	Table 5-15
	1995 IRIS assessment	Mouse, male	Liver		7.5×10^{-3}	

[a]GST-T1$^{+/+}$ = homozygous, full enzyme activity; Mixed = genotypes based on a population reflecting the estimated frequency of genotypes in the current U.S. population: 20% GST-T1$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$ (Haber et al., 2002).
[b]Bolded value is the basis for the recommended oral slope factor of 2×10^{-3} per mg/kg-day.

6.2.5. Cancer Inhalation Unit Risk

The recommended cancer inhalation unit risk is 1×10^{-8} $(\mu g/m^3)^{-1}$ for the development of liver and lung cancers based on data from male $B6C3F_1$ mice using a tissue-specific GST metabolism dose metric. Data for liver and lung tumors in male and female $B6C3F_1$ mice following exposure to airborne dichloromethane were used to develop inhalation unit risks for dichloromethane (Mennear et al., 1988; NTP, 1986). This study was selected as the principal study to derive an inhalation unit risk for dichloromethane because of the completeness of the data, adequate sample size, and clear dose response. In the NTP (1986) study, significant increases in incidence of liver and lung adenomas and carcinomas were observed in both sexes of $B6C3F_1$ mice exposed 6 hours/day, 5 days/week for 2 years.

The PBPK model of Marino et al. (2006) for dichloromethane in the mouse was used to calculate long-term daily average internal liver doses. The selected internal dose metrics for liver tumors and lung tumors were long-term average daily mass of dichloromethane metabolized via the GST pathway per unit volume of liver and lung, respectively. This approach was taken based on evidence that GST-pathway metabolites produced from dichloromethane are primarily responsible for dichloromethane carcinogenicity in mouse liver. The multistage dose-response model (BMDS version 2.0) was used to fit the mouse liver tumor incidence and PBPK model-derived internal dose data and to derive a mouse internal BMD and $BMDL_{10}$. Because the metric is a rate of metabolism rather than the concentration of putative toxic metabolites, and data pertaining to the reactivity or clearance rate of the relevant metabolite(s) are lacking, the human $BMDL_{10}$ was derived by multiplying the mouse $BMDL_{10}$ by a $BW^{0.75}$ allometric scaling factor (operationalized as $[BW_{human}/BW_{mouse}]^{0.25} \approx 7$) to account for the potential slower clearance per volume tissue in the human compared with the mouse. A linear extrapolation approach using the internal human $BMDL_{10}$ for liver and lung tumors was used to calculate human tumor risk factors by dividing the BMR of 0.1 by the human BMDL for each tumor type. Currently, there are no data from chronic inhalation cancer bioassays in mice or rats providing support for a nonlinear dose-response relationship.

The human PBPK model (adapted from David et al. (2006); see Appendix B) provided distributions of human internal dose metrics of daily mass of dichloromethane metabolized via the GST pathway per unit volume of liver and lung resulting from chronic inhalation exposure to a unit concentration of 1 $\mu g/m^3$ dichloromethane (0.00029 ppm). As with the oral slope factor, the cancer inhalation unit risk was derived for a sensitive population: a population composed entirely of carriers of the GST-T1 homozygous positive genotype (that is, the group that would be expected to be most sensitive to the carcinogenic effects of dichloromethane). In addition, cancer values derived for a population reflecting the estimated frequency of GST-T1 genotypes in the current U.S. population (20% GST-$T1^{-/-}$, 48% GST-$T1^{+/-}$, and 32% GST-$T1^{+/+}$) were also presented. The distributions of inhalation unit risks for liver or lung tumors were generated by multiplying the human tumor risk factor for each tumor type and sex by the distribution of

internal doses from chronic exposure to 1 μg/m^3 dichloromethane. A procedure to combine risks for liver and lung tumors using different dose metrics for the different tumors (i.e., liver-specific and lung-specific metabolism for liver and lung tumors, respectively) was used to derive the recommended inhalation unit risk of 1×10^{-8} (μg/m^3)$^{-1}$ based on what is assumed to be the most sensitive of the populations, the GST-T1$^{+/+}$ group.

The current recommended inhalation unit risk value of 1×10^{-8} (μg/m^3)$^{-1}$ is approximately 1.5 orders of magnitude lower than the previous IRIS value of 4.7×10^{-7} (μg/m^3)$^{-1}$. An inhalation unit risk derived from the liver tumor data of the NTP (1986) study using applied concentration dosimetry rather than PBPK modeling, 3.6×10^{-7} (μg/m^3)$^{-1}$, is approximately 1.3 orders of magnitude higher than the currently recommended value of 1×10^{-8} (μg/m^3)$^{-1}$ (Table 6-2). There is approximately one to two orders of magnitude difference among the values based on different dose metrics, scaling factors, and populations.

The recommended inhalation unit risk value of 1×10^{-8} (μg/m^3)$^{-1}$ is based on a tissue-specific GST-internal dose metric with allometric scaling. Although the involvement of the GST pathway in carcinogenic response has been established, some uncertainty remains as to the metabolite(s) involved and the rate at which those metabolites are cleared. The value derived specifically for the GST-T1$^{+/+}$ population is recommended to provide protection for the population that is hypothesized to be most sensitive to the carcinogenic effect. Application of ADAFs to the cancer inhalation unit risk is recommended when assessing risks associated with early-life exposure (see Section 5.4.4 for more details).

Table 6-2. Comparison of inhalation unit risks derived by using various assumptions and metrics

Population[a]	Dose metric	Species, sex	Tumor type	Scaling factor	Inhalation unit risk[b] $(\mu g/m^3)^{-1}$	Source (table)
GST-T1$^{+/+}$	**Tissue-specific GST-metabolism rate[c]**	**Mouse, male**	**Liver and lung**	**7.0**	**1.3×10^{-8}**	**Table 5-20**
GST-T1$^{+/+}$	Tissue-specific GST-metabolism rate	Mouse, male	Liver	7.0	8.5×10^{-9}	Table 5-19
GST-T1$^{+/+}$	Tissue-specific GST-metabolism rate	Mouse, male	Lung	7.0	5.6×10^{-9}	Table 5-19
GST-T1$^{+/+}$	Tissue-specific GST-metabolism rate	Mouse, male	Liver and lung	1.0	1.9×10^{-9}	Table 5-20
GST-T1$^{+/+}$	Tissue-specific GST-metabolism rate	Mouse, male	Liver	1.0	1.2×10^{-9}	Table 5-19
GST-T1$^{+/+}$	Tissue-specific GST-metabolism rate	Mouse, male	Lung	1.0	8.0×10^{-10}	Table 5-19
GST-T1$^{+/+}$	Whole-body GST metabolism rate	Mouse, male	Liver and lung	7.0	1.6×10^{-8}	Table 5-20
GST-T1$^{+/+}$	Whole-body GST metabolism rate	Mouse, male	Liver	7.0	5.5×10^{-9}	Table 5-19
GST-T1$^{+/+}$	Whole-body GST metabolism rate	Mouse, male	Lung	7.0	1.2×10^{-8}	Table 5-19
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Liver and lung	7.0	7.4×10^{-9}	Table 5-20
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Liver	7.0	4.8×10^{-9}	Table 5-19
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Lung	7.0	3.2×10^{-9}	Table 5-19
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Liver and lung	1.0	1.1×10^{-9}	Table 5-20
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Liver	1.0	6.8×10^{-10}	Table 5-19
Mixed	Tissue-specific GST-metabolism rate	Mouse, male	Lung	1.0	4.5×10^{-10}	Table 5-19
Mixed	Whole-body GST metabolism rate	Mouse, male	Liver and lung	7.0	9.2×10^{-9}	Table 5-20
Mixed	Whole-body GST metabolism rate	Mouse, male	Liver	7.0	3.1×10^{-9}	Table 5-19
Mixed	Whole-body GST metabolism rate	Mouse, male	Lung	7.0	6.9×10^{-9}	Table 5-19
	Administered concentration (HEC)	Mouse, male	Liver		3.6×10^{-7}	Table 5-21
	Administered concentration (HEC)	Mouse, male	Lung		8.1×10^{-7}	Table 5-21
	1995 IRIS assessment[c]	Mouse, male	Liver and lung	12.7	4.7×10^{-7}	

[a]GST-T1$^{+/+}$ = homozygous, full enzyme activity; Mixed = genotypes based on a population reflecting the estimated frequency of genotypes in the current U.S. population: 20% GST-T1$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$ (Haber et al., 2002).
[b]Based on mean value of the derived distributions.
[c]Bolded value is the basis for the recommended inhalation unit risk of 1×10^{-8} $(\mu g/m^3)^{-1}$.

6.2.6. Uncertainties in Cancer Risk Values

The database of animal bioassays identifies the liver and lung as the most sensitive target organs for dichloromethane-induced tumor development, and there is high confidence that the dose-response data for liver and lung cancer in mice represent the best available data for derivation of human cancer risks. A dose-response relationship was seen with liver cancer in mice exposed orally and with liver and lung cancer in mice exposed by inhalation. Statistically significant increases in benign mammary gland tumors were observed in one study of F344 rats exposed by inhalation to 2,000 or 4,000 ppm (Mennear et al., 1988; NTP, 1986); evidence for a tumorigenic mammary gland response in Sprague-Dawley rats was limited to increased numbers of benign mammary tumors per animal at levels of 50–500 ppm (Nitschke et al., 1988a) or 500–3,500 ppm (Burek et al., 1984). A gavage study in female Sprague-Dawley rats reported an increased incidence of malignant mammary tumors, mainly adenocarcinomas (8, 6, and 18% in the control, 100, and 500 mg/kg dose groups, respectively), but the increase was not statistically significant. Data were not provided to allow an analysis that accounts for differing mortality rates (Maltoni et al., 1988). The toxicokinetic or mechanistic events that might lead to mammary gland tumor development in rats are unknown, although CYP2E1 (El-Rayes et al., 2003; Hellmold et al., 1998) and GST-T1 expression have been detected in human mammary tissue (Lehmann and Wagner, 2008). Rare CNS tumors were observed in one study in rats spanning a relatively low range of exposures (0–500 ppm) (Nitschke et al., 1988a). These cancers were not seen in two other studies in rats, both involving higher doses (1,000–4,000 ppm) (NTP, 1986; Burek et al., 1984), or in a similar high-dose study in mice (NTP, 1986). The relative rarity of the tumors precludes the use of the low-dose exposure study (Nitschke et al., 1988a) in a quantitative dose-response assessment. The available epidemiologic studies provide evidence of an association between dichloromethane and liver cancer, brain cancer, and some hematopoietic cancers. The available epidemiologic studies do not provide an adequate basis for the evaluation of the role of dichloromethane in breast cancer because there are no cohort studies with adequate statistical power and no breast cancer case-control studies with adequate exposure methodology.

There is uncertainty as to whether the reactivity of dichloromethane metabolites is sufficiently high to preclude systemic distribution. Thus, alternative derivations of cancer risk values were performed under the assumption that high reactivity leads to complete clearance from the tissue in which the active metabolite is formed (scaling factor = 1.0). The difference in scaling factor (7.0 for allometric scaling versus 1.0) results in a sevenfold decrease in estimated cancer toxicity values. Using a whole-body GST metabolism dose metric, the resulting oral slope factor and inhalation unit risk for liver cancer was approximately fivefold lower than when tissue-specific dose metrics were used; however, the inhalation unit risks for lung cancer and for the combined liver and lung cancer risk were higher with the whole-body compared with the tissue-specific metric (Tables 6-1 and 6-2). This difference reflects the lower metabolism that occurs in human versus mouse lung (relative to total): lung-specific metabolism is lower in

humans than mice, so the predicted risk in the lung is lower when based on lung-specific metabolism compared with whole-body metabolism. Mechanistic data support the hypothesis that reactive metabolites produced in the target tissues do not distribute significantly beyond those tissues. Thus, there is less uncertainty in the cancer risk values derived by using a tissue-specific GST metabolism dose metric compared with those derived using a whole-body GST metabolism dose metric.

Uncertainty in the ability of the PBPK models to estimate animal and human internal doses from lifetime low-level environmental exposures may affect the confidence in the cancer risk extrapolated from animal data. Uncertainties in the mouse and human model parameter values were integrated quantitatively into parameter estimation by hierarchical Bayesian methods to calibrate the models at the population level (David et al., 2006; Marino et al., 2006). With the subsequent deterministic application of the mouse model (using the mean value for each parameter distribution), however, the information contained in the mouse parameter uncertainties reported by Marino et al. (2006) is not integrated into the risk estimates described here.

The use of Monte Carlo sampling to define human model parameter distributions allowed for derivation of human distributions of dosimetry and cancer risk, providing for bounds on the recommended risk values. A sensitivity analysis was performed to identify model parameters most influential on the predictions of dose metrics used to estimate oral and inhalation cancer risks. For inhalation exposures in mice, the PB, followed closely by the first-order GST-mediated metabolism rate, had the greatest impact on the dose metric for liver cancer (mg dichloromethane metabolized via GST pathway/L liver/day). For drinking water exposures in mice, the first-order GST-mediated metabolism rate, followed by the CYP-mediated maximum reaction velocity (V_{maxC}) affected the liver cancer dose metric to the greatest extent. For mice inhaling dichloromethane, the lung cancer dose metric, like the liver cancer metric, was highly affected by the first-order GST-mediated metabolism rate and the PB. However, the lung cancer dose metric was most sensitive to the proportional yield of liver GST-mediated metabolic activity attributed to the lung. The PB was experimentally determined, lending high confidence to its value. In contrast, values for the three metabolic parameters were determined by computational optimization against data sets not directly measuring dichloromethane or its metabolites in the target/metabolizing tissues. It is uncertain how alternative values for these three parameters would affect the estimation of animal $BMDL_{10}$ values and, ultimately, inhalation unit risks and oral slope factors. In addition, specific uncertainty remains concerning the human PBPK parameter distributions (see discussion on k_{fC} in Section 3.5.5).

Finally, while the existing model's structure and equations have been extensively described in peer-reviewed publications, uncertainty remains concerning the model structure. An alternative (dual-binding-site) CYP metabolic rate equation (Korzekwa et al., 1998) may better describe CYP2E1-mediated GST metabolism for dichloromethane. This hypothesis requires further laboratory testing and integration into the PBPK modeling.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The Toxicological Review of Dichloromethane (dated March 2010) has undergone a formal external peer review performed by scientists in accordance with the U.S. EPA guidance on peer review (2006b, 2000b). An external peer-review workshop was held September 23, 2010. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and the EPA's responses to these comments arranged by charge question follow. In many cases, the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. When the external peer reviewers commented on decisions and analyses in the Toxicological Review under multiple charge questions, these comments were organized under the most appropriate charge question. The EPA also received scientific comments from the public. These comments and the EPA's responses are included in a separate section of this appendix.

EXTERNAL PEER REVIEWER COMMENTS

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

General Charge Questions

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Comments: Most reviewers considered the Toxicological Review to be comprehensive, clear, concise, and well written. One reviewer considered the presentation of material to be repetitive. Other comments provided in response to General Charge Question 1 were repeated by the peer reviewers in response to other charge questions, and are summarized and discussed under the relevant question.

Response: The *Toxicological Review of Dichloromethane* was revised to reduce redundancy, and information of lesser relevance was removed where appropriate. Collections of more detailed descriptions of studies have been moved to appendices, while the synthesis of these studies and the relevant summary tables have been retained in the main body of the Toxicological Review.

G2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

Comments: One reviewer identified 15 references, most of which related to toxicokinetics and to factors affecting variability in CYP2E1 expression. None of the references specifically addressed dichloromethane.

Response: Relevant references provided by the reviewer were added to appropriate sections in the Toxicological Review; however, citations to secondary sources of material already covered by a primary source were not added.

A. PBPK Modeling

A1a. A rat PBPK model was used for calculating the internal dosimetry for the RfD and RfC. EPA evaluated several versions of previously published rat PBPK models and modified the Andersen et al. (1991) model for use in the reference value calculations. Does the chosen model with EPA's modifications adequately represent the toxicokinetics? Was the model applied properly? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model structure appropriately considered and discussed?

Comments: Three reviewers supported the chosen model for rat PBPK toxicokinetics; one specifically noted the clear presentation and discussion of the model assumptions, parameters, and uncertainties. One of these reviewers suggested two additions to the PBPK modeling section of the Toxicological Review: a table describing the changes from the rat model compared with the model used in the previous assessment, and information regarding how CYP2E1 variability was incorporated into the model (information that was included in Appendix C). Two reviewers did not comment on this question because it was outside their area of expertise.

Response: EPA considered these suggestions but found that the first suggestion was not feasible because a PBPK model for the rat was not used in the previous assessment, and the second suggestion was not necessary because a summary of EPA's consideration of CYP2E1 variability is included in Section 3.5.2 [Probabilistic Human PBPK Dichloromethane Model (David et al., 2006)]. Thus, no changes were made in response to these suggestions.

Comments: One reviewer questioned the choices of models used in the derivation of the rat PBPK model, noting that model A was developed using the flux via GST-pathway in lung tissue in the mouse. This reviewer also suggested that the goal of the analysis should be the evaluation

of a published model to determine its usefulness for risk assessment; if it was deficient in some aspect, than modification or alternative models could be evaluated. The comparison between models would ideally be done using a statistical test, rather than the more commonly used method of visual inspection of the fit of a model to various data sets.

Response: EPA agreed with these suggestions. The Toxicological Review was revised to start with a model that is essentially identical to the published model of Andersen et al. (1991) (formerly "Model B," changed to "Model A"), with the exception that CYP- and GST-mediated metabolism were added to the lung compartment. This change was made to make the model consistent with the mouse and human PBPK models and to correctly apportion metabolism between the two tissues, while holding the total CYP and GST activity constant at the value reported by Andersen et al. (1991). Also, a clear inadequacy of the Andersen et al. (1991) model is that it does not have an oral exposure (gastrointestinal tract) submodel that is needed to describe dosimetry for oral exposures. Andersen et al. (1991) appear to have only used inhalation data, or perhaps inhalation and intravenous data, to estimate their metabolic constants (V_{maxC} and K_m for the CYP pathway, k_{fC} for the GST pathway, and P1, the CO yield factor). While it would be possible to keep these metabolic parameters unchanged and only fit an oral kinetic constant to appropriate data, fitting the oral data was challenging and not entirely satisfactory, suggesting that adjustments in the metabolic parameters should also be considered. The potential for improving the fit of the Andersen et al. (1991) model by numerical optimization was tested by performing a statistical comparison between the fit to the inhalation and intravenous data using the original parameters versus numerical fits, and the latter were found to provide a significant improvement in the goodness of fit. Therefore, the metabolic parameters (V_{maxC}, K_m, k_{fC}, and P1) along with an absorption constant (k_a) for uptake from the gastrointestinal tract were globally fit to a larger data set that included oral toxicokinetic data as well as the inhalation and intravenous data used for initial model testing.

Comments: One reviewer asked about saturation of the CYP2E1 pathway in humans at 400 ppm dichloromethane in the study by Ott et al. (1983c), and how this finding compares to that of the rat based on McKenna and Zembel (1981), with inhibition of CYP2E1 pathway when dichloromethane exposure was increased from 1 to 50 mg/kg in rats.

Response: These findings are not directly comparable. Ott et al. (1983c) examined the oxygen-half-saturation pressure (P_{50}) for O_2 binding to hemoglobin, which is reduced due to COHb resulting from dichloromethane exposure. The saturation described by McKenna and Zempel (1981) is that of CYP-metabolism of dichloromethane to CO under precisely-controlled laboratory conditions. The level of CO formed, hence impact on O_2-Hb saturation, should saturate as dichloromethane exposure increases, due to the saturation of CYP metabolism. But

the data from Ott et al. (1983c) are summary statistics from groups of workers exposed to fairly broad dichloromethane ranges (0, <100, 100–299, and >300 ppm). Given that in nonsmoking men the reduction was only from P_{50} = 26.4 ± 0.8 to 22.7 ± 1 mm Hg between 0 and >300 ppm dichloromethane (501 ppm TWA), it would be difficult to estimate the nonlinearity due to CYP-metabolism saturation in those data (and hence the concentration at which half-saturation is reached), or to compare it to that observed in the McKenna and Zempel (1981) rat studies. In addition, the human exposures of Ott et al. (1983c) were by inhalation and those of McKenna and Zembel (1981) were gavage. Since gavage exposures give a brief but high body burden for the same total dose compared to an exposure regimen that spreads out the exposure over a period of hours (inhalation specifically), even at the same total dose in the same species, one may see saturation from the oral exposure but not in the time-distributed exposure.

Comments: One reviewer postulated that CO binding to CYP2E1 would prevent further metabolism of dichloromethane, leading to increased metabolism through the GST pathway. The reviewer noted the strong binding of CO to CYPs. The reviewer was concerned that any spillover to the GST pathway that resulted from interference of CO:CYP2E1 binding would result in errors in the estimation of the contribution of each pathway to total metabolism, and suggested that the impact of this potential binding be discussed in the Toxicological Review.

Response: In examining this issue, limited data relevant to CO and CYP2E1 activity were identified. In a controlled experimental study, Benowitz et al. (2003) found that cigarette smoking resulted in an *induction* of CYP2E1 activity (measured by chlorzoxazone clearance), while exposure to pure CO at a level that yields the same blood COHb as the cigarettes had no effect on that activity. Given that CYP2E1 metabolic saturation has been observed for many volatile organic compounds in the concentration range at which it appears to occur for dichloromethane, and the observation of a negligible impact on CYP2E1 activity by direct CO exposure of Benowitz et al. (2003), it seems unlikely that the effect of any CO:CYP2E1 inhibition would be significant. A more quantitative analysis could be performed if the binding affinity (Kd) for CO-CYP2E1 were available; however, no data pertaining to this value were identified. However, it should be noted that after a fairly high bolus of dichloromethane (526 mg/kg), the peak COHb seen by Pankow et al. (1991a) was about 9%. Model simulations indicate that much higher COHb levels are not likely because the CYP-mediated metabolism to CO becomes saturated. If one then assumes that CO binds to CYP2E1 with an affinity similar to COHb, this suggests that the maximum level of CYP2E1 inhibition one would then see is around 10. In contrast, when increasing the dose from 1 to 50 mg/kg dichloromethane, more than an order of magnitude lower than the dose used by Pankow et al. (1991a), McKenna and Zempel (1981) observed a 62% reduction in fraction of total dose exhaled as CO, and in increasing the dose from 50 to 200 mg/kg, Angelo et al. (1986b) observed a reduction of over 50% in that

fraction. The changes in dichloromethane gas uptake rates observed by Gargas et al. (1986) are similarly much >10%. Thus, any error introduced by ignoring a relatively small level of CYP2E1 inhibition (expected to be <10%) is not expected to be significant.

If a metabolite had an effect on an enzyme activity, one would expect that effect to be time-dependent: as more metabolite is produced (over the minutes and hours after the beginning of an exposure), metabolite-induced inhibition would lead to a decrease in enzyme activity, and hence a decrease in the rate constant (k) or V_{max}. The model assumes that these constants are not time-dependent, however, and for the most part, appears to be consistent with the toxicokinetic data. In short, if there was a strong time-dependence (due to inhibition) in the rate constants, the model would not fit as well as it does. This is particularly true of the fits to the gas-uptake data of Gargas et al. (1986) shown in Figure C-3. That the models can describe those data well without explicitly including time-dependent inhibition suggests that the impact of such inhibition is not significant.

An additional point to consider is that as long as the model accurately describes the shift in metabolism between the two pathways, the specific mechanism by which the shift occurs (i.e., saturation versus inhibition) is not consequential. For the above reasons, and in the absence of supporting data for the postulated inhibitory effect of CO on CYP2E1 metabolism of dichloromethane, the Toxicological Review was not revised to include a discussion of this issue.

Comments: One reviewer noted a twofold difference in K_m value for dichloromethane oxidative metabolism to CO, and suggested that the potential reasons for the discrepancy (i.e., twofold difference) and the overall impact on the dichloromethane PBPK model should be discussed. This reviewer asked about the influence of CO-CYP2E1 formation on the high affinity and low affinity K_m values, on the overall K_m, and noted that both in vitro and in vivo estimates involve some unrealistic dichloromethane exposure concentrations.

Response: One correction to the reviewer's comment should be noted: the difference in K_m between that estimated by Reitz et al. (1989a) and that obtained from the in vivo analysis is not twofold, but rather, is over two orders of magnitude. Reitz et al. (1989a) did not observe dichloromethane oxidation to CO. CYP2E1 converts dichloromethane to formyl chloride, from which CO may be produced by subsequent reactions. Reitz et al. (1989a) observed the appearance of aqueous-soluble radiolabeled products, likely a combination of multiple products, after extracting the unreacted radiolabeled substrate. At the in vivo dichloromethane concentrations, the high K_m observed by Reitz et al. (1989a) would be essentially indistinguishable from a first-order pathway, since the in vivo dichloromethane concentrations remain well below that K_m.

The concentration range studied by Reitz et al. (1989a) was 1–10 mM, while the K_m estimated from in vivo toxicokinetic data was <10 uM. Thus, Reitz et al. (1989a) were not able

to detect a saturation constant in the range that is consistent with the in vivo data. Reitz et al. (1989a) *did* observe BW saturation in the millimolar range, consistent with a K_m in that range. Evans and Caldwell (2010) discuss the possibility that CYP2E1 may have two active binding sites, which could then be consistent with both a high and low K_m. The model that they consider, however, has only been tested (i.e., using in vitro data where the role of other enzymes can be ruled out) against data for CYP isozymes other than CYP2E1, and not using dichloromethane.

If the reviewer is addressing another difference in K_m values (e.g., from two different studies or between two species) then such a difference is expected given the methods and data used to estimate K_m and inter-species differences in CYPs. In either case, as discussed in detail in response to similar issues raised in PBPK Charge Question A1a, the largest expected impact from CO-CYP binding is about 10%, whether two different CYPs or two binding sites on the same CYP are involved. An effect of this magnitude was not considered significant.

Comments: One reviewer asked about the impact of formaldehyde produced during the metabolism of dichloromethane on the oxidative metabolism of dichloromethane, and whether formaldehyde affects the GST metabolic pathway.

Response: As depicted in Figure 3-1, formaldehyde is a product of GST-mediated metabolism, not the CYP2E1 oxidative pathway. However, EPA is not aware of any quantitative data on the amount of formaldehyde so produced, either in vitro or in vivo, or of any quantitative data on the effect of formaldehyde on either CYP2E1 or GST-T1. Therefore, the potential impact of formaldehyde on either pathway cannot be evaluated.

A1b. The internal dose metric used in the RfD and RfC derivations was based on total hepatic metabolism via the CYP2E1 pathway. Because the metric is a rate of metabolism, and the clearance of metabolites is generally expected to be slower in the human compared with the rat (assuming clearance scales as body weight$^{0.75}$), the rat internal dose metric is adjusted by dividing by a toxicokinetic scaling factor to obtain a human-equivalent internal dose. Are the choices of dose metric and toxicokinetic scaling factor appropriate and scientifically supported? Is the rationale for these choices clearly described? Are the uncertainties in the dose metric selection and calculations appropriately considered and discussed?

Comments: Four reviewers noted agreement with the choice of the dose metric (one indicating the choice the the justification for the choice was limited, however), and one reviewer did not comment directly on these questions. Two other reviewers did not comment on this question because it was outside their area of expertise.

Response: The dose metric was retained.

Comments: Three reviewers raised questions regarding the use of the toxicokinetic scaling factor to account for potential differences between the rat and the human in the rate of clearance of metabolites. The reviewers agreed on the underlying foundation for the use of the scaling factor (i.e., the uncertainty that arises from the need to use a rate of metabolism rather than the effective concentration in the PBPK modeling, and the unknown interspecies differences that preclude prediction of tissue metabolite concentrations). The reviewers disagreed, however, on the optimal approach for addressing this uncertainty, as described below.

Recommendations 1: One reviewer suggested that an UF rather than a scaling factor should be used to address the uncertainty regarding the effect of interspecies differences in metabolite clearance. The reviewer suggested that an alternative derivation using an interspecies toxicokinetic UF of 3 should be presented.

Response: This issue concerns a specific mechanism—metabolic clearance—for which there is information on animal-human differences (i.e., clearance is expected to vary as $BW^{0.75}$ based on data from various compounds). Thus, a scaling factor is used instead of a more general UF because some information is available to support the magnitude of interspecies toxicokinetic difference, rather than general uncertainty. In contrast, an UF of 3 was applied to address toxicodynamic extrapolation in the absence of information to characterize the magnitude of interspecies differences in toxicodynamics.

Recommendations 2: One reviewer suggested that the application of the scaling factor would have the effect of introducing an additional source of uncertainty into the PBPK modeling. This reviewer noted the metric used (i.e., the rate of metabolism) was a highly feasible metric given the available data, but was one with "low desirability" based on relevance and closeness to the postulated mode of action. In contrast, the more "highly desirable" metric, tissue metabolite concentration, is not a metric that can be feasibly estimated and used. The use of the BW scaling factor addresses the residual uncertainty that comes from using the rate of metabolism metric, but is itself a source of uncertainty. The reviewer suggested that EPA should evaluate two approaches, one based on the PBPK-derived metric without the scaling factor and one that includes the use of the scaling factor. From these two approaches, EPA would then choose based on residual uncertainty (level of confidence) and relevance to mode of action (i.e., closeness to the "desired" measure of internal dose).

Response: EPA's approach is consistent with this reviewer's suggestion. In individuals with lower metabolism, the parent dichloromethane concentration will be higher at a given level of

exposure compared with those with higher metabolism. Since the mode of action is that dichloromethane is metabolically activated, higher risk is expected for populations with higher metabolism (i.e., in populations with *lower* parent dichloromethane concentration). Hence, parent concentration is inversely correlated with risk, so use of the parent concentration has poor relevance to the mode of action. Allometric scaling is commonly used because it represents the expected, or most likely, variation in metabolic clearance across species. Therefore, the use of the rate of metabolism metric with the scaling factor is believed to have the lowest uncertainty (i.e., represents the most likely relationship for the concentration of the active metabolite) while using a metric that is relevant to and consistent with the mode of action.

Recommendations 3: One reviewer agreed with the use of the scaling factor, but noted that further clarification regarding its use, consideration of other approaches, and overlap between use of this factor any of the UFs that were also used should be included in the Toxicological Review.

Response: A discussion of these points was added to Section 5.1.2 (Derivation Process for Noncancer Reference Values).

Recommendation 4: One reviewer noted that "Clearance of unidentified CYP metabolites in rodents versus humans remains an unknown," but questioned the use of the scaling factor for the dose metric, suggesting that the use of "experimentally-derived or model predicted, species-specific metabolic rate constants" would be preferable. This reviewer noted that use of this scaling factor would yield a HED that is excessive (i.e., an RfD that is lower than necessary).

Response: It is because of the absence of experimentally-derived, species-specific data pertaining to the relationship between clearance of CYP metabolites in rodents and humans that the scaling factor based on $BW^{0.75}$ scaling is warranted. EPA agrees that if these types of data were available, it would be preferable to use them to evaluate and potentially modify or eliminate the scaling factor.

Comments: Two reviewers questioned the scientific rationale for using $BW^{0.75}$ rather than a scaling factor based on a different value (e.g., $BW^{0.9}$); one of these reviewers noted a publication (Mahmood and Sahajwalla, 2002) and a textbook (Mahmood, 2005) that indicated the $BW^{0.75}$ scaling was not supported by the literature. This reviewer also indicated a high degree of uncertainty in the model due to the lack of data on metabolite kinetics, which otherwise could be used to validate or calibrate the scaling factor used.

Response: Lindstedt and Schaeffer (2002) reviewed the use of allometry for anatomical and physiological parameters. Overall metabolic rates were assessed by resting oxygen uptake and found to vary with an exponent of 0.725 (r^2 = 0.997) using data for 11 species, from a 4 g shrew to a 4,000 kg elephant. When the regression was performed with data from only mice, rats, dogs, and humans, the exponent obtained was 0.766 (r^2 = 0.997). Thus, an exponent of 0.75 is consistent with this measure of overall metabolism in multiple species, including those most commonly used for toxicity testing and health assessments. That exogenous metabolism will scale in parallel with endogenous metabolism has been an assumption made in PBPK modeling, but ventilation and cardiac output (hepatic blood flow) are also key determining factors in overall metabolism and data discussed below on elimination of drugs also supports a value of 0.75.

Other PBPK modelers have used 0.7 or 2/3 for the scaling exponent. For example, Gearhart et al. (1994) used an exponent of 0.7 to scale from rats to humans. This assumption is typically hard-coded into PBPK models with the multiplicative constant (VmaxC for saturable metabolism) reported instead of the actual VmaxC. This was done in the dichloromethane models by Marino et al. (2006) and David et al. (2006), who reported VmaxC normalized to $BW^{0.7}$ (units of mg/h/$kg^{0.7}$). Because the first-order constant, k_F, is a rate per unit tissue volume (total rate is k_f*V_L*C_{VL}, where V_L is liver volume and C_{VL} the dichloromethane concentration), the normalization to units of $kg^{0.3}$/h reflects the same built-in assumption. That this scaling captures observed mouse:human differences in dichloromethane metabolism is reflected by the fact that the mean VmaxC obtained for the mouse by Marino et al. (2006) was 9.2 mg/h/$kg^{0.7}$ (final posterior value) and that obtained for the human by David et al. (2006) was 9.42 mg/h/$kg^{0.7}$: nearly identical numbers. That k_{FC} was estimated to be somewhat less in the human than the mouse, 0.852 vs. 1.42 $kg^{0.3}$/h, respectively, indicates that the total rate of glutathione conjugation increases at a rate less than $BW^{0.7}$, considerably less than scaling as BW^1. The magnitude of the scaling factor would increase if an exponent value less than 0.75 is used. Thus, use of 0.75 in the absence of chemical-specific data is a reasonable choice.

Mahmood and Sahajwalla (2002), cited by the one reviewer, estimated scaling coefficient values for biliary clearance of 8 drugs. This elimination route is not likely to be relevant for dichloromethane metabolites, given that dichloromethane has a lower molecular weight than most drugs, but the data in fact support the scaling used by EPA in this assessment. Of these 8 compounds analyzed, 3 did have coefficient values >1. But four had coefficient values between 0.716 and 0.791, and one had a coefficient value of 0.633. Thus the data appear to be clustered into at least two subsets, and contrary to the reviewers' statement, the default coefficient of 0.75 is well within the range of observation and represents one cluster of the data well. Tang and Mayersohn (2005) evaluated total clearance data for a much larger set of compounds, 61, for which coefficient values ranged from 0.35 to 1.2, with mean +/- standard deviation = 0.748 +/- 0.024. In Mahmood (2005), Table 5.1 provides allometric exponents fit to clearance data for 50 drugs; exponent values ranged from 0.418 to 1.196 with a median of 0.79. The range of these

results indicates the possible range of coefficient values and hence uncertainty in the scaling for clearance of dichloromethane metabolism. Given that the mean coefficient value obtained for the Tang and Mayersohn (2005) data set was 0.748, however, suggests that use of 0.75 is not health-protective on average, but rather, it is an average value. Relative to the lowest coefficient value reported by Tang and Mayersohn (2005), 0.35, use of 0.75 could underestimate metabolite clearance and hence overestimate risk in humans by as much as 10-fold for scaling from the rat and 22-fold for scaling from the mouse.

The results for a range pharmaceutical compounds is in agreement with the comment that the lack of data to evaluate or calibrate clearance of the metabolite creates a potentially large uncertainty in model predictions. However, these data and the physiological scaling data discussed above also are consistent with $BW^{0.75}$ scaling as a most-likely estimate of clearance in humans compared to rodents.

A2a. The mouse PBPK model used in deriving the cancer risk estimates was based on the published work of Marino et al. (2006). Does the chosen model adequately represent the toxicokinetics? Was the model applied properly? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model structure appropriately considered and discussed?

Comments: Four reviewers supported the chosen mouse PBPK model. As in response to PBPK Charge Question A1a, one of these reviewers suggested two additions to the Toxicological Review: a table describing the changes in the mouse model (compared with the model used in the previous IRIS assessment) and information regarding how CYP2E1 variability was incorporated into the model (information that was included in the appendix). Two reviewers did not comment on this question because it was outside their area of expertise.

Response: No additional changes were made based on these suggestions. Table 3-5 provides a comparison of parameters used in the previous assessment and those used in the current mouse model. A summary of EPA's consideration of CYP2E1 variability is included in Section 3.5.2 (Probabilistic Human PBPK Dichloromethane Model) (David et al., 2006).

Comments: One reviewer noted that since tumor formation is thought to result from the GST metabolic pathway, dichloromethane levels below the level of CYP saturation should result in no cancer risk or a highly attenuated risk relative to that seen above the saturation of the CYP pathway.

Response: The predicted risk is highly attenuated below the exposure levels where CYP is saturated, compared to those occurring above it, because CYP metabolism predominates over

GST metabolism at the lower levels. This attenuation is built into the PBPK modeling. EPA does not consider, however, the assumption of zero risk to be supported.

A2b. The internal dose metric used in the cancer quantitation was based on tissue-specific GST metabolism. To account for potential clearance rate differences, the mouse internal dose metric was adjusted by dividing by a toxicokinetic scaling factor to obtain a human-equivalent internal dose. Are the choices of dose metric and toxicokinetic scaling factor appropriate and scientifically supported? Is the rationale for these choices clearly described? Are the uncertainties in the dose metric selection and calculations appropriately considered and discussed?

Comments: Two reviewers specifically noted support for the use of the tissue-specific GST metabolism dose metric, and the other reviewers did not specifically address this question.

Response: The dose metric was retained.

Comments: One reviewer stated that the use of the scaling factor was appropriate and clearly explained. Four reviewers did not provide comments in response to this charge question (two of these noting that it was outside their area of expertise). Two reviewers indicated their response to the scaling factor question was similar to that of the scaling factor addressed in PBPK Modeling Charge Question A1b, specifically raising questions about the justification of this factor. One reviewer noted that "the application of the toxicokinetic scaling as done would be appropriate when the chemical entity (metabolite) itself is the active moiety (which is the case), further metabolism/reaction renders it inactive (which is likely the case), and the rate of the metabolism/reaction process is proportional to the liver perfusion rate, cardiac output or to the body surface (which is not known to be the case)." This reviewer stated that additional support for the use of the scaling factor addressing these points should be provided in the Toxicological Review.

Response: As noted by the reviewer, the first two elements justifying the use of the scaling factor are met. With respect to the third element, it is not known that the rate of reaction is proportional to the liver perfusion rate, cardiac output, or body surface area. It is also important to note that it is not known that the rate of reaction is not proportional to these factors. Hence, no information is available to inform the choice for this element; i.e., whether the clearance of the metabolite is proportional to liver perfusion (allometric scaling) or proportional to liver volume (no scaling factor, or scaling factor = 1). Given this uncertainty, use of the scaling factor is warranted. The discussion of these points was expanded in Sections 5.1.2 (Derivation Process for Noncancer Reference Values), 5.4.1.4 (Dose Conversion and Extrapolation Methods: Cancer

Oral Slope Factor) and 5.4.2.4 (Dose Conversion and Extrapolation Methods: Cancer Inhalation Unit Risk).

Comments: One reviewer raised two questions about the extrapolation of the animal results to humans. One issue concerns the potential contribution of CYP2E1 metabolism, which is much greater in humans than in mice, to genotoxicity. The other issue concerns target tissue concordance and the potential relevance to the observation of leukemia and other types of cancers that were not observed in mice. This reviewer noted the uncertainties arising from these issues as another justification for the use of the scaling factor, and suggested that additional discussion of the potential underestimation of exposure to reactive metabolites should be added. This reviewer reiterated and expanded on this issue in response to PBPK Charge Question A3a and Carcinogenesis Charge Questions C1, C4, and C5.

Response: Responses to this issue are discussed in more detail in response to PBPK Charge Question A3a and Carcinogenesis Charge Questions C1.

A3a. A probabilistic human PBPK model (David et al., 2006) was used to estimate a distribution of human equivalent doses and concentrations for the points of departure (PODs) for the RfD and RfC, respectively. The 1st percentile of these distributions was selected to represent the most sensitive portion of the population. For the derivation of the oral and inhalation cancer risk estimates, the probabilistic human PBPK model was used to calculate the distribution of human internal doses (mg dichloromethane metabolized via the tissue-specific GST pathway per unit volume of tissue) that would be expected from a 1 mg/kg-day oral dose or a 1 μg/m^3 inhalation concentration. This distribution of human internal doses was used with the tumor risk factor to generate a distribution of oral slope factors or inhalation unit risks. Does the chosen model adequately represent the toxicokinetics? Was the model applied properly? Are the model assumptions clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

Comments: Three reviewers supported the selection of the model. Two reviewers did not comment on this question because it was outside their area of expertise. One reviewer asked for the scientific evidence supporting the assumption that some dichloromethane is metabolized by the GST pathway at all levels of exposure.

Response: Below ~20% of the K_m (which has units of concentration), the rate of reaction becomes indistinguishable from a first-order reaction, as it depends on the probability that a substrate molecule collides with an unoccupied active site on the enzyme. Thus, at low

concentrations ([Substrate] << K_m) the rate of enzyme-catalyzed reactions becomes proportional to the concentration of the substrate(s) and enzyme. As long as GSH, GST, and dichloromethane are present at non-zero concentrations, the reaction will proceed at a non-zero rate; that is, the probability of collision with the active site is non-zero, and the rate of reaction will be non-zero. EPA is not aware of any data for single-enzyme/single-substrate reactions that are not consistent with this fundamental theory of enzyme kinetics. This discussion was added to Sections 3.3 and 4.7.3.2.

Comments: One reviewer, in response to PBPK Modeling Charge Question A3b, noted that the choice of the first percentile to account for population variability was supported. Another reviewer, in response to RfD Question B4, noted that the first percentile was chosen to be protective of sensitive individuals, and that this decision, in conjunction with the use of the toxicodynamic variability UF, was adequate. Another reviewer did not agree with use of the first percentile of distribution of the human internal doses, observing that the PBPK model already incorporated measures of variability, and that use of the first percentile in addition to use of the most sensitive GST genotype and the toxicokinetic scaling factor in the cancer potency derivations "over adequately accounts for human variability."

Response: The last comment described above is based on a misunderstanding of the procedures used by EPA. Cancer risk values were based on the mean of the most sensitive GST genotype, not on the upper or lower tail of the distribution. With respect to the noncancer values (RfD and RfC); however, the first percentile of the distribution of HEDs or HECs for the full population (i.e., all genotypes combined) was used. Thus, the reviewer's interpretation of the procedure used in the RfD and RfC derivation was mistaken. The reviewer was correct that the human PBPK model incorporates variability in the estimations through statistical sampling of metabolic and physiological parameters from various distributions. This procedure explicitly accounts for variability that results from these known factors that influence toxicokinetics and dosimetry and so allows for the generation of dosimetric distributions based on population variability. However, one must then select a point on the dosimetric distribution corresponding to a portion or percentile of the population one effectively wishes to protect. The first percentile of the distribution was selected for derivation of the RfD and RfC as a low but non-zero population percentile that can be estimated by computational statistical sampling in a reasonable amount of time (i.e., with a reasonably stable estimate achieved through a finite number of iterations).

Comments: One reviewer noted that GST activity is greater in mice than rats, and greater in rats than the most sensitive human (i.e., those who are GST-$TI^{+/+}$), and noted the relative lack of carcinogenic response seen in the rat bioassays. This reviewer then asked for clarification regarding the assumption of higher human responsiveness as discussed in the justification of the

use of the scaling factor in the 1987 dichloromethane assessment, and differences between this assumption and the use of the scaling factor in the current assessment.

Response: The explanation of what was done in the 1987 dichloromethane assessment was clarified in Section 3.5 (Physiologically Based Pharmacokinetic Models). Overall cancer risk associated with exposure to dichloromethane can be viewed as dependent on two toxicokinetic terms: rate of GST metabolism and rate of GST-metabolite clearance. For cancer, it is assumed that given the same average tissue concentration of active metabolite, humans would have the same average cancer risk as rodents (i.e., that humans are as "responsive" per unit of active metabolite tissue concentration as the most sensitive animal species/sex/strain). The concentration of active metabolite in humans relative to the mouse or rat is approximated as:

[relative human concentration] = [relative GST metabolism]/[relative metabolite clearance].

Because metabolism and other clearance mechanisms (blood perfusion, respiration, renal filtration) are all expected to be about sevenfold slower in a 70-kg human than a 30-g mouse, the second term (relative metabolite clearance), which is in the denominator, is assumed to be 1/7 (one over the toxicokinetic scaling factor).

In the 1987 assessment, the scaling factor was applied to adjust for both interspecies differences in processes that lead to differences in internal doses (e.g., clearance of a given daily amount of dichloromethane metabolically activated/L of tissue) and differences in toxicodynamics or response, whereas the scaling factor used in the current assessment specifically accounts for expected differences in clearance between species. The factor in the current assessment is based on scaling as $[BW]^{3/4}$, reflecting current practices for interspecies extrapolation (U.S. EPA, 2005a, 1992); the 1987 assessment applied the scaling factor of $[BW]^{2/3}$ that was current practice at that time. These differences in scaling resulted in the different applied factors of 7.0 (current assessment) versus 12.7 (1987 assessment).

Comments: One reviewer supported the use of the Bayesian Markov-Chain Monte-Carlo analysis to address human variability, but raised one question regarding the blood:air partition coefficient (PB) for the mouse based on experimentally-derived values from Clewell et al. (1993). This value (23) is higher than the value (8.3) used in previous models. The value for the human (9.7) is closer to the previous value, however, and is based on the values from Andersen et al. (1987). The reviewer raised the question of whether the PB value for humans could be in error, reflecting an underlying difference in the methods of Clewell et al. (1993) and Andersen et al. (1987).

Response: As noted in Section 3.5.1 (Probabilistic Mouse PBPK Dichloromethane Model), the value for the mouse PB used in the current assessment is consistent with that measured in rats (19.4) and hamsters (22.5). The difference between the (new) values for rat, hamster, and mouse and human PB value in this case is typical of rodent-human differences seen for other compounds (e.g., see Wiester et al., 2002). The difference between rodent and human values of PB for volatile organic compounds is believed to result from differences in lipid content. The difference between the earlier measured value (8.3) and the more recent value (23) for mice is likely the result of improvements in the method for measuring PB in the 7 years that passed between the two studies.

Comments: One reviewer suggested that non-Michaelis-Menten kinetics of CYP2E1 metabolism of dichloromethane and uncertainty regarding site concordance and dose metrics be added to the discussion of the model. (A similar comment was made in response to PBPK Modeling Charge Question A2b.)

Response: A discussion of the alternate CYP kinetics is included in Section 3.5.5 (Uncertainties in PBPK Model Structure for the Mouse, Rat, and Human), Section 5.3 (Uncertainties in the Oral Reference Dose and Inhalation Reference Concentration), and Section 5.4.5 (Uncertainties in Cancer Risk Values). GST-T1 is expressed in tissues other than the liver and lung, including the human brain (Juronen et al., 1996), breast tissue (Lehmann and Wagner, 2008), and rat olfactory epithelium (Banger et al., 1994). While the extent of GSH conjugation in these other tissues may not be significant to the overall dosimetry of dichloromethane, they can be significant in understanding the sites of action of dichloromethane as these vary among species.

A3b. EPA modified the parameter distributions in the published David et al. (2006) model. Does the set of model parameter distributions adequately account for population variability and parameter uncertainty in estimating human equivalent doses? Are the human parameter values and distributions clearly presented and scientifically supported?

Comments: One reviewer considered the development and inclusion of the parameter distributions that reflected both parameter uncertainty and interindividual variation to be an important addition to the earlier published version of this model, and that these distributions and their development were clearly explained and scientifically justified. This reviewer specifically noted support for the use of the data from Lipscomb et al. (1997) to characterize the distribution of CYP enzymes activities in humans. Two reviewers did not comment on this charge question because it was outside their area of expertise.

Response: No response required.

Comments: One reviewer asked why the geometric standard deviations were based on the trichloroethylene model rather than the distribution of CYP protein.

Response: The geometric standard deviations for CYP were not based on the trichloroethylene model per se, but on measures of metabolic activity using trichloroethylene as the substrate. Protein activity is not necessarily proportional to protein levels, and protein levels are more difficult to quantify accurately. CYP2E1 activity is often measured using p-nitrophenol hydroxylation activity; in the dichloromethane assessment, an alternate CYP2E1-specific substrate was used to quantify the range in activity.

Comments: One reviewer questioned the BW adjustment for CYP2E1 activity, and suggested an adjustment based on liver volume as an alternative.

Response: CYP activity was scaled by BW because that is the scaling relationship used by David et al. (2006). Further, EPA does not consider scaling by liver weight to be better supported. In addition, BWs, but not liver weights, are available for the individual subjects from which human toxicokinetic data were taken. (Liver weight data would not be obtainable in this type of study, since it involved healthy individuals.) An assumed relationship between liver weight and BW could be used, but then one would implicitly be using BW.

Comments: One reviewer asked if the distribution of K_fC for the U.S. population derived from the Swedish study took into account the ethnic make-up of the respective populations.

Response: The proportion of the three genotypes in the U.S. population, as reported by Haber et al. (2002) and used here, represents the ethnicity distribution of the U.S. population, based on U.S. census data. This point is provided in Section 3.3 (Metabolism).

Comments: One reviewer asked how the mass balance of the flows and volumes was ensured during the Monte Carlo iterations.

Response: As indicated in the last column of Table B-3, after each set of Monte Carlo samples for fractional blood flows; for example, the sampled values were divided by the sum of the sampled values, so that the sum of the resulting fractions equal one. Volume fractions were normalized to sum to 0.9215, allowing for 7.85% of the BW as bone, teeth, nails, and hair.

Comments: One reviewer asked about the basis upon which the normal distribution for GST was set.

Response: A normal (i.e., Gaussian) distribution, rather than some other distribution form, was used because that is what was used by David et al. (2006) and it is consistent with the data of Warholm et al. (1994). Warholm et al. (1994) state that their data are consistent with Hardy-Weinberg equilibrium: the expected long-term population distribution for a binary polymorphism in a population assuming no selection advantage for one of the three genotypes.

B. Noncancer Toxicity of Dichloromethane

Oral reference dose (RfD) for dichloromethane

B1. A chronic RfD for dichloromethane has been derived from a 2-year oral (drinking water) study in the rat (Serota et al., 1986a). Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comments: Six reviewers supported the selection of the Serota et al. (1986a) as the principal study. One reviewer also suggested that the choice of the principal study would be strengthened by inclusion of a graphical presentation of the different endpoints based on internal dose metrics. One reviewer did not comment on this question because it was outside this reviewer's area of expertise.

Response: EPA agrees that an exposure-response array such as that described by the reviewer could be an effective way to compare study findings across studies and endpoints; however, a figure based on internal dose metrics was not developed for the following reasons:
(1) equivalent types of dose metrics (i.e., tissue-specific concentrations or rates of metabolism) cannot be generated for all of the endpoints that were considered (specifically neurotoxicity and reproductive/developmental toxicity) and (2) if the surrogate blood AUC concentration was used from the PBPK model, the internal doses would be proportional to the administered dose unless the endpoints were from different species.

Comment: One reviewer suggested that EPA confirm whether the Agency had access to the original Hazleton report in the rat to check for any discrepancies between the original report and the published study (Serota et al., 1986a).

Response: EPA was unable to obtain the original Hazleton report.

B2. An increase in the incidence of liver lesions (foci/areas of alteration) was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Comments: Six reviewers supported the selection of liver lesions (foci/areas of alteration) as the critical effect for the RfD. One of these reviewers reiterated the idea of presenting an exposure response array based on internal dose metrics to strengthen the selection of the critical effect. One reviewer did not comment on this question because it was outside this reviewer's area of expertise.

Response: A response that addresses the recommendation for an exposure response array based on internal dose metrics is provided under RfD Charge Question B1.

B3. Benchmark dose (BMD) modeling was applied to the incidence data for liver lesions to derive the POD for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in incidence of liver lesions) scientifically supported and clearly described?

Comments: Six reviewers generally agreed that the application of BMD analysis to derive the POD for the RfD was appropriate and clearly described. One of the reviewer who agreed with the modeling noted the approach and several assumptions result in a "conservative" RfD (i.e., a value that is lower than is necessary). One of these reviewers specifically stated that a BMR of 10% increase in the incidence of liver lesions is scientifically supported and consistent with EPA guidelines. One reviewer commented that selection of a 10% BMR is not appropriate if the UF that would be applied to a NOAEL (i.e., $UF_L = 1$) is used. This reviewer indicated that a 5% BMR more closely corresponds to a NOAEL for this type of data (animal study and quantal response). One reviewer did not comment on this question because it was outside this reviewer's area of expertise.

One reviewer indicated that using a first percentile HED as well as a subset of the GST-$T1^{+/+}$ population for the RfD derivation was not justified. One reviewer disagreed with applying a toxicokinetic scaling factor to the internal dose. This reviewer also suggested that it would be useful to show alternative RfDs based on other dose metrics.

Response: An UF for LOAEL-to-NOAEL extrapolation was not used because the Agency's current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. A 10% extra risk of increased foci/areas of alterations was applied under the

assumption that it represents a minimal biologically significant degree of change. Therefore, an UF for extrapolation from a LOAEL to a NOAEL was not applied. There are no additional data to suggest that the severity of the critical effect or the power of the study would warrant a lower BMR (see Sections 5.1.4 and 5.2.3).

The GST-T1$^{+/+}$ population subset was not selected as a sensitive group for the RfD (or RfC) derivation. The reasoning behind the use of the first percentile HED is discussed in response to comments under PBPK Modeling Charge Question A3a. The use of the toxicokinetic scaling factor is addressed in the response to comments under PBPK Modeling Charge Questions A1b and A2b. The rationale for the use of the chosen dose metric is explained in Section 5.1.3 (Evaluation of Dose Metrics for Use in Noncancer Reference Value Derivations). The GST metabolism, AUC, and combined GST and the CYP dose metrics did not present reasonable choices based on model fit and consistency of response across studies at comparable dose levels, and as such, development of RfDs based on these dose metrics was not supported.

Comments: One reviewer asked for the rationale for selection of the model, among the suite of BMD models available, used to derive the RfD and RfC. This reviewer asked how these models relate to the available scientific data.

Response: There are currently no mechanistic data to support or derive a biologically-based model or to inform the model selection from among the available empirical models. The BMD models selected for the reference value derivations were based on the best statistical fit to the data as described in Sections 5.1.4 and 5.2.3 and Appendix F.

B4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described in the document? Please provide a detailed explanation. If changes to an UF are proposed, please identify and provide a rationale.

Comments: Reviewers generally agreed with application of the UFs for animal to human extrapolation (i.e., UF_A of 3 to account for the toxicodynamics portion of the interspecies UF) and for human variability in sensitivity (i.e., intraspecies UF_H of 3). One reviewer reiterated a comment on the LOAEL to NOAEL UF, noting that use of an UF that would be applied with a NOAEL (i.e., $UF_L = 1$) is not appropriate with a BMR of 10%. Two reviewers did not offer comments on the UFs used to derive the RfD, one noting that the calculation of RfDs was outside this reviewer's area of expertise.

Response: The issues of the choice of a 10% BMR and related UF pertaining to LOAEL to NOAEL extrapolation, are discussed in response to RfD Charge Question B3.

Comments: Two reviewers agreed with the selection of a database UF of 3, one of the reviewers noting the importance of reflecting data deficiencies in the area of neurodevelopmental effects. Three reviewers did not think that a database UF of 3 was necessary (one noting that there is a great deal of research on a wide variety of endpoints in several species) or well justified (i.e., that the case had not been made for uncertainty associated with lack of a neurodevelopmental assessment). One of these reviewers suggested running a PBPK model to determine the CO levels expected at low dichloromethane doses and to compare these levels to CO levels that are known to produce CNS effects in animals and humans.

Response: As recommended, EPA compared CO levels associated with neurotoxicity and predicted CO levels (via PBPK modeling) from the HED used to derive the RfD using the human dichloromethane PBPK model (David et al., 2006). The human PBPK model predicted relatively low CO exposures at dichloromethane levels at the RfD, levels that would not be expected to result in a substantial health risk. This analysis assumes that CO is the only agent responsible for the observed CNS and potential developmental neurotoxicity effects. This assumption is not supported by the available data. This point was made by one of the reviewers who noted (in the "additional comments" section) the potential for dichloromethane (the parent compound) to induce CNS dysfunction. In addition, mechanistic studies and studies comparing CO and dichloromethane exposures in rats (Karlsson et al., 1987; Rosengren et al., 1986; Putz et al., 1979; Stewart et al., 1972a, b) indicate the parent compound can pass through the placenta. Thus, the database UF was applied because the available data are consistent with neurodevelopmental toxicity potential for dichloromethane, but it is not known if neurodevelopmental toxicity would be a more or less sensitive endpoint than the critical endpoint (liver lesions). The database UF was also applied because a two-generation oral exposure reproductive toxicity study is not available and the two-generation inhalation exposure study by Nitschke et al. (1988a) did not dose the animals continuously during gestation and lactation. This aspect of the design represents a limitation of the study. In consideration of these deficiencies in the database, a database UF of 3 is supported. The discussion of the database UF (Section 5.1.5) was revised to more clearly describe the rationale for application of a database UF of 3.

Comments: One reviewer suggested that an additional UF be included to account for uncertainties in dichloromethane metabolism.

Response: As discussed in EPA's *A Review of the Reference Dose and Reference Concentration Process* (U.S. EPA, 2002), UFs are applied to account for uncertainties in animal to human extrapolation (UF_A), human variability (UF_H), subchronic to chronic extrapolation (UF_S), LOAEL to NOAEL extrapolation (UF_L), and database deficiencies (UF_D). The interspecies scaling factor accounts for some of the uncertainty in overall dichloromethane metabolism. Using the first percentile internal HED rather than the mean of the population distribution, as predicted by the human PBPK model, also explicitly accounts for uncertainty and variability in the metabolic activity among humans. Thus, EPA concluded that using these two factors in combination sufficiently accounts for toxicokinetic uncertainties, which include those from metabolism.

Comments: One reviewer indicated that where the metric is the rate of metabolism (production) rather than the more relevant metric of metabolite concentration as is the case with dichloromethane, both the toxicokinetic component of the interspecies UF (= 3) and the scaling factor were, in effect, accounting for interspecies differences in clearance of the parent compound. The reviewer raised concerns about the "repetitive application" of the scaling factor within this context, and how this compares to a situation where no PBPK modeling is applied (the "default" approach) in which either a toxicokinetic UF (= 3) or a body surface area scaling factor (but not both) is used to account for toxicokinetic uncertainty.

Response: The conceptual difficulty with the dichloromethane PBPK model is that it is an incomplete model regarding both the parent dichloromethane and the presumed toxic metabolite(s). Ideally there would be two (or more) linked PBPK models, or submodels, the first being the existing model that describes parent dichloromethane absorption, distribution, metabolism, and elimination (ADME) processes, and the downstream submodels describing the these processes for each toxic metabolite. A linked submodel would track a metabolite's distribution throughout the body, its metabolism and elimination, and therefore, a linked submodel could be used without a scaling factor. Text was added to Section 5.1.2 to clarify those factors applied to account for the toxicokinetic and dynamic components of interspecies extrapolation and intraspecies variability.

Inhalation reference concentration (RfC) for dichloromethane

B5. A chronic RfC for dichloromethane has been derived from a 2-year inhalation bioassay in rats (Nitschke et al., 1988a) Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comments: Five reviewers supported the selection of Nitschke et al. (1988a) as the principal study. One reviewer did not consider the inhalation study by Nitschke et al. (1988a) an appropriate basis for RfC derivation because of limitations in the liver lesion data (hepatic vacuolization) reported in this study. One reviewer suggested that a graphical display of endpoint data based on internal dose metrics would strengthen the choice of the principal study. One reviewer did not comment on this question because it was outside this reviewer's area of expertise.

Response: A response that addresses the critical effect (hepatic vacuolization) is provided under RfC Charge Question B6. A response that addresses the recommendation for an exposure response array based on internal dose metrics is provided under RfD Charge Question B1.

Comments: Two reviewers noted the value of the findings from epidemiological studies of neurological effects in workers exposed to dichloromethane as supportive data for the RfC derived from the animal data. One reviewer suggested that consideration be given to studies that identified elevated levels of COHb (an effect identified in humans) or the Savolainen et al. (1981) neurological study in rats as possible principal studies.

Response: EPA agrees that the potential for a significant health risk due to dichloromethane-generated CO should be evaluated to assure that the final RfC is protective for that mode of action. Increased CO and COHb have implications for neurotoxicity as well as cardiovascular disease risk. In examining this question, inaccuracies in the CO submodel representing endogenous CO production of the David et al. (2006) PBPK model were noted. Since protection against CO-induced effects should consider all sources of CO, determining an RfC for dichloromethane based on CO would need to account for the range of ambient CO levels likely encountered, further complicating the analysis. Thus, a simpler evaluation was performed involving a comparison of CO exposure rates at the CO National Ambient Air Quality Standard versus that produced from dichloromethane metabolism at the candidate RfC based on liver effects in the rat. This evaluation indicated that an RfC based on CO synthesis would likely be much higher than the one based on rat liver effects, and hence not sufficiently protective against liver lesions. Therefore, development of an alternative RfC based on CO synthesis was not warranted.

Savolainen et al. (1981) observed neurochemical changes in the cerebrum and cerebellum of rats following a 2 week (6 hours/day, 5 days/week) exposure to 500, 1,000, and 1,000 TWA ppm dichloromethane. EPA did not consider this study as a principal study because of the acute nature of the exposure conditions, the type of effect examined, and the fact that the effects seen were not as sensitive as the liver effects seen in Nitschke et al. (1988a). EPA considered the

comparison RfC developed based on the Lash et al. (1991) study of neurological effects among retired dichloromethane-exposed workers to be of higher quality than the Savolainen et al. (1981) study in rats for the evaluation of neurological effects.

B6. An increase in the incidence of hepatic vacuolation was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Comments: Five reviewers supported the selection of hepatic vacuolation as the critical effect for the RfC. One reviewer questioned the biological significance of hepatic vacuolation as the critical effect, noting that hepatic vacuolation appeared to be a high-dose effect in female rats only, was incompletely reported in the male rat, and had no human correlate. This reviewer suggested that these limitations should be noted in the Toxicological Review. One reviewer did not comment on this question because it was outside this reviewer's area of expertise.

Response: A discussion of biological relevance of hepatic vacuolation was added to the discussion of the selection of the critical effect in Section 5.2.1 (Choice of Principal Study and Critical Effect—with Rationale and Justification). These lesions were noted to be indicative of fatty changes in the liver by the study authors, and thus EPA considered this endpoint to be a precursor of toxicity. In addition, a discussion of the dose-response pattern seen in the Nitschke et al. (1988a) study, and the comparison between this pattern and the pattern seen in the higher dose study of Burek et al. (1984) (also in female Sprague-Dawley rats) was also added to Section 5.2.1. Although a linear dose-response is not seen across the experimental dose range (0–500 ppm) in Nitschke et al. (1988a) study, an increase is seen at the highest dose, consistent with the dose-related increase observed beginning at 500 ppm in the Burek et al. (1984) study. The lowest concentration in Burek et al. (1984), 500 ppm, was the LOAEL; this study does not inform the shape of the curve below this value. The incomplete reporting of the male response data (i.e., only the incidence rates in the controls and the highest exposure group were reported) in Nitschke et al. (1988a) is also noted in Section 5.2.3.

B7. BMD modeling was applied to the incidence data for hepatic vacuolation to derive the POD for the RfC. Has the BMD modeling been appropriately conducted and clearly described? Is the BMR selected for use in deriving the POD (i.e., a 10% increase in incidence of hepatic vacuolation) scientifically supported and clearly described?

Comments: Six reviewers generally agreed that the application of BMD analysis to derive the POD for the RfC was appropriate and clearly described; one of these reviewers noted that the

modeling was "reasonable, but conservative" (i.e., results in a RfD that is lower than necessary). One of these reviewers specifically noted that the BMR was scientifically supported, clearly described, and consistent with EPA guidelines. One reviewer did not comment on this question because it was outside this reviewer's area of expertise. Three reviewers reiterated comments that had also been offered in response to other charge questions. One of these reviewers commented that selection of a 10% BMR is not appropriate if the UF that would be applied to a NOAEL (i.e., $UF_L = 1$) is used. This reviewer indicated that a 5% BMR more closely corresponds to a NOAEL for this type of data (animal study and quantal response). One reviewer questioned the use of the first percentile human equivalent in the RfC derivation, and one reviewer questioned the use of a toxicokinetic scaling factor.

Response: A response that addresses the application of a LOAEL-to-NOAEL UF where BMD modeling was conducted is provided under RfC Charge Question B3.

The reasoning behind the use of the first percentile HED is discussed in response to comments under PBPK Modeling Charge Question A3a. The use of the toxicokinetic scaling factor is addressed in the response to comments under PBPK Modeling Charge Questions A1b and A2b and RfD Charge Question B4.

B8. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs scientifically supported and clearly described in the document? Please provide a detailed explanation. If changes to an UF are proposed, please identify and provide a rationale.

Comments: Reviewers generally agreed with application of the UFs for animal-to-human extrapolation (i.e., UF of 3 to account for the toxicodynamics portion of the interspecies UF) and for human variability in sensitivity (i.e., intraspecies UF of 3).

One reviewer offered a specific comment on the LOAEL-to-NOAEL UF, noting that use of an UF that would be applied with a NOAEL (i.e., UF of 1) is not appropriate with a BMR of 10%. One reviewer did not comment on this question because it was outside this reviewer's area of expertise.

Response: A response that addresses the application of a LOAEL-to-NOAEL UF where BMD modeling was conducted is provided under RfC Charge Question B3.

Comment: Two reviewers agreed with the selection of a database UF of 10. One reviewer did not think that a database UF of 10 was necessary because of the relatively large database for dichloromethane, and suggested that either a value of 3 or 1 would be more appropriate. Another reviewer stated that the database UF of 10 was not well justified, noting the relatively large

database for dichloromethane and that it is inconsistent to have different database UFs for different routes of exposure for a systemic toxicant such as dichloromethane. Another reviewer also disagreed with the database UF of 10 because, although neurodevelopmental effects have not been the focus of studies of dichloromethane exposure, other structurally related volatile organic compounds have not been shown to result in this type of effect. This reviewer also noted that maternal CO levels would not be raised at the proposed value of the RfC.

Response: EPA agreed with the recommendation to change the RfC database UF to 3, the same value as is used for the RfD database UF. The inhalation database for dichloromethane is large, but several concerns raised by specific studies support a database UF of 3. Specifically, the database UF of 3 for the RfC is applied because of concerns with respect to the limitations of the study protocol of the two-generation reproductive toxicity study, and concerns regarding the neurodevelopmental and immunotoxicity studies that are not adequately addressed by the available studies. As one reviewer suggested, EPA determined the levels of CO at the HEC used for deriving the RfC using the human PBPK model (David et al., 2006). The predicted levels of CO generated by the model were lower than levels at which neurodevelopmental toxicity has been observed. Therefore, EPA agrees that it is unlikely that the CO generated from dichloromethane metabolism would result in neurodevelopmental toxicity. However, this analysis does not account for potential concerns of neurodevelopmental toxicity associated with the parent compound or possibly other metabolites. Dichloromethane exposure is known to produce neurotoxicity in humans and adult animals (see Sections 4.1.2.2, 4.1.2.3, 4.1.2.5, and 4.4.2), and these effects cannot be explained solely by CO (Karlsson et al., 1987; Rosengren et al., 1986; Putz et al., 1979; Stewart et al., 1972a, b). The parent compound can pass through the placental barrier (Withey and Karpinski, 1985; Anders and Sunram, 1982). Data from Aranyi et al. (1986) demonstrated evidence of portal-of-entry (i.e., pulmonary) immunosuppression following a single 100 ppm dichloromethane exposure for 3 hours in CD-1 mice, an exposure level that is lower than the POD for the liver effects that serve as the critical effect for the RfC. Increased risk of pulmonary infectious diseases, particularly bronchitis-related mortality, is also suggested by some of the cohort studies of exposed workers (Radican et al., 2008; Gibbs et al., 1996; Lanes et al., 1993; Gibbs, 1992; Lanes et al., 1990), providing additional support for the potential importance of this localized immunosuppression. In consideration of these deficiencies in the database, a database UF of 3 is supported. The discussion of the database UF in Section 5.2.4 was revised to more clearly describe this rationale.

Comments: One reviewer repeated a suggestion (also made in response to RfD Charge Question B4) that an additional UF be included to account for uncertainties in dichloromethane metabolism.

Response: A response to this comment is provided under RfD Charge Question B4. Briefly, the interspecies scaling factor and the use of the first percentile internal HEC rather than the mean of the population distribution, as predicted by the human PBPK model, sufficiently account for toxicokinetic uncertainties, which include those from metabolism.

Comments**:** One reviewer reiterated points previously presented pertaining to the use of PBPK modeling to account for interspecies differences in toxicokinetics and a scaling factor to account for interspecies differences in clearance of metabolites, and questions concerning limitations of the dose metric that is available for use in the PBPK models (i.e., a metric based on the rate of metabolite production, rather than tissue concentration).

Response: Responses to these comments are provided under PBPK Charge Questions A1b and A2b and RfD Charge Question B4.

C. Carcinogenicity of Dichloromethane

C1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgrd.html), dichloromethane is likely to be carcinogenic to humans by all routes of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described?

Comments: Three reviewers indicated that the descriptor of dichloromethane as "likely to be carcinogenic to humans by all routes of exposure" was scientifically justified and clearly described; a fourth reviewer noted this was not a primary area of expertise but that the document provided a clear description of the information.

Three reviewers disagreed with the "likely" cancer weight-of-evidence categorization. One of these reviewers stated that the limited evidence of animal carcinogenicity and largely negative epidemiology data more appropriately supported a descriptor of "possible human carcinogen." The second reviewer stated that existing data better supports a descriptor of "suggested to be carcinogenic to humans" and the third reviewer considered dichloromethane "unlikely to be carcinogenic to humans via the oral exposure pathway." Reasons provided by these reviewers for considering that the available data did not support a descriptor of "likely to be carcinogenic to humans" included the following:

(1) Two reviewers questioned the relevance of liver tumors in male $B6C3F_1$ mice to humans, noting the high incidence of spontaneous liver tumors in male mice of this strain and the greater GST metabolic activity in the mouse that should result in much greater susceptibility of the mouse to dichloromethane-induced hepatocarcinogenicity than humans. One of these reviewers also commented that the higher alveolar ventilation rate, cardiac output, and dichloromethane blood:air partition coefficient in the mouse would

lead to a greater systemic absorption of inhaled dichloromethane, and thus higher internal doses, in mice compared with rats and in rats compared with humans.

(2) One reviewer noted that there is a greater prevalence of Clara cells, which contain relatively high levels of CYP2E1 and GST-T1, in the bronchioles of the mouse compared with rats and humans, and that this difference should be considered when evaluating the relevance of lung tumors seen in the mouse bioassay.

(3) One reviewer noted the negative results from the rat oral exposure study (Serota et al., 1986a), and one reviewer noted that "no evidence of carcinogenicity" was seen in two animal species (in oral and inhalation studies in rats, and in an inhalation study in hamsters). This reviewer stated that the findings that at least two species were negative for tumors is suggestive that the agent may not be a carcinogenic concern to humans, and that a classification of "suggested to be carcinogenic to humans" would be better supported by the data.

(4) One reviewer characterized EPA's analysis of the Serota et al. (1986b) drinking water study in the $B6C3F_1$ mouse as a "reanalysis" using a different statistical approach and control groups than had been used by Serota et al. (1986b), which leads to a small but statistically significant increase in liver tumors. This reviewer did not agree with EPA's approach, but instead agreed with the interpretation by the authors that dichloromethane was negative for carcinogenicity by the oral route of exposure. Another reviewer also noted that the dose-response pattern seen in mice was considered negative by Serota et al. (1986b), and that presence of statistically significant findings in "some, but not all of doses" is a weak finding. This reviewer also pointed to the similarity between the results in the exposed groups and data from historical controls as an additional factor that weakens the findings.

(5) Two reviewers characterized the epidemiological data as generally negative or indicated that more weight should be given to the epidemiology studies that "did not provide clear, statistically significant evidence of hepatic and/or lung tumors" in dichloromethane-exposed workers.

Response: Responses to the reviewers' who disagreed with the cancer descriptor of "likely to be carcinogenic to humans" follow. An analysis of the reviewers comments led to clarifications to Toxicological Review, and a summary of these points was added to Section 4.7.2. After evaluating the peer reviewers comments, the cancer weight-of-evidence descriptor for dichloromethane of "likely to be carcinogenic to humans" was retained based on evidence presented in Section 4.7.2.

(1) $B6C3F_1$ mice are relatively susceptible to liver tumors and mouse liver tumors can occur with a relatively high background. For these reasons, use of mouse liver tumor data in risk assessment has been a subject of controversy (King-Herbert and Thayer,

2006). In the absence of mode-of-action data or other information that establishes lack of human relevance, EPA considers mouse liver tumors (as well as other rodent tumors) to be relevant to humans. Interspecies differences in the metabolic activity of GST (which is relatively higher in the mouse compared with humans) do not indicate a lack of human relevance but rather species differences; however, the PBPK modeling accounts for interspecies differences in the amount of the relevant metabolite(s) formed (which is relatively higher in the mouse). A discussion of these issues related to mouse liver tumor relevance was expanded in the Toxicological Review Section 4.7.3.2 (General Conclusions About the Mode of Action for Tumors in Rodents and Relevance to Humans).

(2) The net difference in total lung CYP activity (versus liver) in humans and rats versus mice, which is at least partly due to differences in Clara cell prevalence, is accounted for by the PBPK model. The role of Clara cells per se in the development of mouse lung tumors is not known and reliably quantifying or predicting the micro-dosimetry in and around individual Clara cells is beyond the current state of knowledge. (At least one PBPK model exists, for styrene, that attempts to predict dosimetry in the Clara cell, but the data to validate such a model are not available.)

(3) The EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that negative data from two animal species can suggest a lack of carcinogenic concern for humans. This guidance document also notes, however, that the descriptor is based on the synthesis of all available data, that is data from positive and negative animal assays, mode of action information, and data from epidemiological studies. Thus, the results from the rat and hamster studies do not in themselves serve as a basis for a classification of "suggested to be carcinogenic to humans," as they must be viewed within the context of the other data, as described in Section 4.7 (Evaluation of Carcinogenicity). With respect to the interpretation of the rat data, although EPA agrees that the oral rat study (Serota et al., 1986a) is negative with respect to liver carcinogenicity, it is not clear that all of the rat data should be categorically described in this way. As noted by another reviewer, the potential relevance of the rat leukemia and mammary tumor data should also be considered.

(4) The analysis that was described by a reviewer as a EPA reanalysis was contained in the original full report of the 2-year mouse bioassay prepared by Hazleton Laboratories (1983). The paper based on these data, published by Serota et al. (1986b), inaccurately reported that the cut-point for statistical significance testing was 0.05, rather than the value of 0.0125 that was used. Although it is true that the increase in the liver tumor incidence in the 60 mg/kg-day group was not statistically significant, the presence of statistically significant increases in the 100-, 125-, and 250-mg/kg-day exposure groups does not suggest a random pattern of response. A plateau in the response at the highest

dose is seen, as was described by EPA, but this plateau does not negate the pattern across the dose groups. With respect to comparisons with historical controls, the incidence of liver tumors in the control groups (19%) was almost identical to the mean seen in the historical controls from this laboratory (17.8% based on 354 male $B6C3F_1$ mice). This comparison to historical control data provides no indication that the observed trend is being driven by an artificially low tumor rate in controls and no indication that the experimental conditions resulted in a systematic increase in the incidence of hepatocellular adenomas and carcinomas. For these reasons, EPA considers the characterization of the Serota et al. (1986b) results in Section 4.7.2 to be accurate and transparent.

(5) As indicated in the synthesis of the epidemiological studies of lung cancer in Section 4.1.3.2.3, EPA agrees that the available epidemiologic studies do not provide evidence for an association between dichloromethane and lung cancer, although it should be noted that this conclusion is based on a relatively limited database. EPA does not agree, however, with the characterization of the data from epidemiological studies pertaining to cancer and dichloromethane as being generally negative. The concerns raised by this collection of data, specifically with respect to the observations regarding liver and biliary tract cancer, brain cancer, and non-Hodgkin lymphoma, are summarized in Section 4.1.3.2. Although this collection of studies in itself does not establish dichloromethane as a human carcinogen, these studies do not provide support for the position that the cancer risk to humans from dichloromethane is negligible. There are several limitations in the available cohort studies that EPA considered in evaluating the point estimates and the lack of statistical significance of some of the results. These limitations, summarized in Section 4.1.3.2, include the low statistical power, particularly within the context of relatively rare cancers (including liver cancer and hematopoietic cancers), the lack of women and thus lack of data pertaining to breast cancer, the use of mortality rather than incidence data (of particular concern for cancers with a relatively high survival rate, such as non-Hodgkin lymphoma), missing job history data for a majority of cohort members in one study (Lanes et al., 1993), and the inability to create an inception cohort in another study (Gibbs et al., 1996).

Comment: One reviewer who supported the cancer classification of "likely to be carcinogenic to humans" recommended additional discussion of the range of sites observed in human studies (specifically, hematopoietic cancers). In particular, this reviewer noted the focus of the assessment on lung and liver tumors could be too narrow and has implications for comparisons of potency based on the animal data.

Response: Additional epidemiological studies of leukemia and lymphoma risk in workers exposed to dichloromethane were identified and added to Appendix D (Section D.5.6, Case-control studies of lymphoma, leukemia, and multiple myeloma), and the discussion of the NTP (1986) mononuclear cell leukemia results was expanded in Section 4.2.2.2.1 [Chronic inhalation exposure in F344/N rats (Mennear et al., 1988; NTP, 1986)]. In addition, as noted in response to PBPK Charge Question A3a, the issue of site concordance and implications with respect to toxicokinetic modeling and uncertainties were also added to Section 4.7.3.1 (Hypothesized Mode of Action) and Section 5.4.5 (Uncertainties in Cancer Risk Values), respectively.

C2. A mutagenic mode of carcinogenic action is proposed for dichloromethane. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for dichloromethane that may support an alternative mode of action.

Comments: Five reviewers agreed that a mutagenic mode of carcinogenic action is supported. One reviewer, whose area of expertise focuses on genotoxicity, commended the presentation of the series of tables of genotoxicity data and also suggested that a table(s) be added that would summarize, for a particular rodent species, target tissue, and exposure route, data outlining key events in the mode-of-action timeline to support analysis of temporality and dose-response concordance with respect to genotoxic and nongenotoxic modes of action. This reviewer described a framework for assessing the evidence pertaining to a mutagenic mode of action, with a hierarchy of types of evidence from (1) assays detecting primary DNA damage (e.g., DNA breakage, unscheduled DNA synthesis, sister chromatid exchanges); (2) assays detecting chromosomal breakage (e.g., chromosomal aberrations, micronuclei tests); and (3) gene mutation assays. This reviewer observed that the in vitro data are probably sufficient to conclude that dichloromethane is an in vitro mutagen, but there are no in vivo data in rodents to address whether dichloromethane can induce mutations either in target or nontarget tissues, and concluded that the data are therefore insufficient to prove that dichloromethane acts via a mutagenic mode of carcinogenic action. The same reviewer indicated that there is no evidence to strongly conclude that dichloromethane acts via a nonmutagenic mode of action and that one must therefore conclude that the mode of action for tumor induction is unknown. Another reviewer noted that the evidence pertaining to DNA damage comes from studies of very high exposures (inhalation or oral) to dichloromethane, and would be expected to be minimal or negligible at very low exposures or in species with low GST-T1 activity. This reviewer suggested that discussion of these points within the mode of action section would result in a more balanced presentation of the positive and negative findings, specifically with respect to the relevance of the mode of action to humans.

Response: EPA agrees with the majority of the reviewers who stated that a mutagenic mode of carcinogenic action is supported, and has followed the recommendations of the reviewers for improviing the summary and presentation of the evidence pertaining to this conclusion. To address the suggestions pertaining to additional tabular presentation of the data, four new tables were added to Section 4.7.3.1.1 (Experimental support for the hypothesized mode of action). Three of these new tables (Tables 4-35 to 4-37) present summaries of the relevant genotoxicity data by type of assay, species, and target organ, and the fourth new table (Table 4-38) presents a summary of the data discussing the strength of the evidence, the target-tissue specificity, dose-response concordance, and temporality. EPA did not apply this type of detailed summary to the nongenotoxicity data (Section 4.7.3.1.2) because these data are very limited and provide no indication that cell proliferation is a relevant mode of action with respect to dichloromethane and the observed liver and lung tumors in mice.

EPA also revised the discussion to better address the suggestion pertaining to the presentation of the framework used to evaluate all of the available evidence. The database for dichloromethane provides support for the mutagenicity of dichloromethane and the key role of GST metabolism and the formation of DNA-reactive GST-pathway metabolites along each of these lines: 1) in vivo evidence of chromosomal mutations (chromosomal aberrations and micronuclei) in the mouse lung and peripheral red blood cells (but not in the more bone marrow, a site that would be expected to be much more limited in terms of degree of dichloromethane metabolism; liver tissue, another site of tumor response, has not been examined in these assays 2) in vitro chromosomal instability evidence in human cells, other mammalian cells (i.e., CHO), and in bacterial systems; and 3) positive DNA damage indicator assays in numerous vivo and in vitro studies. EPA concluded that the overall weight of the evidence supported the conclusion that dichloromethane induces cancer by a mutagenic mode of action. This weight-of-evidence analysis includes explicit acknowledgement of the lack of in vivo demonstration of mutations in critical target genes for carcinogenesis (see Section 4.7.3.1.1). In addition, EPA concluded that the available studies provide sufficient data to indicate that the hypothesized mutagenic mode of action is relevant to humans. Section 4.7.3 (Hypothesized Mode of Action) was revised consistent with the reviewer's suggestions, specifically using the suggested framework for the evaluation (describing the types of evidence, and the relative strength of the different types in terms of the hierarchy of evidence), noting key limitations of the available evidence (e.g., a lack of studies demonstrating induction of specific mutations in vivo, inability to detect highly reactive DNA adducts in vivo, that much of the evidence comes from high-dose studies in mice, and the greater GST activity in mice compared with humans), and making a clearer distinction between measures of mutagenicity (chromosomal mutations) and measures of genotoxicity (indicator assays of DNA damage). The relative weighting of different types of evidence (i.e., the greater weight given to data pertaining to chromosomal instability compared with genotoxicity indicator assays of DNA damage) is also explicitly described in this revision.

Quantitative cancer assessment - oral exposure

C3. A 2-year drinking water study in mice (Serota et al., 1986b) was selected for the derivation of an oral slope factor (OSF) for dichloromethane. Please comment on whether the selection of this study for quantitation is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.
Comments: Four reviewers supported the use of the 2-year drinking water study in mice (Serota et al., 1986b) for quantitation, although one of these reviewers noted the marginal statistical significance of the tumor incidences. One reviewer considered the discussion pertaining to this choice to be clear, but that it was not within the reviewer's primary area of expertise. Two reviewers disagreed with the use of Serota et al. (1986b). One of these reviewers disagreed with the "reanalysis" of the mouse liver tumor findings by EPA, and supported the interpretation by Serota et al. (1986b), i.e., that dichloromethane was negative for carcinogenicity by the oral route of exposure. This reviewer, and one other reviewer, suggested that the chronic inhalation study by NTP be used as an alternative basis for the oral slope factor (through use of route-to-route extrapolation using PBPK modeling). One reviewer also suggested that the analysis of combined datasets from oral and inhalation routes, or analysis based on the arithmetic mean of slope factors for the two exposure routes, should also be considered.

Response: Comments on EPA's analysis of the Serota et al. (1986b) study are addressed under Carcinogenicity Charge Question C1. EPA presented a route-to-route extrapolation in Section 5.4.1.6 (Alternative Derivation Based on Route-to-Route Extrapolation). The rationale for selection of the oral slope factor based on the oral exposure study over the value derived by route-to-route extrapolation was clarified in Section 5.4.5 (Uncertainties in Cancer Risk Values).

C4. The OSF was calculated by linear extrapolation from the POD (lower 95% confidence limit on the dose associated with 10% extra risk for liver tumors in male mice). The OSF is based on an analysis of the most sensitive of the human subgroups, the GST-T1$^{+/+}$ genotype, using mean internal dose predictions for that subgroup. Please comment on whether this approach is scientifically supported and clearly described. Has the modeling been appropriately conducted and clearly described?

Comments: Three reviewers agreed with the extrapolation approach and oral slope factor calculation developed by EPA. Two reviewers disagreed with the use of linear extrapolation, indicating that this approach ignores repair systems and other biological processes that would make a threshold model more appropriate. One of the two reviewers further observed that the series of assumptions resulted in a cancer potency estimate "that is much more health protective

than necessary for dichloromethane." One reviewer did not comment on this question because it was outside this reviewer's area of expertise.

Response: Consideration of repair systems and other biological processes that occur at low doses would require a biologically-based (toxicodynamic) model that accounts for the biological processes involved in a response. However, sufficient data to develop a biologically-based toxicodynamic model for dichloromethane are not available. As noted in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), "In the absence of data supporting a biologically based model for extrapolation outside of the observed range, the choice of approach is based on the view of mode of action of the agent arrived at in the hazard assessment. If more than one approach (e.g., both a nonlinear and linear approach) are supported by the data, they should be used and presented to the decision maker." Dichloromethane is hypothesized to have a mutagenic mode of action for carcinogenicity, which is consistent with application of a linear extrapolation approach. Therefore, consistent with 2005 Cancer Guidelines and in the absence of data to support a toxicodynamic model, linear extrapolation was retained.

Comments: Four reviewers agreed with the use of the mean internal dose predictions based on the GST-T1$^{+/+}$ genotype, the group that is thought to be most sensitive to the carcinogenicity of dichloromethane (mediated through the GST metabolic pathway). One reviewer did not consider derivation of the oral slope factor for the most sensitive population to be clearly justified, indicating the need for clarification regarding the "realism and scientific validity of this approach in light of the use of a probabilistic PBPK model that already accounts for the population distribution of parameters of relevance."

Response: The probabilistic PBPK model allows the prediction of a distribution of internal doses in the U.S. population as a whole. To determine a cancer slope factor, one must select a point or percentile from within that overall distribution at which the cancer risk is to be assessed. In assessments without probabilistic models, one is effectively attempting to determine the overall average population cancer risk, assuming no additional knowledge to inform differentiation of risk among the population. This average population risk would correspond to the mean internal dose, or 50th percentile, of the model-predicted distribution for the population as a whole. Because there is information about subpopulation sensitivity for dichloromethane that is not typically available, the choice was made to estimate risk for the average (i.e., mean) of the GST-T1$^{+/+}$ subpopulation. This issue is also discussed under PBPK Modeling Charge Question A3a.

Comments: One reviewer noted that although the use of the GST-T1$^{+/+}$ genotype as the most sensitive group was justified, the use of the tissue-specific dose level in the liver (for GST-T1$^{+/+}$)

largely ignores the possibility of tumor sites other than liver, which could be proportionally more significant in the human than the mouse. This reviewer suggested that this issue be addressed in the discussion of uncertainties.

Response: As noted in response to PBPK Modeling Charge Question A3a and Carcinogenicity Charge Question C1, discussion of the issues of site concordance and GST activity in various tissues was added to Section 4.7.3.1 (Hypothesized Mode of Action) and Section 5.4.5 (Uncertainties in Cancer Risk Values).

Quantitative cancer assessment - inhalation exposure

C5. A 2-year cancer bioassay in mice (NTP, 1986) was selected for the derivation of an inhalation unit risk (IUR) for dichloromethane. Please comment on whether the selection of this study for quantitation is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

Comments: Six reviewers agreed with the use of the NTP (1986) 2-year inhalation cancer bioassay for the derivation of the inhalation unit risk. One of these reviewers, however, repeated concerns (also expressed in previous charge questions) regarding the relevance of mouse liver tumors to humans, the relatively high number of Clara cells in mouse bronchioles, and the relatively high GST activity in the mouse compared with humans. This reviewer also questioned the relevance of a high-exposure study such as that employed by the NTP (1986) to human exposures in environmental settings. One reviewer reiterated the recommendation that additional discussion of issues regarding interspecies extrapolation at sites other than liver and lung be addressed in the discussion of uncertainties. One reviewer did not comment on this question because it was outside this reviewer's area of expertise.

Response: The NTP (1986) bioassay was retained as the principal study for derivation of the inhalation unit risk. Responses to the previous issues raised by two reviewers are addressed under Carcinogenicity Charge Question 1. Use of high-exposure conditions in animal experiments is a standard and accepted practice in conducting toxicology studies. The doses used in the NTP (1986) mouse bioassay did not appear to exceed the maximum tolerated dose based on body weight gain and survival. For example, the mean body weight of high-dose males was comparable to the controls until week 90 of the study, and mean body weight of high-dose female mice was 0–9% lower than the control. Reduced survival of treated mice observed during the second year of the study was attributed by NTP to the high incidences of liver and lung neoplasia and not to the use of doses that were excessively high.

C6. The IUR was calculated by linear extrapolation from the POD (lower 95% confidence limit on the dose associated with 10% extra risk for lung or liver tumors in male mice) taking into consideration total cancer risk by determining the upper bound on the combined risk for male lung and liver tumors. The IUR is also based on the analysis of the most sensitive of the human subgroups, the GST-T1$^{+/+}$ genotype, using mean internal dose predictions for that subgroup. Please comment on whether this approach is scientifically supported and clearly described. Has the modeling been appropriately conducted and clearly described?

Comments: Four reviewers generally supported the extrapolation and modeling approach used by EPA, but three of these reviewers posed questions about the procedures used for combining risk across the two tumor sites (comments discussed in more detail below). One reviewer disagreed with the use of the linear extrapolation (as also noted in response to Carcinogenicity Charge Question C4).

Response: A response pertaining to the use of the linear extrapolation is provided under Carcinogenicity Charge Question C4.

Comments: Four reviewers agreed with the use of the mean internal dose predictions based on the GST-T1$^{+/+}$ genotype, the group that is thought to be most sensitive to the carcinogenic capacity of dichloromethane (mediated through the GST metabolic pathway). One reviewer repeated the recommendation offered in response to other charge questions that the uncertainties section should provide additional discussion of issues regarding interspecies extrapolation at sites other than liver and lung. Two reviewers did not comment on this question.

Response: The site concordance issue was addressed in response to PBPK Charge Question A3a and Carcinogenicity Charge Question C1.

Comment: One reviewer did not support the use of the sensitive population (as had also been expressed in response to Carcinogenicity Charge Question C4). This reviewer recommended instead that the mean of the entire population, using a modeling approach that specifically addressed sources of variability in the population, be used rather than the mean in the most sensitive group (the GST-T1$^{+/+}$ genotype). This reviewer also asked why the cancer potency derivations were based on the mean value of the sensitive population, and the noncancer reference values were based on the first percentile of the HEDs or HECs in the full population (all GST-T1 genotypes).

Response: By definition, the RfD and RfC should be protective of the entire population, including subpopulations; this point and the use of the first percentile specifically are discussed at greater length in the response to points under PBPK Charge Question A3a. Cancer risks, however, have historically been estimated based on an average population risk. As discussed in response to comments under Carcinogenicity Charge Question C4, in the case of dichloromethane, average cancer risk was estimated for a sensitive group based on genotype, a group that is estimated to represent approximately one-third of the entire population. Focus on this portion of the population, which would be considered a higher risk population, is warranted based on current understanding of genetic variation, metabolism, and mode of action.

Comments: One reviewer did not think the use of a risk estimation based on the combined risk of lung and liver tumors was justified. Two reviewers raised concerns regarding the assumption of normality of the underlying risk distributions of the liver and lung tumors. One of these reviewers noted that the procedure assumes a normal distribution of the BMD and assumes independence (i.e., no correlation) between the presence of lung and liver tumors. If these assumptions were not met, the 95% confidence limit calculated for the combined lung and liver tumors risk is approximate. This reviewer suggested an alternative approach, based on calculation of the incidence of lung and/or liver tumors in each individual mouse in the NTP (1986) study (using the individual mouse data in the appendices), and using BMD modeling of this combined risk. The other reviewer noted the potential for the lack of normality in the probability distributions to result in an underestimation of the resulting 95% upper confidence level on the summed risk. This reviewer provided a reference (Salmon and Roth, 2010) that discusses this issue in more detail.

Response: In situations in which tumors at multiple sites are seen in an animal bioassay, a unit risk estimate based on tumor incidence at only one of these sites will result in an underestimation of the total carcinogenic potency. Consistent with the recommendations of the NRC (1994) and with EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), a statistically appropriate upper bound on composite risk was estimated to gain understanding of composite risk across multiple tumor sites. This rationale is noted in the discussion of the combined risk (Section 5.4.2.5, Cancer Inhalation Unit Risk). EPA agrees that the procedure used is approximate, and EPA considered the suggestion of using a calculation based on number of tumor-bearing animals for an estimate of combined tumor risk. This type of calculation may not be appropriate, however, if as suggested in the NTP (1986) mouse data, independence between tumor sites is present (NRC, 1994; Bogen, 1990). EPA reviewed the paper by Salmon and Roth (2010), and found that it supports the procedure used by the EPA in the situation presented by the dichloromethane assessment. Specifically, the combined risk was generated from the first order models for male mice. As noted by Salmon and Roth (2010), "In simple cases where the

majority of the variation in the data is explained by a linear model, the resulting distribution shape is a single peak, although this may have a long tail at the high end of the estimate range depending on how much unexplained variation is present. Where the separate tumor incidences to be added together all fit this description, the procedure used by U.S. EPA (2002) of assuming a normal shape of the likelihood density function in order to predict a 95% confidence limit on the combined distribution will produce reasonable, if not exact, results."

Additional Reviewer Comments

Comment: One reviewer asked why the PBPK model development in humans included ages <18 years as a specific group for scaling V_{max}, given available data indicating that CYP2E1 expression increased only up to age 3 months, and given the larger liver size (normalized to BW) in young children.

Response: Use of the age group categorized as <18 years was chosen because this represents the age range during which growth occurs. While Johnsrud et al. (2003) showed that the activity per mg microsomal protein in infants (<3 months) was not significantly different from adult levels, assuming the mg protein/g liver is about constant, this means that the total CYP2E1 activity in a person will increase with age, as the size of the liver increases. Rather than assuming direct proportionality with liver weight or BW, a power-function was fit to the data of Johnsrud et al. (2003) and found that the relationship was more closely represented by direct proportionality (coefficient = 0.88) than the allometric coefficient that was otherwise used in the model by David et al. (2006) (coefficient = 0.7). The relationship used by David et al. (2006), which yields a higher activity per g liver in the child than the adult, overpredicted the data of Johnsrud et al. (2003). The equation was adjusted to match those data. Figure B-11 (upper panel) shows that liver size decreases with age up to 17 years, based on data. Lacking data on changes in the fraction of blood flow to the liver with age, the distribution function used to describe that fraction has no dependence on age of the individual.

Comments: One reviewer also raised the question of the reversibility of the neurological effects seen with dichloromethane, as well as other solvents, noting the relatively short recovery periods observed post-exposure in animal studies.

Response: Lash et al. (1991) is the only human study of dichloromethane that supports an examination of neurotoxicity endpoints following a period of no exposure to the chemical. Lash et al. (1991) examined the effects of dichloromethane among retired workers (mean length of retirement among the study participants was 5 years) and found decrements in attention and reaction time in complex tasks among the retired workers. Although limited by small sample

size and low statistical power of the study, the findings of Lash et al. (1991) suggest that neurological effects of dichloromethane may not be fully reversible. EPA has not identified other studies of dichloromethane or other chlorinated solvents (e.g., trichloroethylene and tetrachloroethylene) that were designed specifically to examine the long-term effects after cessation of a solvent exposure. EPA does not consider the available database adequate to support conclusions about the reversibility of neurologic effects of dichloromethane.

PUBLIC COMMENTS

Comments: A commenter supported the application of the human probabilistic PBPK model used in the assessment, including incorporation of variability in CYP2E1 activity and variation in GST-T1 activity that results from the different GST-T1 genotypes. This commenter also supported use of cancer potency values derived for the population presumed to have the greatest sensitivity to carcinogenic effects via the GST-mediated metabolic pathway, i.e., the GST-$T1^{+/+}$ genotype. This commenter raised several issues, some of which were also addressed in comments by the review panel, as delineated below:

(1) In situations where the dose metric is a rate of metabolism (production) rather than a tissue concentration, the commentator asked if a PBPK model could be used to provide the interspecies conversion instead of using the scaling factor.

Response: The rationale for the use of the scaling factor was discussed in response to PBPK Charge Questions A1b and A2b. It is because of the absence of experimentally-derived, species-specific data pertaining to the relationship between clearance of CYP metabolites in rodents and humans that the scaling factor based on $BW^{0.75}$ scaling is warranted. EPA agrees that if these types of data were available, it would be preferable to use them to evaluate and potentially modify or eliminate the scaling factor.

(2) A commenter questioned the use of liver effects in rats for the derivation of reference values, stating that these effects were of low toxicological concern and that evidence of liver effects in humans was lacking. The commenter suggested that COHb (resulting from CYP2E1 metabolism of dichloromethane) should be evaluated as a relevant health effect, using the standards for CO. The commenter also supported the use of the human neurotoxicological data from Cherry et al. (1983) and Lash et al. (1991) for derivation of the RfC, and suggested that a route-to-route extrapolation from these data be used for to derive RfD.

Response: As noted in response to RfC Charge Question B5, increased CO and COHb have implications for neurotoxicity as well as cardiovascular disease risk, and EPA agrees that the

potential for a significant health risk due to dichloromethane-generated CO should be considered. EPA's evaluation is described in response to Charge Question B4. EPA maintains, however, that the liver effects observed in the rat are of toxicological concern. Although the three epidemiological studies examining serologic measures of liver function (Soden, 1993; Kolodner et al., 1990; Ott et al., 1983a) do not provide clear evidence of hepatic damage in dichloromethane-exposed workers, these data are limited and the absence, presence, or extent of hepatic damage has not been established. EPA agrees that the Cherry et al. (1983) and Lash et al. (1991) neurological effects data are quite relevant, since these are studies examining effects in currently exposed workers and persistent effects in retired workers (mean 5 years post-retirement). The potential RfC derived based on these are slightly higher than the RfC derived from rat hepatic lesions (0.6 mg/m^3); these differences are not unexpected given the uncertainties in both the exposure measures and the effect size measures of the epidemiologic data.

As noted in response to Carcinogenicity Charge Question C3, EPA presented a route-to-route extrapolation in Section 5.4.1.6 (Alternative Derivation Based on Route-to-Route Extrapolation). The basis for selection of the oral slope factor based on the oral exposure study over the value derived by route-to-route extrapolation was clarified in Section 5.4.5 (Uncertainties in Cancer Risk Values).

Within that framework, EPA explored the suggested option of estimating an RfD using a route-to-route extrapolation from the Lash et al. (1991) data. This procedure required several steps and assumptions. To calculate an average internal dose for the exposed worker population, the dose metric used was AUC of the blood concentration of parent dichloromethane. Since the effects observed in Lash et al. (1991) were long-term, it was assumed that this average AUC was a better predictor of effect than peak concentration. It was also assumed that the workers were exposed 8 hours/day, 5 days/week, and the AUC was averaged over a full week. The distribution of ages over which the workers were exposed was estimated from the demographic details provided by Lash et al. (1991), and the internal dose for each sample individual was estimated at the mid-point of his exposure period. The average age of the simulated worker population at time-of-exposure was 50.3 years. All other model parameters were then sampled randomly from the distributions otherwise used for the PBPK model. The estimated mean blood dichloromethane AUC for the exposed workers was 11.1 mg-hour/L. The corresponding distribution of oral exposures for the population as a whole was then estimated, using the model distributions for the full U.S. population and the assumed oral ingestion pattern (total intake divided among six boluses over the course of the day), for a sample of 10,000 individuals. The mean, median, fifth, and first percentiles of that distribution were 12.4, 11.1, 5.7, and 4.3 mg/kg-day, respectively. Using the resulting first percentile exposure rate (4.3 mg/kg-day) allows the PBPK model to account for toxicokinetic variability and uncertainty. A total UF of 100 would then be applied: 3 for intrahuman toxicodynamic uncertainty; 10 for LOAEL to NOAEL extrapolation; and 3 for database uncertainty [as used for the RfD based on rat lesions as reported

by Serota et al. (1986a)]. The resulting alternative RfD of 0.04 mg/kg-day is approximately sevenfold higher than the RfD based on rat liver lesions (0.006 mg/kg-day). The primary uncertainty in this analysis is that it is based on effects seen an average of 5 years post-exposure, and so does not capture what is likely to be stronger effects seen at the time of exposure. Other uncertainties in this analysis are introduced by attempting to simulate the demographics of the exposed workers, the choice of exposure-period mid-point (versus age in last year of exposure, for example), and the choice of dose metric (dichloromethane AUC rather than metabolite production). The latter two uncertainties stem from lack of knowledge of the mechanism for the neurological effect. This analysis demonstrates that the RfD based on rat liver lesions should also be protective of potential neurological effects. Given the uncertainty in the level of exposure and degree of response evaluated by Lash et al. (1991), as well as the approximation of worker demographics, this analysis was not added to the Toxicological Review.

(3) A commenter questioned the approach to the derivation of the RfD and RfC, including the use of the $BMDL_{10}$ (rather than the BMD) in conjunction with the scaling factor to account for interspecies differences in metabolite clearance, use of the first percentile of the HED (or HEC), and use of UFs to account for interspecies and intraspecies toxicodynamic variability.

Response: The methodology used to derive the RfD and RfC is consistent with EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002). The scaling factor is used in place of the toxicokinetic component of the default UF for interspecies extrapolation because there is no PBPK submodel for the toxic metabolite(s) to otherwise extrapolate the metabolite dose-rate from rodents to humans. The first percentile of the distribution of human exposures (rather than an average human equivalent exposure) is used instead of the toxicokinetic component of the default UF for intraspecies variability. In the absence of chemical-specific information, it is appropriate to use UFs to account for interspecies and intraspecies toxicodynamic variability.

(4) A commenter provided a set of comments that had also been submitted as a public comment on the draft IRIS assessment of trichloroethylene relating to the use of the first percentile of the HED (and HEC) for the derivation of the RfD and RfC. The commenter stated that the allowance for inter-human toxicokinetic variability double counts and misconstrues the nature of the dose-response curve. In particular, the commenter stated if one assumes a tolerance distribution-based dose-response curve for quantal endpoints, an experimental animal dose corresponding to a low (e.g., 1%) response may reflect either toxicokinetic or toxicodynamic sensitivity, with no readily available way to split the components out. The commenter stated that the method employed in the assessment seems to implicitly assume that all of the variability in dose-response among rats is attributable to toxicodynamics and that all of the variation in

response at a given dose in humans is attributable to toxicokinetics. The commenter concluded that the net result is to yield an RfC that is overcorrected for human inter-individual variation to a degree that is not possible to know with the available analyses.

Response: The method employed assumes neither that all of the variability in dose-response among rats is due to toxicodynamics, nor that all of the variation among humans is due to toxicokinetics. EPA's approach uses the $BMDL_{10}$ determined from rat dose-response data (i.e., the lower confidence limit on a measure of dose or exposure expected to result in a 10% response in rats), not a 1% response level. As described in greater detail in EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000a), the $BMDL_{10}$ is used as a POD for reference value derivation in place of a NOAEL or LOAEL; hence, the same suite of UFs and potential replacements apply. In particular, these include an UF_A of 10 for animal-to-human extrapolation and an UF_H of 10 for variability among humans. The commenter is in part suggesting that some of the uncertainty and variability accounted for by these UFs is already accounted for by use of the BMDL instead of the BMD. This is not the case; however, use of the lower bound on the BMD accounts for uncertainty inherent in a given study (e.g., a smaller number of animals used in a given study results in a relatively lower BMDL than a study with a larger number of animals).

For the noncancer assessment, the probabilistic human PBPK model is assumed to explicitly account for **toxicokinetic** variability and uncertainty among the human population; distributions of HECs and HEDs are thereby calculated for humans. Use of the first percentile rather than the mean of a HEC or HED distribution replaces the toxicokinetic portion of the UF_H so this UF is reduced from 10 to 3, with the remaining UF of 3 accounting for toxicodynamic uncertainty. An alternative would be to use the mean HEC or HED from the distribution calculated and to then apply the full UF_H of 10; this alternative would result in lower RfD and RfC values than those derived in the current assessment, all other factors being kept the same. Thus, the total correction for interindividual human variation is less than would be obtained with a non-probabilistic PBPK analysis.

The comment that the RfC overcorrects for human interindividual variation assumes that the commenter knows that the variation is less than the correction actually used. While EPA recognizes that the probabilistic human PBPK model may overpredict the toxicokinetic variability to a small extent, given the extensive data incorporated into this model any such error is expected to be small. Since the actual extent of human **toxicodynamic** variability is unknown, it could be larger than the remaining UF_H of 3. The commenter's conclusion that the RfC is overcorrected is therefore unsupported by any data.

(5) A commenter stated that application of a database UF >1 was not justified because ATSDR did not identify developmental neurotoxicity as a priority data need for dichloromethane, and

because the Aranyi et al. (1986) data pertaining to lung resistance to infection and bactericidal activity should not be considered an effect because it was based on an acute exposure (3-hour, 100 ppm exposure) and the concurrent controls had a low mortality rate.

Response: In the 2000 ATSDR Toxicological Profile for Methylene Chloride, data needs are identified by category. The categories that were evaluated were: genotoxicity, reproductive toxicity, developmental toxicity, immunotoxicity, neurotoxicity, epidemiological, and human dosimetry studies. Specific data needs were identified by ATSDR (2000) for reproductive/ developmental, neurotoxicological, and immunotoxicological endpoints, which is in agreement with this Toxicological Review. Developmental neurotoxicity was not a category that was specifically evaluated in the ATSDR Toxicological Profile (2000).

The variability in mortality rates and bactericidal activity in the Aranyi et al. (1986) study was discussed in the Section 4.4.5 (Immunotoxicity Studies in Animals). A recent study by Selgrade and Gilmour (2010) used a similar method to Aranyi et al. (1986). Susceptibility of CD-1 mice to respiratory infection and mortality due to *Streptococcus zooepidemicus* exposure and the ability of pulmonary macrophages to clear bacterial infection were examined in mice exposed for 3 hours to trichloroethylene at concentrations of 5, 10, 25, 50, 100, or 200 ppm or chloroform at concentrations of 100, 500, 1000, or 2000 ppm. This study supports the methodology used by Aranyi et al. (1986), and suggests that these types of immunosuppressive endpoints may represent sensitive effects of chemical exposure.

(6) A commenter stated that EPA did not adequately value the contribution of cohort studies and also did not fully acknowledge limitations of case-control studies; in particular, possible dichloromethane exposure misclassification in the Heineman et al. (1994) study. A paper describing these issues by Dell et al. (1999)[9] was referenced by the commenter.

Response: EPA expanded a discussion of exposure assessment issues in the description of the Heineman et al. (1994) case-control study (Appendix D, Section D.5.1).

(7) A commenter noted that the genotoxic effects of dichloromethane are due to the GST-T1 metabolic pathway, and that these effects are not likely to be relevant to humans because of the higher activity of this pathway in mice compared to humans.

Response: As noted in response to Charge Question C1, interspecies differences in the metabolic activity of GST-T1 do not establish a lack of human relevance. The PBPK modeling accounts for interspecies differences in the amount of the relevant metabolite(s) formed.

[9] The commenter referred to "Dell et al. (2003)", but the correct reference for the paper is Dell et al. (1999).

(8) A commenter disagreed with EPA's interpretation of the Serota et al. (1986b) oral exposure bioassay data. The commenter did not believe that this study should be used to derive the oral slope factor, recommending instead route-to-route extrapolation of data from the NTP (1986) inhalation study.

Response: Please refer to response Carcinogenicity Charge Question C1 regarding EPA's evaluation of the Serota et al. (1986b) data, based on the full report of this study prepared by Hazleton Laboratories (1983).

APPENDIX B. HUMAN PBPK DICHLOROMETHANE MODEL

B.1. HUMAN MODEL DESCRIPTION

The basic model structure used by David et al. (2006) was that of Andersen et al. (1987) with the addition of the CO submodel of Andersen et al. (1991), refinements from the Marino et al. (2006) mouse model, and an inclusion of CYP metabolism in rapidly perfused tissue (see Figure B-1).

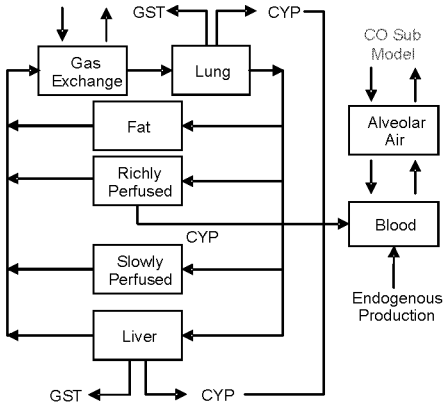


Figure B-1. Schematic of the David et al. (2006) PBPK model for dichloromethane in the human.

In order to incorporate known variability in human physiology and metabolism of dichloromethane into internal dosimetry, Monte Carlo analysis of the human model was performed to derive probability distributions of internal dose, as reported in David et al. (2006), but with changes in some of the key distributions as described below. The shape of the resulting dose distribution can be used to quantify the variability and uncertainty in internal dose with respect to variability in human physiology and variability and uncertainty in dichloromethane metabolism. The human model was run repeatedly using a random sample of each parameter from its respective parameter distribution in each iteration. Internal doses predicted for all iterations collectively defined a distribution for internal dose. The Monte Carlo analysis was run for 10,000 or 20,000 iterations. Repeated Monte Carlo analyses (at 10,000 iterations each) yielded 99th percentile values of internal dose in the liver or lung that differed by <2%. Normal or log-normal distributions of physiological and metabolic parameter and partition coefficient values were described by a mean, SD, and in most cases upper and lower truncation bounds. Physiological parameter and partition coefficient values were initially taken from the literature as described in David et al. (2006) and their distributions were assumed to be true variability (physiological parameters) or a level of uncertainty and variability (partition coefficients), neither of which could be meaningfully informed by the dichloromethane pharmacokinetic data.

Hence, the distributions for physiological parameters and partition coefficients were not updated in the Bayesian analysis of David et al. (2006).

The first-order oral absorption rate constant, k_a = 5.0/hour, was used in conjunction with human drinking water exposures simulated as six discrete drinking water episodes for specified times (25, 10, 25, 10, 25, and 5% of total daily intake at hours 0, 3, 5, 8, 11, and 15 of each day) (Reitz et al., 1997). Metabolic parameter distributions were derived from multiple human data sets by using MCMC calibration, also described in David et al. (2006).

The variability of genotypic expression of GST-T1 activity (the mechanism for GST-mediated metabolism of dichloromethane) was simulated as a uniform discrete distribution of the three GST-T1 genotypes (+/+, +/-, -/-) with varying activities in the liver and lung. The genotype frequency was based on data from Haber et al. (2002), with a frequency of genotypes of 32, 48, and 20% in the +/+, +/-, and -/- groups, respectively. GST activities measured by Warholm et al. (1994) for the three genotypes in a group of 208 healthy male and female subjects from Sweden were scaled by David et al. (2006) to obtain distributions of k_{fC} for each genotype that, when weighted by estimated frequencies of the genotypes in U.S. populations, would result in a distribution of k_{fC} activities with a mean equal to 0.852/hour-$kg^{0.3}$, which is the mean estimate of the population-mean value of k_{fC} obtained from the Bayesian analysis. The resulting distributions of internal lung and liver dose in human populations would have a theoretical probability of 20% for zero exposure to GST-mediated metabolites, and hence zero cancer risk for that 20% of the population. The final parameter distributions used by David et al. (2006) are summarized in Table B-1.

Table B-1. Parameter distributions used in human Monte Carlo analysis for dichloromethane by David et al. (2006)

Parameter		Distribution		Source
		Mean (arithmetic)	SD	
BW	Body weight (kg)	70.0	21.0	Humans[a]
QCC	Cardiac output (L/hr-kg$^{0.74}$)	16.5	1.49	Humans[a]
VPR	Ventilation:perfusion ratio	1.45	0.203	Humans[a]
QFC	Fat	0.05	0.0150	Humans[a]
QLC	Liver	0.26	0.0910	Humans[a]
QRC	Rapidly perfused tissues	0.50	0.10	Humans[a]
QSC	Slow perfused tissues	0.19	0.0285	Humans[a]
Tissue volumes (fraction BW)				
VFC	Fat	0.19	0.0570	Humans[a]
VLC	Liver	0.026	0.00130	Humans[a]
VLuC	Lung	0.0115	0.00161	Humans[a]
VRC	Rapidly perfused tissues	0.064	0.00640	Humans[a]
VSC	Slowly perfused tissues (muscle)	0.63	0.189	Humans[a]
Partition coefficients				
PB	Blood:air	9.7	0.970	Humans[b]
PF	Fat:blood	12.4	3.72	Rats[b]
PL	Liver:blood	1.46	0.292	Rats[b]
PLu	Lung:arterial blood	1.46	0.292	Rats[b]
PR	Rapidly perfused tissue:blood	1.46	0.292	Rats[b]
PS	Slowly perfused tissue (muscle:blood)	0.82	0.164	Rats[b]
Metabolism parameters				
V_{maxC}	Maximum metabolism rate (mg/hr-kg$^{0.7}$)	9.34	1.73	Calibration[c]
K_m	Affinity (mg/L)	0.433	0.146	Calibration[c]
A1	Ratio of lung V_{max} to liver V_{max}	0.000993	0.000396	Calibration[c]
A2	Ratio of lung KF to liver KF	0.0102	0.00739	Calibration[c]
FracR	Fractional CYP2E1 capacity in rapidly perfused tissue	0.0193	0.0152	Calibration[c]
First order metabolism rate (/hr-kg$^{0.3}$)				
k_{fC}	Homozygous (-/-)	0	0	Calibration[c]
	Heterozygous (+/-)	0.676	0.123	Calibration[c]
	Homozygous (+/+)	1.31	0.167	Calibration[c]
Oral absorption				
k_a	First-order oral absorption rate constant (/hr)	5.0		Reitz et al. (1997) (point estimate)

[a]U.S. EPA (2000d) human PBPK model used for vinyl chloride.
[b]Andersen et al. (1987). Blood:air partition measured by using human samples; other partition coefficients based on estimates from tissue measures in rats.
[c]Bayesian calibration based on five data sets (see text for description); posterior distributions presented in this table.

Source: David et al. (2006).

B.2. REVISIONS TO PARAMETER DISTRIBUTIONS OF DAVID ET AL. (2006)

An evaluation of the David et al. (2006) model and parameterization was undertaken, focusing on the adequacy of the characterization of parameter distributions in the full human population. EPA's conclusion is that the reported distributions for physiological parameters in particular, but also key metabolic parameters, only represented a narrow set of adults (with the exception of BW) or failed to include the parameter uncertainty from the Bayesian analysis. Therefore, supplemental data sources were chosen to define a number of the physiological parameter distributions in a way that should fully characterize the variability in the human population for individuals between 6 months and 80 years of age. Since many physiological parameters vary with age and gender, a structured approach will be used where an individual's age and then gender may be selected from the overall population distribution for these characteristics (the male:female ratio in the population declines with age, for example). Dosimetry simulations can then be run for each such individual to obtain an overall population distribution of internal doses. Thus, each dosimetry distribution represents a "snapshot" of the dosimetry in a given individual at a given age and age- and gender-appropriate sampled body composition (fraction of BW in each tissue group). Finally, the sampling for two key metabolic parameters representing metabolism by CYP2E1 (i.e., V_{maxC}) and GST-T1 (i.e., k_{fC}) was adjusted to explicitly account for both the interindividual variability and the parameter-value uncertainty among humans.

In estimating the human equivalent exposure levels for noncancer endpoints, some of which can be presumed to be relatively short-term effects possibly occurring from exposures of several weeks or months, using the distribution of such dosimetry "snapshots" should provide precisely the correct distribution to estimate overall population risk. For estimating cancer risk where risk is due to the cumulative exposure over months or years, however, the ideal approach would be to simulate the time-course of internal doses in a given individual tracked over a lifetime or significant portion thereof. But doing so would require estimating time-courses for each physiological and metabolic parameter in the individual over that time-period, a task that would be far more complicated than the structured "snapshot" approach used here. For example, while the CYP2E1 activity in an individual at age 12 is probably predictive of the activity in that individual at age 70 (e.g., someone who has above-average CYP2E1 activity when younger may well continue to be above average throughout his or her lifetime), we simply do not have the information or model structure to predict the time-dependences. Further, we know, for example, that some individuals who are lean in their youth may become obese by middle-age, while others (through lifestyle-changes) change in the opposite direction; and these changes may be reversed by the time the individual reaches 70 or 80 years of age.

Therefore a "life-course" dosimetry for specific individuals has not been calculated. For calculating HECs for noncancer effects, however, this means that the exposure level is identified

such that 99% of the population at a given time is predicted to have an internal dose at or below a POD (defined as an internal dose level). It then seems highly likely that if one were to track individual exposure over time, one would also find that this equivalent exposure keeps 99% of the population from exceeding the POD. In short, if 99% of individuals' snapshot-internal doses are below the POD (i.e., 99% of the internal doses in a cross-section of society on a given day), it can be anticipated that no more than 1% of all people will exceed that POD at any point in their life-times, even though the model simulations did not specifically track the changes in internal dose with age. For cancer risk, it can likewise be assumed that the average internal dose per unit exposure in the population as a whole (or the GST-T1 +/+ portion of the population) at a given point in time is a good estimate of the average one would estimate if the internal dose was tracked over the lifetime in the same population—where the distribution of physiological and metabolic characteristics at a given age is the same as used to estimate the average internal dose distribution for a cross-section of society.

B.3. CY2E1 AND GST-T1

This evaluation incorporated additional data concerning the variability in CYP2E1 activity among humans, based on Lipscomb et al. (2003). The Lipscomb et al. (2003) study was based on in vitro analysis of liver samples from 75 human tissue donors (activity towards trichloroethylene and measurements of protein content) to estimate a distribution of activity in the population. The distribution used by David et al. (2006) for hepatic CYP2E1 activity for dichloromethane (V_{maxC}) was a truncated log-normal distribution with GM = 9.34 mg/hour-kg$^{0.7}$, GSD = 1.14, lower bound = 6.33 (68% of mean), and upper bound = 13.8 (218% of mean). However, Lipscomb et al. (2003) (Table IV), analyzing data from a larger set of human tissue donors, derived an ultimate distribution for CYP2E1 activity with trichloroethylene (in units of pmol oxidized/minute/g liver) with GSD = 1.7274, 5th percentile = 40.7% of the mean, and 95th percentile = 245.8% of the mean. These data support a wider distribution in CYP2E1 activity than had been used in the David et al. (2006) model, with approximately a sixfold range between the upper and lower bounds in Lipscomb et al. (2003) and a twofold range in David et al. (2006). Since the distribution for V_{maxC} (CYP2E1) parameterized by the posterior-distribution parameters in Table 4 of David et al. (2006) represents the population mean and uncertainty in that mean, that uncertainty is not reduced or replaced by the knowledge of variability gained from the data of Lipscomb et al. (2003). Therefore, in EPA's Monte-Carlo simulations, a two-dimensional sampling process was used for V_{maxC}. First the population-mean value of V_{maxC}, $V_{maxC,mean}$, was sampled from the range of uncertainty represented by a log-normal distribution with GM = 9.34 mg/hour-kg$^{0.7}$ and GSD = 1.14 mg/hour-kg$^{0.7}$ [values converted from linear-space mean [SD] of 9.42 [1.23] mg/hour-kg$^{0.7}$ reported by David et al. (2006)] and upper/lower bounds of 7.20 and 12.11 mg/hour-kg$^{0.7}$, respectively (± 2 SD in log-space). (The linear space or arithmetic mean is shown in Table B-1.) After obtaining the sample population mean

($V_{maxC,mean}$), an individual V_{maxC} value was then obtained by sampling from the log-normal distribution with that mean but GSD = 1.73 as obtained from the data of Lipscomb et al. (2003). Further, since even the data available to Lipscomb et al. (2003) were limited, and the log-normal distribution is naturally bounded to be greater than zero, a nontruncated distribution was used for this second dimension (step) of parameter sampling.

For GST-T1-mediated metabolism characterized by the rate coefficient, k_{fC}, David et al. (2006) replaced their estimates of population mean, uncertainty, and variability (the latter is not reported by David et al. (2006) but would have been estimated along with the uncertainty as part of the Bayesian analysis) with a measure of population variability alone. The population variability was obtained by using the known distribution of GST-T1 genotypes in the U.S. population (from Haber et al., 2002) and the genotype-specific activity distributions from Warholm et al. (1994), scaled to have the same mean value as the overall mean estimate of the population mean obtained by David et al. (2006): 0.852 $kg^{0.3}$/hour. This treatment, however, fails to incorporate the uncertainty in the population mean characterized by the CV for k_{fC} in Table 4 of David et al. (2006), which is 0.711. Therefore, like V_{maxC}, EPA chose to use a two-dimensional sampling technique for k_{fC}. First, $k_{fC,mean}$ is sampled from a log-normal distribution with GM = 0.6944 $kg^{0.3}$/hour and GSD = 1.896 $kg^{0.3}$/hour (converted from the linear-space mean and CV of 0.852 $kg^{0.3}$/hour and 0.711, respectively) with upper/lower bounds of 0.193 $kg^{0.3}$/hour and 2.50 $kg^{0.3}$/hour, respectively (± 2 SD in log-space). After obtaining the sample population mean ($k_{fC,mean}$), an individual's genotype was sampled from the discrete incidence distribution [32% chance to be GST-T1 +/+, 48% chance to be +/-, and 20% chance to be -/-; Haber et al. (2002)]. Given those genotype frequencies, the interindividual variability was then characterized by rescaling the activity distributions from Warholm et al. (1994), but with the upper and lower bounds set to zero and mean + 5 SDs, respectively. David et al. (2006) also used zero for the lower bounds, but set upper bounds to mean + 3 SDs. However, in keeping with the decision to use an unbounded (log-normal) distribution for CYP2E1, the GST-T1 upper bounds were set at 5 SDs above the mean to assure characterization of the upper end of the human distribution. This rescaling and choice of bounds yields:

$$k_{fC} = \begin{cases} 0 \quad for\ GST\text{–}T1\ \text{–/–}, \\ k_{fC,mean} \times N(0.8929,\ 0.1622 \mid 0 \le x \le 1.704) \quad for\ GST\text{–}T1\ \text{+/–}, \\ k_{fC,mean} \times N(1.786,\ 0.2276 \mid 0 \le x \le 2.924) \quad for\ GST\text{–}T1\ \text{+/+}, \end{cases}$$

where $N(\mu, \sigma \mid LB \le x \le UB)$ is the truncated-normal distribution with mean = μ and SD = σ, bounded between LB and UB. To be clear, when the GST-T1 genotype is sampled as indicated above, the mean value for the tri-model distribution defined here for k_{fC} is $k_{fC,mean}$, the mean for the GST-T1 +/- subpopulation is one-half that for the GST-T1 +/+ subpopulation, and the CV for

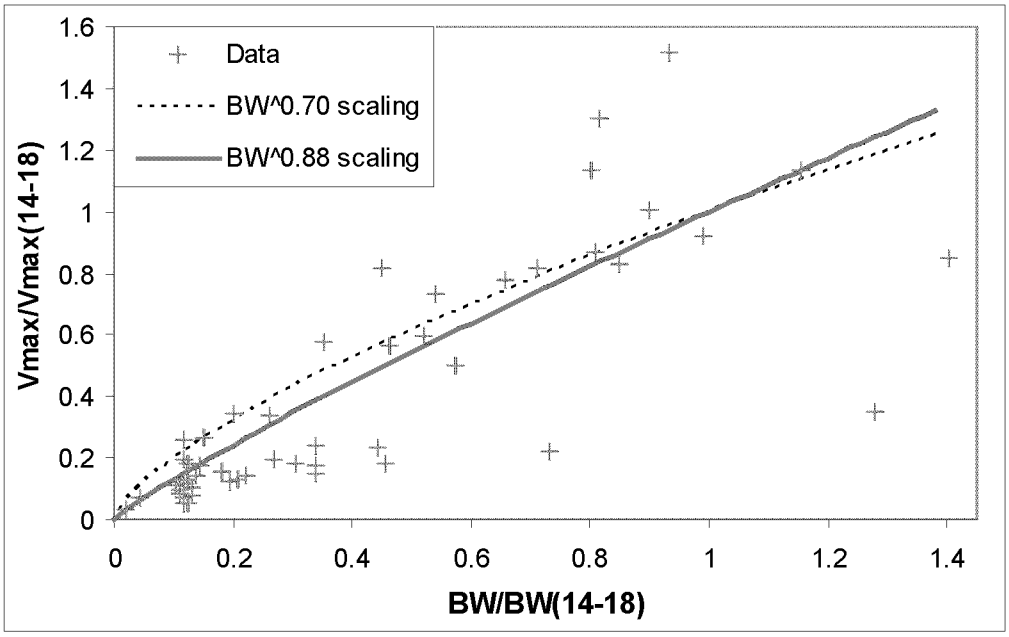
each subpopulation is the same as used by David et al. (2006) [from the results of Warholm et al. (1994)].

To assure that the age-dependence of CYP2E1 is properly characterized, particularly for children, the data of Johnsrud et al. (2003)[10] was analyzed. This study measures CYP2E1 activity and other parameters from individuals up to 18 years of age. For a significant subset of the individuals in that study, values were available for the liver CYP2E1 content (activity/mg microsomal protein), liver weight, and BW. For individuals 14–18 years old, there appeared to be no significant trend in the CYP2E1 activity, and the average BW was 69.6 kg, essentially identical to the value of 70 kg used as a representative adult. Therefore, the data from the 14- to 18-year-old individuals were assumed to represent adult values, and hence, an evaluation of the change versus adult could be made by normalizing to these data. In particular, assuming that the microsomal content per gram of liver is constant across the age-range considered here (6 months–18 years), the total activity for an individual relative to that in an adult could be estimated as:

$$V_{max}(\text{individual})/V_{max}(14\text{–}18) = \text{CYP2E1(individual)}\cdot\text{LW(individual)}/[\text{CYP2E1(14–18)}\cdot\text{LW(14–18)}]$$

where V_{max}(individual), CYP2E1(individual), and LW(individual) are the individual's total activity, activity (per mg microsomal protein), and liver weight, respectively, while V_{max}(14–18), CYP2E1(14–18), and LW(14–18) are the respective average values for individuals 14–18 years old. (If not normalized, V_{max} = CYP2E1·msp·LW, where msp is the microsomal content [mg/kg liver], but if msp is the same in all individuals, it drops from the equation when dividing by the 14–18-year-old average.) These normalized activities are plotted against the relative BW, BW(individual)/BW(14–18) in Figure B-2.

[10]Individual data supplied by the corresponding author D. Gail McCarver, to Paul Schlosser, U.S. EPA.



Source: Johnsrud et al. (2003).

Figure B-2. Total CYP2E1 activity (V_{max}) normalized to the average total activity in 14–18-year-old individuals (V_{max}[14–18]) plotted against normalized BW for individuals ranging from 6 months to 18 years of age.

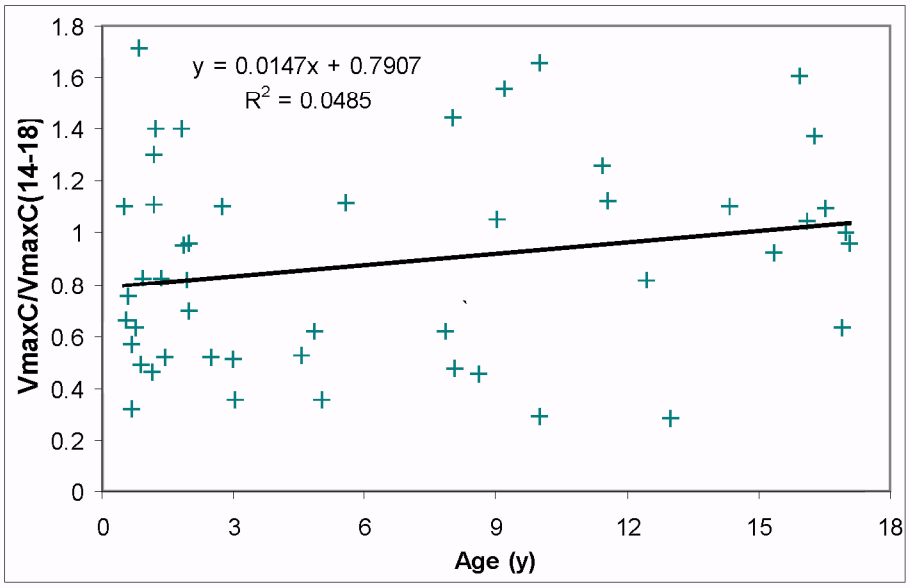
The data in Figure B-2 are compared to two model predictions: the allometric-based prediction used by David et al. (2006) that V_{max} will scale as $BW^{0.7}$, and an alternate scaling obtained by fitting to these data, $BW^{0.88}$. Both alternatives do a fairly good job of representing the average trend in the data, but the scaling by $BW^{0.7}$ tends to under-predict the data in the range of 0.4–0.8 for BW/BW(14–18). Therefore, this fitted coefficient will be used when estimating the total activity for individuals <18 years of age. However, since these data indicate a slower trend (rise in V_{max} with BW) for normalized BW ~0.7 and above, and there are no data to indicate that total activity continues to increase so rapidly in adults over 70 kg, the coefficient will be kept at 0.7 for individuals >18 years of age.

Using the allometric coefficient of 0.88, the normalized V_{maxC} values were computed in the same group of individuals as:

$$V_{maxC}(\text{individual})/V_{maxC}(14–18) = [V_{max}(\text{individual})/BW(\text{individual})^{0.88}]/[V_{max}(14–18)/BW(14–18)^{0.88}].$$

To test the revised allometric function versus the data, these values were then plotted against individual age, and a linear regression was performed, as shown in Figure B-3. While the slight trend in the regression indicates that not all of the age-dependence may be captured by this function, the low R^2 and small value of the slope indicate that the observation is not

statistically significant and that further attempts to explicitly account for age dependence would lead to minimal improvement. This representation of the data also clearly shows that the overall variability in the scaled activity (V_{maxC}) is fairly constant across ages: approximately sixfold, ranging from ~0.3 to 1.8. This is the same range observed in adults by Lipscomb et al. (2003), as noted above. Thus, these data support the use of a constant variability (GSD) in simulating population variability in CYP2E1 activity for children above 6 months of age, as well as adults.



Source: Johnsrud et al. (2003).

Figure B-3. Body-weight scaled CYP2E1 activity (V_{maxC}) normalized to the average scaled activity in 14–18-year-old individuals (V_{maxC}[14–18]) plotted against age individuals ranging from 6 months to 18 years of age.

Note that scaling CYP2E1 activity by $BW^{0.88}$ for children and by $BW^{0.7}$ for adults, rather than per liver weight (which is expected to scale as $BW^{1.0}$ with only a 5% CV in liver fraction), leads to a lower range in CYP2E1 than scaling by BW^{1} would indicate. In particular, the distribution predicted by the model for total CYP2E1 activity (mg/hour, upper/lower bound, given BW between 7 and 130 kg) will be about twofold less than if one assumed the activity varied as liver weight and simply multiplied the two sets of upper/lower bounds (BW and activity/g liver). However, given the database for this analysis from adults and children, the resulting distribution is expected to provide a good prediction of variability in the overall population.

Unfortunately, there is not a rich data set for the age-dependence of GST-T1 such as is available for CYP2E1. Strange et al. (1989) examined the developmental patterns for two other GST classes, mu and pi, and found that the child:adult activity for mu followed a similar pattern as CYP2E1, increasing from a low level near birth over time, but generally being higher than the

ratio for CYP2E1 in a given age-range. GST-pi, however, was expressed at 21 times adult values in children up to 1 year of age, then declined with age, so it is difficult to draw specific conclusions regarding age-dependent variation in GST-T1 from these data (i.e., a quantitative analysis of the activity data for other GST classes will not be used to estimate the variation in GST-T).

As is, the model uses a first-order rate constant for GST-T1 which is scaled as $BW^{-0.3}$. While the more recent "standard" for scaling of first-order constants is as $BW^{-0.25}$, the difference between these two is small and $BW^{-0.3}$ was used in the Bayesian model calibration. Because first-order constants are multiplied by tissue volumes in calculating total metabolism rates, and tissue volume scales approximately as BW^1, scaling the GST-T1 constant by $BW^{-0.3}$ is equivalent to scaling a V_{max} by $BW^{0.7}$. Even though the results cited above for GST-pi have the opposite trend, data on CYP2E1 activity discussed above and the trend in GST-mu activity are both at least qualitatively consistent with this scaling. Therefore, this scaling is assumed to appropriately account for age-dependent changes in GST-T1 activity for individuals over 6 months of age via the explicit dependence on BW.

B.4. ANALYSIS OF HUMAN PHYSIOLOGICAL DISTRIBUTIONS FOR PBPK MODELING

While the BW distribution in the David et al. (2006) PBPK model used ranges from 7 to 130 kg, thus covering 6-month-old children to obese adults, there are age-dependent changes and gender-dependent differences in ventilation rates and body fat that are not explicitly included. To more accurately reflect the distribution of physiological parameters in the entire population, the unstructured distributions of David et al. (2006) for certain primary or key parameters were replaced with distributions based on available information that specifically accounts for the population distributions of age and gender and the age- and gender-specific distributions or functions for BW, QCC, alveolar ventilation, body fat (fraction), and liver fraction. In the following, $v \sim U[0,1]$ indicates that v is a random sample from the uniform distribution from 0 to 1.

B.4.1. Age

U.S. Census Bureau statistics[11] for 6 months to 80 years of age were normalized (population for 6 months to 1 year assumed to be one-half of 0–1-year population), and the resulting quantiles were plotted against the corresponding ages with a polynomial function fit, as shown in Figure B-4. A sample individual's age can be determined by using the polynomial, given $v \sim U[0,1]$. (Alternately, age can be specified.)

[11]Available at http://factfinder.census.gov/servlet/DatasetMainPageServlet; use "enter a table number" or "list all tables" to select tables QT-P1 and QT-P2 for the (entire) United States.

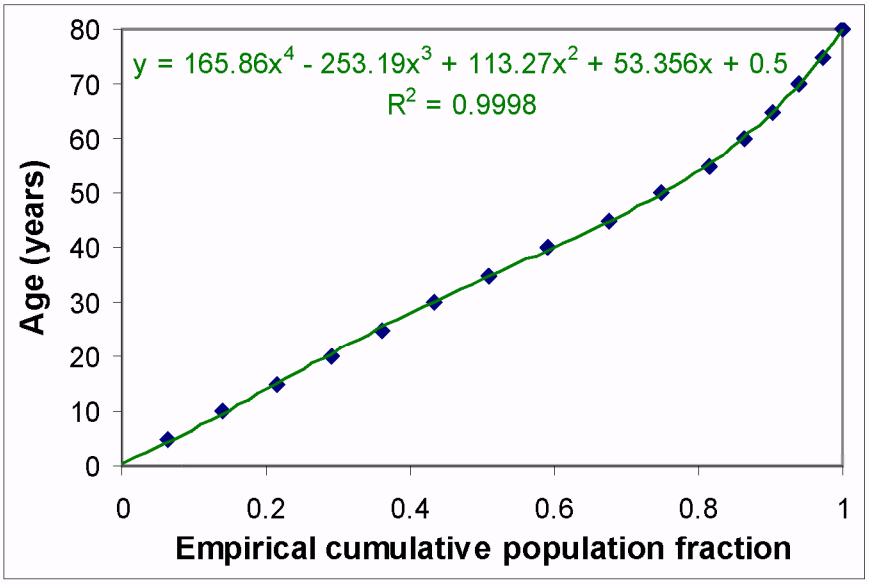


Figure B-4. U.S. age distribution, 6 months to 80 years (values from U.S. Census Bureau).

B.4.2. Gender

U.S. Census Bureau statistics for fraction of males versus age in 5-year intervals were plotted and an empirical function was fit, as shown in Figure B-5. Given the individual's age, the gender is randomly selected as male if v ~ U[0,1] is less than or equal to the polynomial and, otherwise female. (Alternately, it can be specified.)

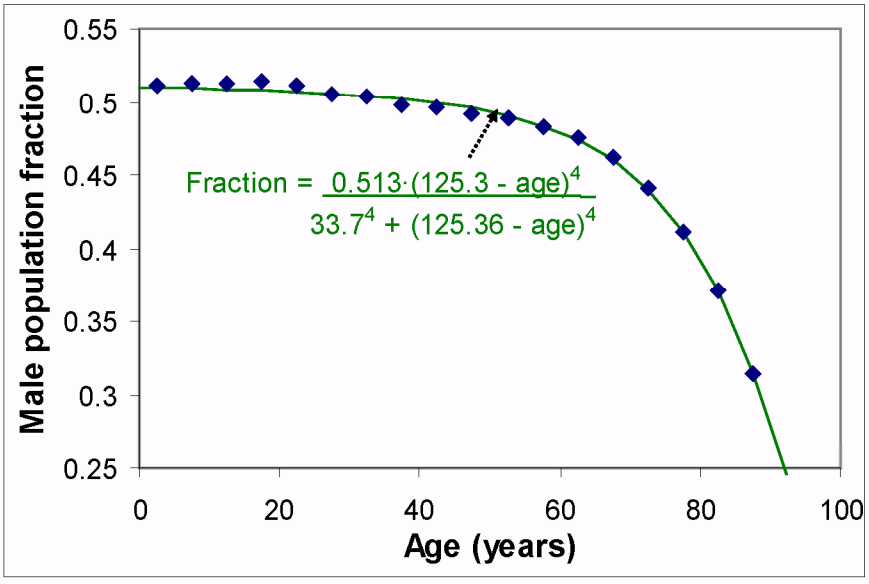


Figure B-5. U.S. age-specific gender distribution (values from U.S. Census Bureau).

B.4.3. BW

Portier et al. (2007) reported statistical mean and SD for BW as a function of age and gender based on the National Health and Nutrition Examination Survey (NHANES) IV. Portier et al. (2007) also described empirical functions fit to these results; however, those functions were found to be exquisitely sensitive to the fitted parameters to the point that entering the functions by using the four significant figures for parameters given by Portier et al. (2007) gave results that significantly deviated from the results as shown by the authors in plots at higher age ranges. Therefore, a somewhat different functional form was chosen. In particular for BW mean and SD for each gender, a function of the form was fitted:

$$\text{value} = \begin{cases} \exp[poly_1((age_c - age)/10)], & age \le age_c \\ \exp[poly_2((age_c - age)/10)], & age \ge age_c \end{cases}$$

where $poly_i(x) = a \cdot x + b_i \cdot x^2 + c_i \cdot x^3$, i = 1 and 2, age is in years, age_c is a "cut" age, dividing the early-age function from the later age function, and $poly_i$ (i = 1 or 2). Because there is no explicit constant term in $poly_i$ and the linear coefficient, a, is common to the two functions, the functions will automatically satisfy the condition of being equal and of having equal slope (first derivative) at $age = age_c$, so the overall function will be smooth and continuous.

The functions were fitted to the summarized data based on minimizing a weighted sum of square errors, error = $\sum n_{age} \times [\text{data(age)} - \text{function(age)}]^2$, where n_{age} was the number of observations for the age. The resulting parameter values are listed in Table B-2, and the curve fits are shown in Figure B-6. Given the age and gender from Sections B.4.1 and B.4.2, the BW is then randomly selected from a normal distribution, with the resulting mean and SD truncated at the 1st and 99th percentile.

Table B-2. Parameters for BW distributions as functions of age and gender

Parameter	Male BW (kg)		Female BW (kg)	
	Polynomial parameters for mean[a]	Polynomial parameters for SD[a]	Polynomial parameters for mean[a]	Polynomial parameters for SD[a]
age_c	21	16	16	13
A	4.406	2.87	4.146	2.574
b_1	-0.0285	0.06	-0.147	-0.358
c_1	-0.729	-2.56	-1.36	-2.55
b_2	0.115	0.96	0.44	1.16
b_2	0.0048	0.0448	-0.0278	-0.0861

[a]Mean or SD = $\begin{cases} \exp[poly_1((age_c - age)/10)], & age \le age_c \\ \exp[poly_2((age_c - age)/10)], & age \ge age_c \end{cases}$

where $poly_i(x) = a \cdot x + b_i \cdot x^2 + c_i \cdot x^3$, i = 1 and 2

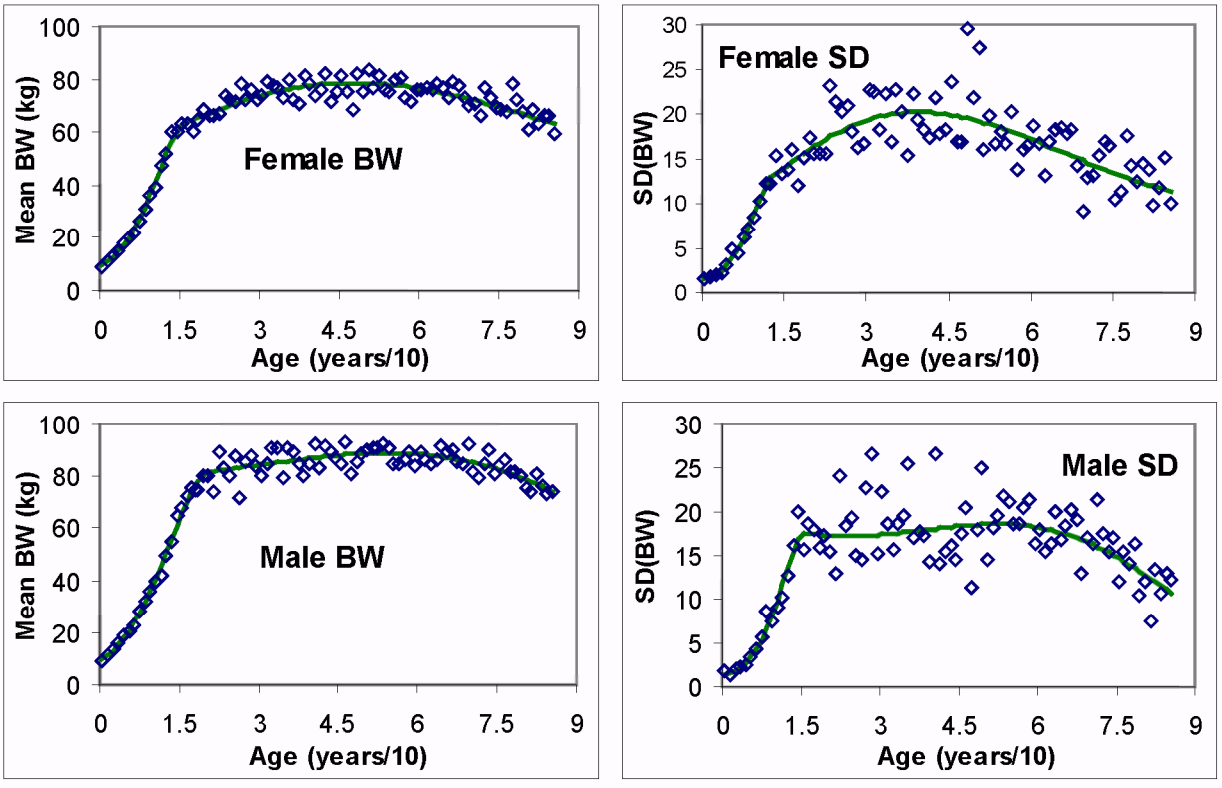


Figure B-6. Function fits to age-dependent data for BW mean and SDs for males and females in the United States [values from Portier et al. (2007)].

An example output BW distribution, from a Monte Carlo simulation for ages 0.5–80 years, both genders, is shown in Figure B-7. The range, 6.6–131.4 kg, is only slightly larger

than that set by David et al. (2006) (i.e., 7–130 kg). However, the bimodal form is an unexpected but reproducible result, presumably occurring because the fraction of a given life span spent at intermediate BW values is smaller, as evidenced by the most rapid growth rate occurring between ~7 and 18 years of age (Figure B-6).

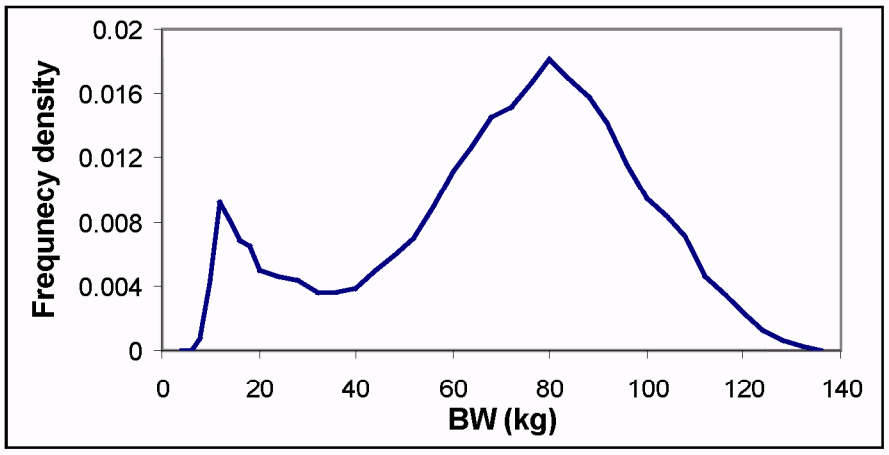


Figure B-7. Example BW histogram from Monte Carlo simulation for 0.5–80-year-old males and females in the United States (simulated n = 10,000).

B.4.4. Alveolar Ventilation

Clewell et al. (2004) tabulated values for the alveolar ventilation constant QAlvC (L/hour-kg$^{0.75}$) for males and females at different ages (QAlvC is multiplied by BW$^{0.75}$ to obtain the total rate). Smooth functions of age were fitted to those results to use as age- and gender-specific mean values, shown in Figure B-8. Arcus-Arth and Blaisdell (2007) reported GSD values for respiration rates for 0–18 years of age; a smooth function was fit to those results and the value at 18 years was assumed to apply for all adults greater than age 18 (Figure B-9). An individual's QAlvC was then selected from a log-normal distribution with the resulting mean and GSD (given age and gender) truncated between the 5th and 95th percentile.

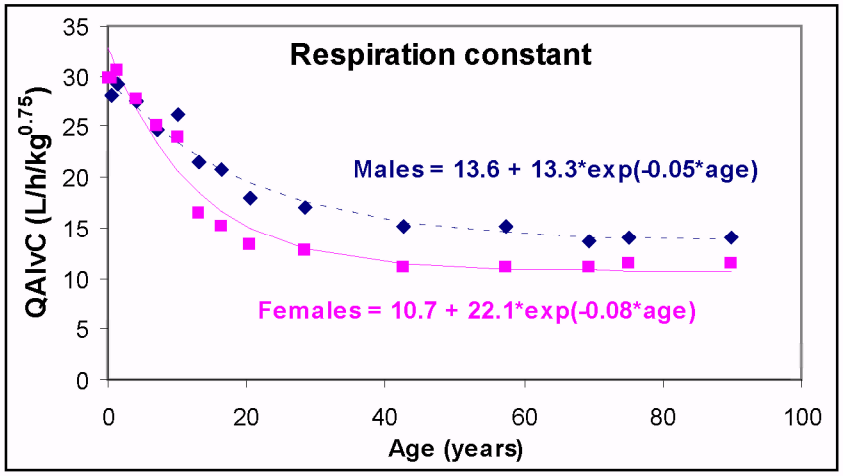


Figure B-8. Mean value respiration rates for males and females as a function of age [values from Clewell et al. (2004)].

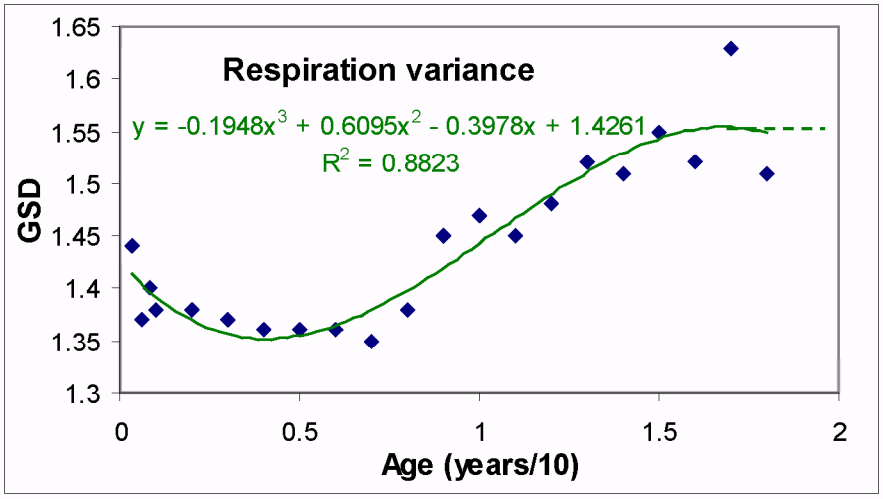


Figure B-9. GSDs for respiration rates for males and females as a function of age [values from Arcus-Arth and Blaisdell (2007)].

B.4.5. QCC

Clewell at al. (2004) provide a function that will be taken to represent the mean for the QCC, $QCC_{mean} = 56.906 \times (1.0 - e^{-0.681 \times \exp[0.0454 \times QAlvC]}) - 29.747$. However, using this function alone will tightly link cardiac flow and ventilation rates, rather than using a distribution in the VPR (VPR = QAlv/QC = QAlvC/QCC) as was done by David et al. (2006). Since a distribution is already defined for QAlvC, above, a VPR subject to variability will be estimated but renormalized to match the ratio of QAlvC and QCC_{mean} as defined above. In effect, this means $QCC_{sample} = QCC_{mean} \times VPR_{mean}/VPR_{sample}$ will be chosen, where VPR_{mean} and VPR_{sample} are the mean and a random sample from the distribution defined by David et al. (2006). Clewell et al.

(2004) suggest scaling QCC and alveolar ventilation as $BW^{0.75}$, while David et al. (2006) used $BW^{0.74}$. While virtually identical, it is noted that the implementation here uses $BW^{0.75}$.

B.4.6. Fat Fraction

Tabulated values from Clewell et al. (2004) show indistinguishable values for the fraction of BW as fat (VFC) for males and females up to 7 years of age, after which they diverge. Polynomial functions were fit separately for 0–7, 7–20, and 20–80 years, with the latter two ranges being gender-specific, as shown in Figure B-10. The ratio of the resulting gender- and age-specific value to the VFC_{mean} from David et al. (2006) was then used to scale the bounded normal distribution as specified by David et al. (2006) for the selected age and gender.

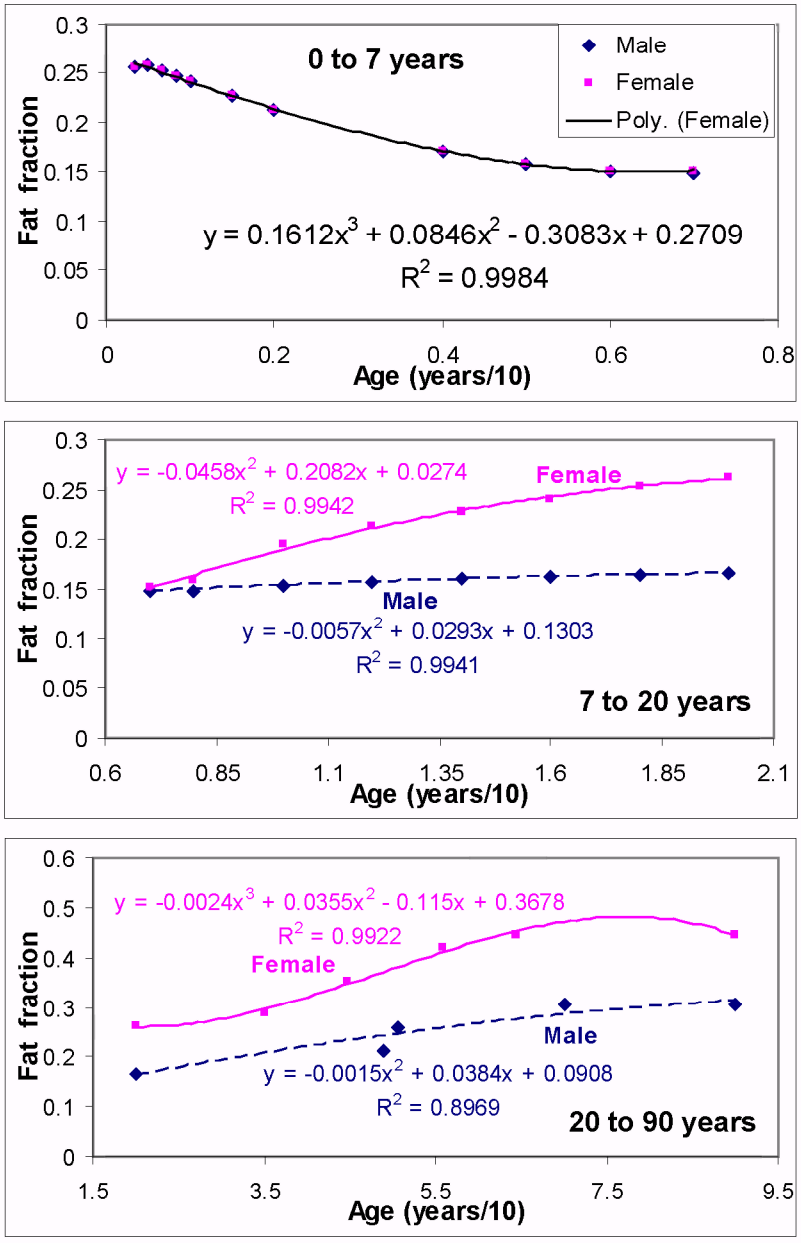


Figure B-10. Fraction body fat (VFC) over various age ranges in males and females [data from Clewell et al. (2004)].

B.4.7. Liver Fraction

Tabulated values from Clewell et al. (2004) showed an interesting age dependence for the liver fraction of BW, VLC, as shown in Figure B-11. While female VLC values were somewhat higher than males between 18 and 40 years, and the difference may be statistically significant, the overall variation is not large; the two were indistinguishable during other age ranges, and model predictions are not overly sensitive to VLC. Therefore, only the male data were used. Smooth functions were fit separately for 0–18 and 18–80 years and used to scale the distribution from David et al. (2006) for the given age, as was done for body fat.

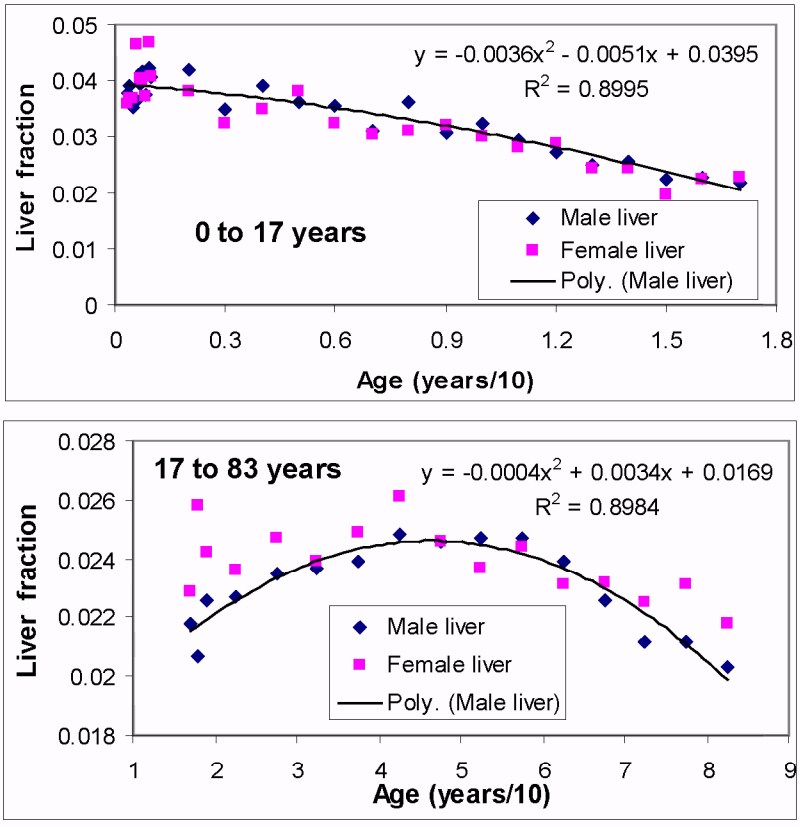


Figure B-11. Fraction liver (VLC) as a function of age [data from Clewell et al. (2004)].

B.4.8. Tissue Volume Normalization

While not explicitly stated by David et al. (2006), total tissue volume must remain roughly the same as a fraction of BW. While this fraction could also change with age, gender, and other characteristics, it was assumed that any change in it would be modest and would not significantly affect model predictions, given the fairly broad distribution implemented for total BW. This normalization was applied irrespective of those factors. Therefore, after drawing sample values of the tissue volume fractions from each of their respective distributions, the fractions were then normalized to a total fraction of 0.9215, which is the sum of the mean values for the fractions for the distributions as described by David et al. (2006). The remaining body mass is taken to be bone, teeth, hair, nails, and any other minimally or nonperfused components.

B.5. SUMMARY OF REVISED HUMAN PBPK MODEL

The resulting set of parameter distribution characteristics, including those used as defined by David et al. (2006), are described in Table B-3. The metabolic parameter statistics reported in Table 4 of David et al. (2006) are summary statistics of the converged parameter chains obtained in that analysis for the population mean of each parameter. As such, those statistics (means and

CVs) are assumed to represent the most likely value of the mean and the degree of uncertainty in that mean. However, for the metabolic parameters other than V_{maxC} (representing CYP2E1 activity) and k_{fC} (GST-T1 activity), EPA considers it reasonable to assume negligible variability among the population compared to the estimated uncertainties. So while EPA's objective is to account for both variability and parameter uncertainty in the population, the statistics for those other parameters (K_m, A1, A2, and FracR) were used as is to define population distributions. For V_{maxC} and k_{fC}, two-dimensional sampling routines were used, as described in detail above, to explicitly account for both uncertainty and the known high degree of interindividual variability. Distributions for a number of the physiological parameters, which are assumed to represent a well known degree of variability, were also revised from those used by David et al. (2006).

Table B-3. Parameter distributions for the human PBPK model for dichloromethane used by EPA

Parameter		Distribution					Section or source
		Shape	(Geometric) mean[a]	SD/GSD[a]	Lower bound	Upper bound	
BW	Body weight (kg)	Normal	*f (age, gender)*		*1st Percentile*	*99th Percentile*	B.4.3; NHANES IV
Flow rates							
QAlvC	Alveolar ventilation ($L/hr/kg^{0.75}$)	Normal	*f (age, gender)*	*f (age)*	*5th Percentile*	*95th Percentile*	B.4.4; mean: Clewell et al. (2004); SD: Arcus-Arth and Blaisdell (2007)
vprv	Variability in ventilation/perfusion ratio	Log-normal	1.00	0.203	0.69	1.42	VPR/VPR_{mean} of David et al. (2006)
QCC	Cardiac output ($L/hr/kg^{0.75}$)	$QCC_{mean} = f(QAlvC)$		$QCC = QCC_{mean}/vprv$			B.4.5; Clewell et al. (2004) (mean)
Fractional flow rates (fraction of cardiac output)							
QFC	Fat	Normal	0.05	0.0150	0.0050	0.0950	David et al. (2006); *after sampling from these distributions, normalize:* $Qi = \frac{QC \cdot QiC}{\sum QjC}$
QLC	Liver	Normal	0.26	0.0910	0.010	0.533	
QRC	Rapidly perfused tissues	Normal	0.50	0.10	0.20	0.80	
QSC	Slow perfused tissues	Normal	0.19	0.0285	0.105	0.276	
Tissue volumes (fraction BW)							
VFC	Fat	Normal	*f (age, gender)*	0.3 × mean	0.1 × mean	1.9 × mean	B.4.6; fat mean: Clewell et al. (2004); B.4.7; liver mean: Clewell et al. (2004); otherwise David et al. (2006); *after sampling from these distributions, normalize:* $Vi = \frac{0.9215 \cdot BW \cdot ViC}{\sum VjC}$
VLC	Liver	Normal	*f (age)*	0.05 × mean	0.85 × mean	1.15 × mean	
VLuC	Lung	Normal	0.0115	0.00161	0.00667	0.0163	
VRC	Rapidly perfused tissues	Normal	0.064	0.00640	0.0448	0.0832	
VSC	Slowly perfused tissues	Normal	0.63	0.189	0.431	0.829	
Partition coefficients							
PB	Blood/air	Log-normal	9.7	1.1	7.16	13.0	GM and GSD values listed here, converted from arithmetic mean and SD values of David et al. (2006)
PF	Fat/blood	Log-normal	11.9	1.34	4.92	28.7	
PL, PLu, and PR	Liver/blood, lung/arterial blood, and rapidly perfused tissue/blood	Log-normal	1.43	1.22	0.790	2.59	
PS	Slowly perfused tissue (muscle)/blood	Log-normal	0.80	1.22	0.444	1.46	

(Table B-3; page 1 of 2)

Table B-3. Parameter distributions for the human PBPK model for dichloromethane used by EPA

Parameter		Distribution					Section or source
		Shape	(Geometric) mean[a]	SD/GSD[a]	Lower bound	Upper bound	
Metabolism parameters (based on Monte Carte calibration from five human data sets)							
$V_{maxC,mean}$/ V_{maxC}	Population mean / individual maximum metabolism rate ($mg/hr/kg^{Xvmax}$)	Log-normal Log-normal	9.34 $V_{maxC,mean}$	1.14 1.73	7.20 (none)	12.11 (none)	B.3; mean: David et al. (2006); Individual GSD: Lipscomb et al. (2003); Xvmax = 0.88 for age <18. Xvmax = 0.70 for age ≥18.
K_m	Affinity (mg/L)	Log-normal	0.41	1.39	0.154	1.10	GM and GSD values listed here, converted from arithmetic mean and SD values of David et al. (2006)
A1	Ratio of lung V_{max} to liver V_{max}	Log-normal	0.00092	1.47	0.000291	0.00292	
A2	Ratio of lung KF to liver KF	Log-normal	0.0083	1.92	0.00116	0.0580	
FracR	Fractional MFO capacity in rapidly perfused tissue	Log-normal	0.0152	2.0	0.00190	0.122	
First order metabolism rate ($/hr/kg^{0.3}$)							
$k_{fC,mean}$	Population average	Log-normal	0.6944	1.896	0.1932	2.496	Adapted from David et al. (2006); k_{fCmean} is first sampled, then the relative individual value, k_{fC}/k_{fCmean}, given the genotype; k_{fC} is then the product.
$k_{fC}/k_{fC,mean}$	Homozygous (-/-)	N/A	$k_{fC} = 0$	–	–	–	
	Heterozygous (+/-)	Normal	0.8929	0.1622	0	1.704	
	Homozygous (+/+)	Normal	1.786	0.2276	0	2.924	

[a]Arithmetic mean and SD listed for normal distributions; GM and GSD listed for log-normal distributions.

The Monte-Carlo sampling approach used effectively assumes that all of the parameters are distributed independently, ignoring the covariance that was likely represented in the actual posterior chains. This approach will tend to overestimate the overall range of parametric variability and uncertainty and, hence, distribution of dose metrics in the population compared to what one would obtain if the covariance were explicitly included. Thus, if the covariance (i.e., the variance-covariance matrix) for the set of parameters had been reported by David et al. (2006), it could have been used to narrow the predicted distribution of internal doses or equivalent applied doses, but lacking such information, the approach used will not underestimate risk or overestimate lower bounds on human equivalent exposure levels. However, this source of overestimation in variability and uncertainty is probably offset to some extent by the fact that the analysis leaves out the degree of interindividual variability for K_m, A1, A2, and FracR that should have also been estimated by the Bayesian analysis.

APPENDIX C. RAT DICHLOROMETHANE PBPK MODELS

The critical studies and data chosen for derivation of a recommended RfD and RfC were based on nonneoplastic liver lesions in rats inhaling dichloromethane for 2 years (Nitschke et al., 1988a; Serota et al., 1986a). For this reason, a PBPK model for inhaled and orally absorbed dichloromethane in rats was needed to provide estimates of internal dosimetry for dose-response modeling and to extrapolate internal liver doses from rats to humans. Several deterministic PBPK rat models have been reported in the scientific literature (Sweeney et al., 2004; Andersen et al., 1991; Reitz, 1991; Reitz et al., 1989a; Reitz et al., 1988; U.S. EPA, 1988a; Andersen et al., 1987; U.S. EPA, 1987a, b; Gargas et al., 1986). Unlike in the mouse study (Marino et al., 2006), however, no probabilistic models are available in which the uncertainty in model parameters was reduced by utilizing multiple data sets for parameter estimation. Rat data were not available that would allow for MCMC calibration of individual metabolic parameters for the CYP or GST pathways. For example, the MCMC calibration of the mouse model (Marino et al., 2006) relied on inhalation data using trans-1,2-dichloroethylene, a CYP2E1 inhibitor, (not available for the rat) to specifically estimate GST metabolism in isolation of CYP metabolism, thereby improving the estimate of metabolic flux through the competing pathways. Thus, the selected model includes parameter values estimated by deterministic methods only. In order to use the latest data for dichloromethane toxicokinetics in rats, an assessment was conducted of multiple rat models (or modified versions of those models) to select the most appropriate model for use in the derivation of the RfD and RfC.

C.1. METHODS OF ANALYSIS

C.1.1. Selection of Evaluation Data Sets and PBPK Models

Published studies of dichloromethane metabolism and toxicokinetics in rats were reviewed to identify data sets for use in model evaluation and possible calibration. Toxicokinetic data were available for:

- blood levels of dichloromethane, the percent saturation of hemoglobin as COHb, and expired dichloromethane and CO following intravenous injection (Angelo et al., 1986b);
- dichloromethane air concentrations in closed chamber experiments (Gargas et al., 1986);
- dichloromethane and percent COHb blood levels during and after a 4-hour open chamber (constant concentration) inhalation exposure (Andersen et al., 1991; Andersen et al., 1987);

- Percent COHb levels from 30 minutes to 12 hours postexposure following a single oral dose of 526 mg/kg (6.2 mmol/kg in Oleum pedum tauri vehicle) (Pankow et al., 1991a); and

- Cumulative dichloromethane (mg) expired up to 96 hours following gavage doses of 250, 500, 1,000, or 2,000 mg/kg in corn oil or water (Kirschman et al., 1986).

Three variations on the PBPK models of Andersen et al. (1991; 1987) were assessed for the ability to predict these data. The model structure is depicted in Figure C-1. In each model, metabolism involves two competing pathways: the GST pathway, described with a linear first-order kinetic model, and the CYP pathway, described with a saturable Michaelis-Menten kinetic model.

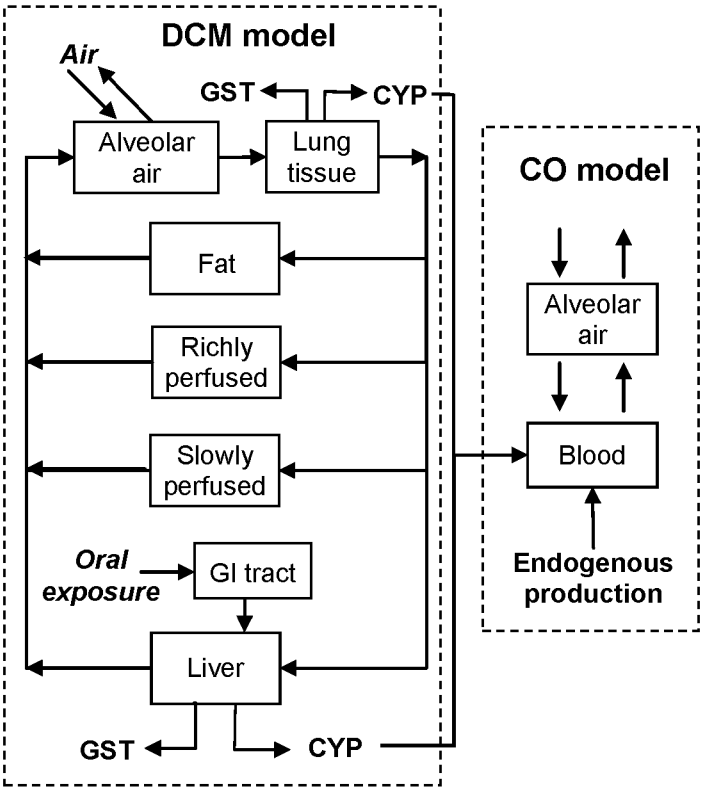


Figure C-1. Schematic of the PBPK model for dichloromethane in the rat.

Variation A is a hybrid of Andersen et al. (1991) and Andersen et al. (1987) in that it included both CO production resulting from CYP-mediated metabolism (Andersen et al., 1987) and lung metabolism of the parent compound (Andersen et al., 1991). Andersen et al. (1987) based the lung-to-liver metabolism ratios (A1 and A2 for CYP- and GST-mediated metabolism, respectively) on the reaction rates reported by Lorenz et al. (1984). Instead EPA used: (1) the reaction rates reported by Reitz et al. (1989a); (2) the levels of microsomal and non-microsomal

(cytosolic) protein reported by Litterst et al. (1975); and (3) the relative lung and liver tissue weights from Andersen et al. (1987).

Specifically for CYP activity, Reitz et al. (1989a) observed metabolic rates of 4.10 and 0.16 nmol/minute/mg microsomal protein with 5 mM dichloromethane using rat liver and lung preparations, respectively. Litterst et al. (1975) measured 0.027 and 0.0094 mg microsomal protein per kg rat liver and lung, respectively, and the fraction of body for rat liver and lung from Andersen et al. (1987) for a 0.3 kg rat is 4.0 and 1.16%, respectively; Andersen et al. (1987) used an allometric relationship for lung mass, $V_{Lung} = 0.0115*BW^{0.99}$, so the lung fraction varies with BW. Based on these values, 99.8 and 0.2% of total CYP activity is estimated to be in rat liver and lung, respectively. Therefore, the liver CYP allometric constant from Andersen et al. (1991), V_{maxC}, was changed from 4.0 to 3.992 mg/hour/$kg^{0.7}$ and A1 was set to 0.002. This value is significantly less than 0.0558, the value estimated and used by Andersen et al. (1987). The ratio of activity per mg microsomal protein in liver versus lung from Reitz et al. (1989a) is 0.039, fairly close to the value of A1 used by Andersen et al. (1987). This suggests that the discrepancy between EPA's value of A1 and that of Andersen et al. (1987) is that the previous value did not account for differences in microsomal protein content and tissue mass.

Similarly for GST activity, Reitz et al. (1989a) observed 7.05 and 1.0 nmol/minute/mg cytosolic protein for rat liver and lung preparations, respectively, at 40 mM dichloromethane. While Litterst et al. (1975) did not report the amounts of cytosolic protein per se in their preparations, they did report protein amounts in the supernatant from separation of microsomal protein that EPA used as effectively being cytosolic: 0.0531 and 0.0556 mg protein/kg tissue in liver and lung, respectively. Using the same liver and lung tissue fractions as for the CYP calculation, one can then estimate that 95.9% of GST activity is in the rat liver, and hence, the liver allometric constant for GST activity, k_{fC}, was changed from 2.0 to 1.917 $kg^{0.3}$/hour. Since the rate constant, $k_f = k_{fC}/BW^{0.3}$, is an activity per volume of tissue, the calculation of A2 (lung:liver activity ratio) is then calculated using the activity per mg cytosolic protein and amount of cytosolic protein per kg tissue to obtain the relative activity per kg tissue: A2 = 0.149. Using k_{fC} = 1.917 $kg^{0.3}$/hour and A2 = 0.149 yields the same total GST activity for a 0.3 kg rat as using k_{fC} = 2.0 $kg^{0.3}$/hour with no activity in the lung. This value of A2 is close to that used by Andersen et al. (1987), 0.136.

Simulations of the intravenous and inhalation data described above with Variation A were visually indistinguishable from simulations with the model set to exactly match the one used by Andersen et al. (1991); i.e., with V_{maxC} and k_{fC} set at the values specified in Table 1 of Andersen et al. (1991) and A1 = A2 = 0, there was no metabolism in the lung compartment (results not shown). Hence, Variation A was thereafter considered to be effectively identical to the original model of Andersen et al. (1991).

Variation B tests the hypothesis that, with the distribution of GST activity between the lung and liver, statistically significant improvements in the fit of the model to the data outlined

above can be achieved. Andersen et al. (1991) explicitly adjusted the three primary metabolic constants, V_{maxC}, K_m, and k_{fC} for CYP- and GST-mediated metabolism. EPA noted that while the text of Andersen et al. (1991) states that the constant for CO yield per unit of CYP metabolism, P1, was set at 70% [the value used by Gargas et al. (1986)], the value listed in Table 1 of Andersen et al. (1991) for the rat was P1 = 0.8 (i.e., 80%). The published figures of Andersen et al. (1991) could be reproduced with P1 = 0.8 but not with 0.7. Further, Gargas et al. (1986) indicated that the value they used, 0.7, was itself fit to data. Thus, it appears that both Gargas et al. (1986) and Andersen et al. (1991) fit P1 to their in vivo pharmacokinetic data, arriving at slightly different values; EPA also included this parameter among those to be numerically fitted.

Variation C: As will be shown, Variation B fits the considered data set significantly better than using the original parameter set (Variation A). In order to also described oral exposures, a gastrointestinal submodel was added comprised of an upper and a lower gastrointestinal compartment, with associated rate constants for transfer from upper to lower gastrointestinal tract (k_{12}) and absorption from the upper (k_a) and lower gastrointestinal tract (k_{a2}). This variation was created by fitting the parameters adjusted in (B) along with the gastrointestinal rate constants to the inhalation and intravenous data used previously along with oral dosimetry data, to obtain a global optimum for the adjusted parameters.

C.1.2. Analysis

While attempting to reproduce the fits to CO-inhalation kinetic data of Andersen et al. (1991), EPA found that the CO submodel constants were not entirely self-consistent. In particular, the background amount of total blood CO given for rats in Table 1 of Andersen et al. (1991) (AB_{COC} = 0.117 mg/kg) corresponds to a (background) blood COHb = 0.7% saturation [value given in text of Andersen et al. (1991)]. But the endogenous production rate constant (REN_{COC} = 0.035 mg/hour/kg$^{0.7}$) is insufficient to support that background level, even with their listed background air concentration (COINH = 2.2 ppm CO). Instead, simulations with this constant level of CO in the air and endogenous production lead to a predicted steady-state CO level of 0.5% rather than 0.7%. Also, some data sets proposed for simulation in this assessment had background COHb levels different from 0.7%. Therefore, in addition to the changes described above, algebraic equations were included in the code ("INITIAL" section in acslX) such that the endogenous CO production rate (REN_{CO}) and initial total CO mass in the blood (A_{BL20}) were then calculated to match specified time-zero percent COHb ($\%COHb_0$) and background CO air concentration (COINH), rather than using a fixed background CO production rate. These equations were subject to a conditional (if-then-else) statement that allows the user to either set the constants exactly as listed by Andersen et al. (1991) or use the new algebraic equations to set REN_{COC} and A_{BL20} to match a specified $\%COHb_0$ and make that level the steady-state COHb level in the absence of dichloromethane exposure. This last change did not

introduce any adjustable parameters, but rather made the choice of endogenous production level easier, with $\%COHb_0$ being the user-set variable rather than having to calibrate the background production rate to match the observed background percent COHb.

Analysis Details

Model variations were implemented using the acslX simulation software (version 3.0.1.6, The Aegis Technologies Group, Huntsville, Alabama). Parameter-estimation was performed using the Nelder-Mead algorithm as implemented in the acslX package. Initial values of V_{maxC}, k_{fC}, P1, A1, and A2 were those described above for Variation A, while that for K_m was the value used by Andersen et al. (1991) (0.4 mg/L).

In attempting to evaluate any differences between model simulations with Variation A compared to the original model of Andersen et al. (1991) (i.e., with no lung metabolism) two small but noticeable internal discrepancies were noted in Andersen et al. (1991) and handled as follows. First, while the model and parameters of Andersen et al. (1991) were nominally designed to include an endogenous background level of CO production and COHb = 0.7% saturation, simulations shown in Figures 3 and 4 of Andersen et al. (1991) appear to begin with COHb = 0% (and in Figure 4, approach 0% at the end of the observation period). If there is endogenous production and background CO exposure consistent with 0.7% COHb, this COHb level should be present at the start of any exogenous dichloromethane exposure and be asymptotically approached after an exposure ends. EPA's simulations were so conducted, including Variation A. Hence, the simulation results (plots) differ slightly from those in Andersen et al. (1991), the difference being noticeable near the start of exposure. While the difference may not appear large in the data plots, it could significantly impact the goodness-of-fit (likelihood) calculations. Since the background CO and COHb levels are set identically for both model variations, this choice should not unfairly bias the comparison between them.

The other slight discrepancy in Andersen et al. (1991) is that, while Table 1 specifies a rat BW = 0.22 kg and V_{maxC} = 4.0 mg/hour/$kg^{0.7}$, these values lead to V_{max} = 1.386 mg/hour. However, the legends of Figures 3 and 4 in Andersen et al. (1991) state that V_{max} was 1.46 mg/hour for those simulations. Since this V_{max} would occur for a 0.237 kg rat with V_{maxC} = 4.0, this discrepancy was resolved by using that BW instead of 0.22 kg, without adjusting V_{maxC}, for those particular simulations. Like the preceding choice, this input parameter specification was made uniformly for both model variations and may contribute to differences between EPA's Variation A simulations and Figures 3 and 4 of Andersen et al. (1991).

Variation B specifically involves comparing the goodness of fit of Variation A, in which metabolism is distributed between lung and liver but the effective parameters are otherwise identical to Andersen et al. (1991), to the goodness of fit obtained when V_{maxC}, K_m, k_{fC}, and P1 are fit to the data, with other parameters as specified in Table 1. For this test, goodness of fit is determined as the log-likelihood function (LLF) value reported by acslX. From a strictly

statistical viewpoint, Variation B involves using the same number of adjustable parameters as set by Andersen et al. (1991); hence, any improvement in fit could be considered significant. However, in deference to the precedent of a previously published scientific paper, EPA used the test described by Collins et al. (1999): 2 times the change in the LLF was compared to the value of the χ^2 distribution with four degrees (since four parameters are numerically fitted) at a *p*-value of 0.05.

Variation B was obtained by fitting first the closed-chamber gas uptake data of Gargas et al. (1986), the intravenous data of Angelo et al. (1986b), and the open-chamber (200 and 1,014 ppm) inhalation data of Andersen et al. (1987). Initial estimates of the gastrointestinal submodel parameters, k_a, k_{12}, and k_{a2}, were then obtained by keeping the metabolic parameters constant at Variation B values while calibrating the oral constants against the oral exposure data of Angelo et al. (1986b): blood and liver dichloromethane levels, expired dichloromethane levels (all time-points), and expired CO levels (24-hour cumulative). Only the 24-hour cumulative CO exhalation data were used to inform the estimate k_a (earlier time-points not used) for reasons explained below. Final parameter estimates were obtained by performing a global optimization in which all parameters were fit simultaneously to the combined intravenous, inhalation, and oral exposure data. Results of a 30-minute exposure to 5,159 ppm dichloromethane reported by Andersen et al. (1991) (data *not* used for EPA's model calibration) were also used to compare Variation A and C predictions. Two other data sets from gavage exposures (Pankow et al., 1991a; Kirschman et al., 1986) were also used in the evaluation of the model's ability to predict oral exposure data.

The statistical error model used by acslX also depends on a coefficient, the hetero-scedasticity, which allows for variation between an absolute error model (e.g., unrelated to the magnitude of the response variable) and a relative error model (e.g., experimental noise is assumed to be proportional to the response variable). The heteroscedasticity for each observed response was also fitted to the data (adjusted by acslX) along with any fitted metabolic parameters.

C.2. RESULTS

Parameter values for Variations A–C are listed in Table C-1. Two versions of the PBPK model for dichloromethane (Variations A and B) were first evaluated for goodness of fit to a common set of inhalation and intravenous pharmacokinetic data. The oral absorption and gastrointestinal transfer constants (k_a, k_{a2}, and k_{12}) were only calibrated as described above for the model that was deemed best with statistical justification: Variation B. The resulting metabolic parameters, Variation C, were almost identical to those obtained for Variation B and simulation results for the two were visually indistinguishable. Therefore, only results for Variations A and C are shown when comparing model predictions to data below. The endogenous level of COHb, $\%COHb_0$, was set to 0.7% and the background CO concentration set

to 2.2 ppm, as stated in the text of Andersen et al. (1991). However, for studies that used radiolabeled dichloromethane and only tracked the radiolabeled CO produced from that dichloromethane, the endogenous levels were not a factor and hence were set to zero.

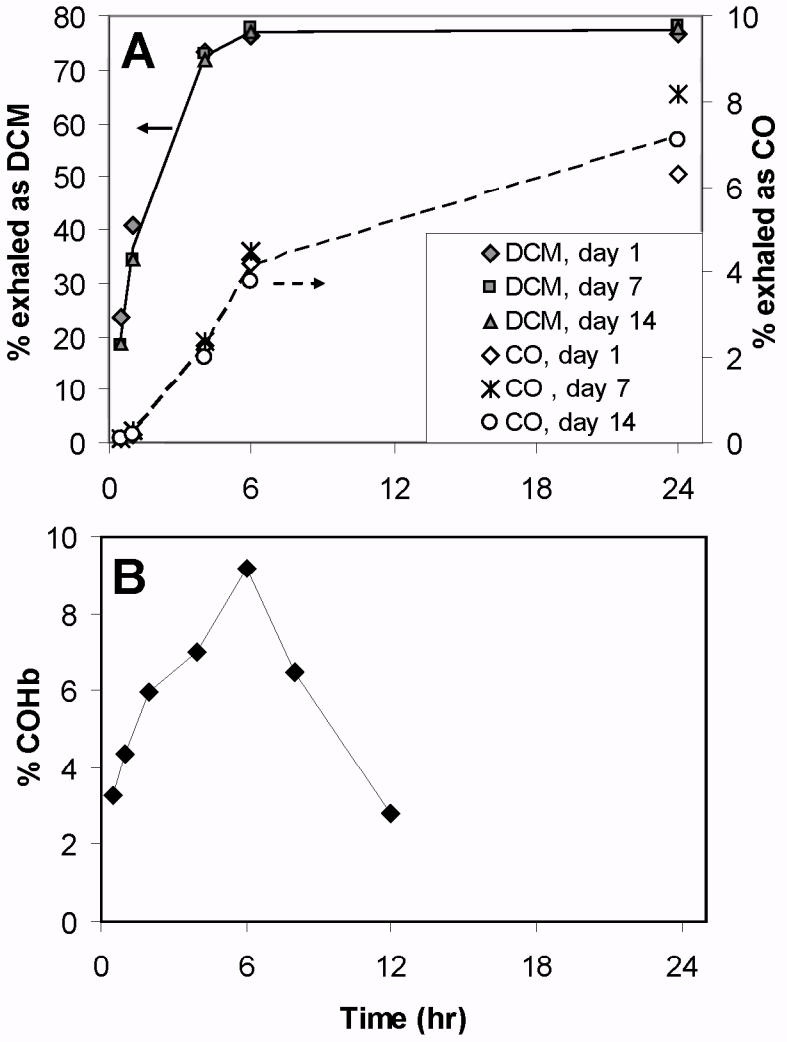
Table C-1. Parameter values used in rat PBPK models

Parameter	Variation A	Variation B	Variation C
Flow rates			
QCC (L/hr-kg$^{0.74}$)	15.9	Same as Variation A	
VPR	0.94		
Fractional flow rates (percent of QCC)			
Fat	9	Same as Variation A	
Liver	20		
Rapidly perfused tissues	56		
Slowly perfused tissues	15		
Tissue volumes (percent BW)			
Fat	7	Same as Variation A	
Liver	4		
Lung (scaled as BW$^{0.99}$)	1.15		
Rapidly perfused tissues	5		
Slowly perfused tissues	75		
Partition coefficients			
Blood:air	19.4	Same as Variation A	
Fat:blood	6.19		
Liver/blood	0.732		
Lung/arterial blood	0.46		
Rapidly perfused tissue/blood	0.732		
Slowly perfused tissue (muscle)/blood	0.408		
Metabolism and absorption parameters			
V_{maxC}, max CYP metabolic rate in liver (mg/hr-kg$^{0.7}$)	3.992	3.97	3.97
K_m, CYP affinity (mg/L)	0.4	0.509	0.510
k_fc, first order GST metabolic rate in liver (kg$^{0.3}$/hr)	1.917	2.46	2.47
P1, yield of CO from CYP metabolism (no units)	0.8	0.68	0.68
F1, correction factor for CO exhalation rate	1.21	1.21	1.21
A1, ratio of lung V_{max} to liver V_{max}	0.002	0.002	0.002
A2, ratio of lung k_f to liver k_f	0.149	0.149	0.149
k_a, oral absorption constant (1/h)	5.0	ND	4.31
LLF, log-likelihood for fit to data in Figures C-3–C-6	-657.9	-591.2[a]	-591.3[a,b]

[a]Difference from Variation A is statistically significant at $p < 0.001$, assuming a χ^2 distribution with four degrees of freedom.
[b]Difference from Variation B is not statistically significant, assuming a χ^2 distribution with four degrees of freedom.

C.2.1. Evaluation of Model Structure for Description of Carboxyhemoglobin Levels

The model of Andersen et al. (1991) (Variation A) calculated the amount of COHb by assuming instantaneous equilibration between the free CO and hemoglobin-bound CO in the blood. However, when Angelo et al. (1986b) observed the kinetics of dichloromethane in blood and exhaled dichloromethane and CO after oral administration, they found that blood dichloromethane peaked rapidly in <30 minutes. The rate of exhalation of dichloromethane, shown by the rate of rise in the exhaled dichloromethane data in Figure C-2A where the plateau in exhaled dichloromethane levels from 6 to 24 hours, indicates that blood dichloromethane levels had dropped to nearly 0 by 6 hours. Exhaled CO, however, shows a more gradual rise than dichloromethane, continuing between 6 and 24 hours. Pankow et al. (1991a) measured COHb in blood after dichloromethane exposure, albeit at a higher dose [526 mg/kg versus 200 mg/kg by Angelo et al. (1986b)], and found that blood COHb levels did not peak until 6 hours after dosing (Figure C-2B), while Angelo et al. (1986b) found that blood dichloromethane had declined to ~4% of the peak level (10-minute concentration) by 6 hours. Some delay between peak dichloromethane and peak (cumulative) CO levels is predicted by the published (Andersen et al., 1991) and modified (Variations A to C) Andersen et al. (1991) models because CO is produced from oxidative dichloromethane metabolism. However, EPA found that none of the PBPK models could simultaneously describe both the very short-time peak in blood dichloromethane with the concurrent rapid rise in exhaled dichloromethane levels (Figure C-2A) and the late peak blood CO (Figure C-2B) and slower rate of CO exhalation (Figure C-2A). The final models were able to adequately predict dichloromethane exhalation data and dichloromethane blood concentration data but overestimated CO exhalation data (these findings are illustrated for Variation C later in the results section).



A: Observations of exhaled [14C]-labeled dichloromethane (DCM) (left y-axis) and CO (right y-axis) after a bolus oral dose of 200 mg/kg [14C]-dichloromethane in rats (data of Angelo et al. [1986b]). Animals were either naive ("day 1") or previously exposed for 6 ("day 7") or 13 days ("day 14") at the same dose to detect possible effects of multi-day exposure on metabolism. Time (x-axis) is time from when the bolus is given on the day when the exhaled breath data were collected. (Lines are plotted through average of all three exposure groups. Arrows indicate corresponding y-axis.) B: Blood COHb (percent of total hemoglobin) from a single gavage dose of 526 mg/kg dichloromethane in rats [data of Pankow et al. (1991a)].

Figure C-2. Observations of exhaled [^{14}C]-labelled dichloromethane (DCM) CO, and blood COHb (percent of total hemoglobin) after a bolus oral or single gavage dose of dichloromethane.

EPA hypothesized that the models' inability to simultaneously fit these data might specifically be due to the assumption that CO only distributes into the blood, rather than other body tissues. Cho et al. (2001) measured CO transport in the perfused rat hindlimb and showed significant distribution into the tissue (compared to albumin or sucrose, for which distribution into tissue was minimal) and estimated a volume of distribution of 2.3 L/kg and permeability-area (PA) product of 4.86 L/(h-kg). Cho et al. (2001) also obtained a fractional recovery of only 45% of the infused CO after 10 minute of collection. Since modeling CO kinetics with a high degree of accuracy was not an objective of the current assessment, EPA conducted only a limited exploration of an alternate CO model that included a tissue compartment with transport between that compartment and the blood set using the permeability-area value of Cho et al. (2001). With this compartment included, the kinetics of CO and COHb were slowed significantly from those obtained with the Andersen et al. (1991) model structure, better matching the shape of the concentration-time toxicokinetic data (results not shown).

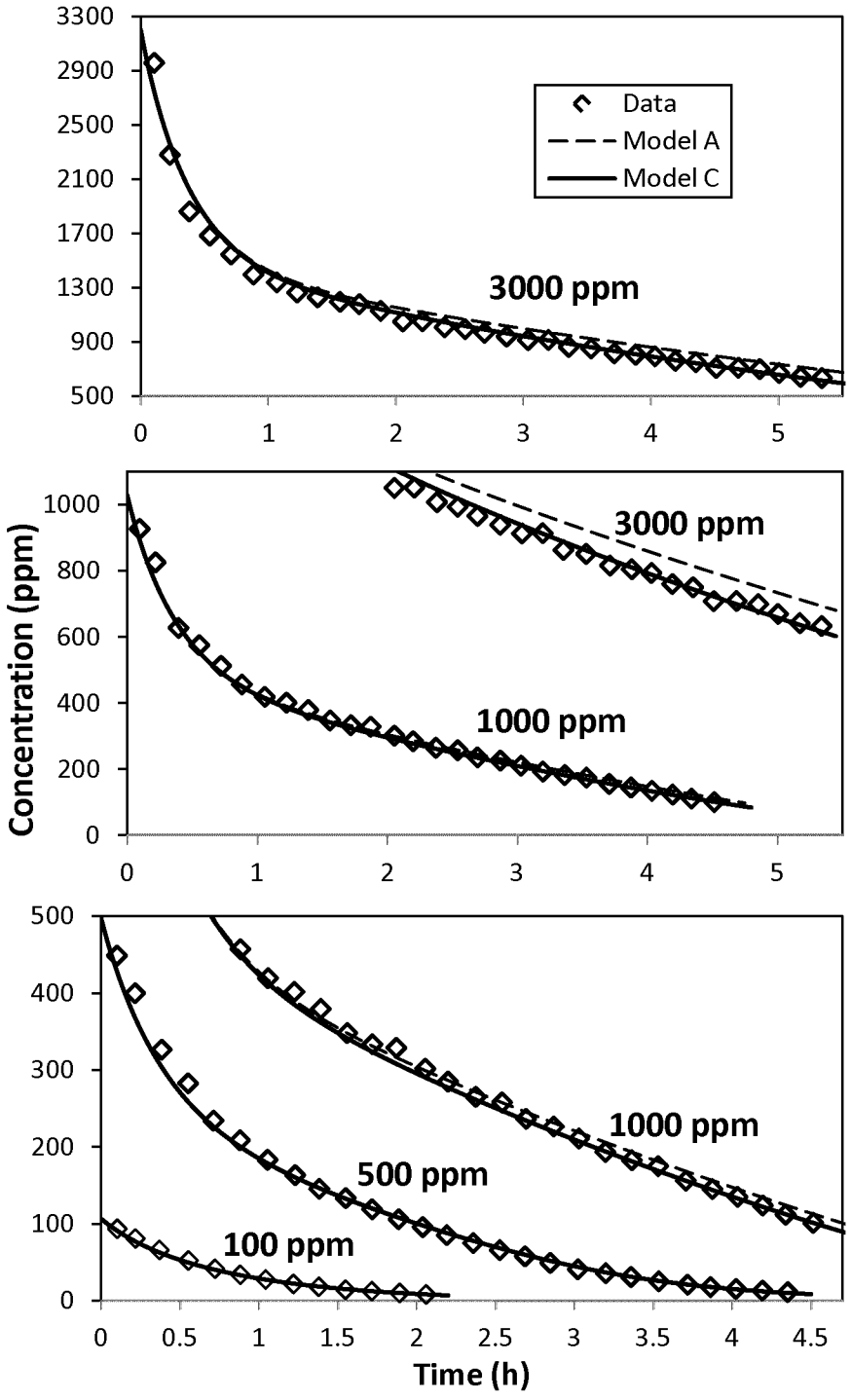
In addition to the lack of tissue-distribution, the Andersen et al. (1991) model structure (set of equations) characterizes the kinetics of free CO and COHb as being at instantaneous equilibrium with one another so that the kinetics of COHb are completely determined by the predicted rate of appearance and elimination of CO. The rate of production of CO is in turn determined by the rate of metabolism of dichloromethane and the rate of elimination of CO is determined by the rate of metabolism of dichloromethane and the CO-yield parameter, P1, while the rate of elimination of CO is determined by physiological parameters and adjustment constant, F1, that multiplies the CO exhalation equation. Andersen et al. (1991) used a 2-hour exposure to 500 ppm CO to estimate F1 and another CO-submodel parameter [results shown in Figure 2 of Andersen et al. (1991)]. EPA found that this fit to the CO-exposure data was highly sensitive to F1, and hence concluded that its value was well-characterized and should not be adjusted. Altering the rate of dichloromethane metabolism would degrade the fit to the dichloromethane data and the primary purpose of the model is to describe dichloromethane kinetics. Adjusting physiological parameters (e.g., cardiac output or respiration rate) in an attempt to fit the CO exhalation would only hide the error and reduce the model's reliability in predicting other aspects of dosimetry, particularly the pharmacokinetics of dichloromethane and its rates of metabolism, which are critical for risk assessment. The only remaining adjustable parameter was then P1 (CO yield from CYP metabolism of dichloromethane); while changing its value could improve the fit to the short-term CO data, it would simultaneously degrade the fit to the long-term data, or total measured CO yield. In summary, it appears that given the model structure and assumptions, one cannot explain the difference between the exhalation time-courses for dichloromethane and CO/COHb shown in Figure C-2 while retaining realistic physiological parameters and matching the measured total CO yield. As described above, a limited analysis indicated that including distribution of CO into body tissues in the model would at least partly resolve this apparent discrepancy (results not shown). Further improvement might be obtained

by also including the actual kinetics of hemoglobin-CO binding and disassociation (i.e., using kinetic constants determined in vitro).

While the analysis of the CO and COHb data above led to the conclusion that the purpose of the model for chronic-exposure risk assessment was best served by not using some of those data (i.e., the short-term dichloromethane and CO exhalation data), the combination of dichloromethane concentration data in various compartments as described below (primarily blood and air) and the more limited long-term CO exhalation data were considered adequate and necessary to estimate model parameters for dichloromethane. Kinetic COHb data were used for model calibration, however; since those were predicted well by the model, and comparisons of model predictions to the CO data are shown to further illustrate the discrepancy in kinetics and the agreement in long-term mass balance. If the model was to be used for evaluation of the risk of acute CO exposure, then it would be necessary to demonstrate adequate reproduction of the COHb data at all times.

C.2.2. Evaluation of Prediction of Uptake, Blood and Liver Concentrations, and Expiration of Dichloromethane

One data set used for model calibration is the closed chamber experiments of Gargas et al. (1986) (100–3,000 ppm dichloromethane). Because actual chamber concentrations can differ from target concentrations, a simple exponential function was fit to the first four data points at each concentration and used to extrapolate back to an actual initial concentration. The initial concentrations so obtained and used in the modeling were 107, 498, 1,028, and 3,206 ppm versus the target concentrations of 100, 500, 1,000, and 3,000 ppm. Predictions using Variations A and C both fit the observed chamber air concentrations reasonably well (Figure C-3), but Variation C fit the 3000 ppm data more exactly from 2 hours on (Figure C-3, middle panel). The difference between Variations A and C is diminished at 1,000 ppm (Figure C-3, middle and bottom panels), but where a difference can be seen (Figure C-3, bottom pane, 2.5 hours and beyond), Variation C again fits the data more exactly. Predictions with the two models at 500 and 100 ppm were essentially identical.



Target initial concentrations were 100, 500, 1,000, and 3,000 ppm. A simple exponential curve was fit to the first four data points of each data set to identify initial conditions for each simulation.

Figure C-3. Observations of Gargas et al. (1986) (data points) and predictions for Variations A and C (curves) for respiratory uptake by three rats of 100–3,000 ppm dichloromethane in a 9-L closed chamber.

Model simulations were next compared to measurements of blood dichloromethane concentration and the fraction (percent) of dichloromethane exhaled after intravenous injections of 10 or 50 mg/kg (Angelo et al., 1986b) (Figure C-4). Intravenous data are considered particularly useful for evaluating metabolic rate constants because the kinetics and complexity of uptake by other routes of exposure have been bypassed. Blood concentration simulations using the two models were almost identical (Figure C-4, upper panel) and match the observed blood dichloromethane levels fairly well: simulations are within a factor of 3 of the data (often closer) and the slope of the model curves after the initial distribution phase (~5 minutes) are close to that of the data. However, both models overpredicted the observed data to some extent, particularly the 50 mg/kg dose, for which all of the observed data points are overpredicted, whereas the 10 mg/kg simulations match the first three data points well. Because the upper panel of Figure C-4 is plotted on a log-y scale, the discrepancy between model simulations and the 50 mg/kg data appears similar to the discrepancy versus the 10 mg/kg data at later time points, but in fact is much larger in an absolute sense.

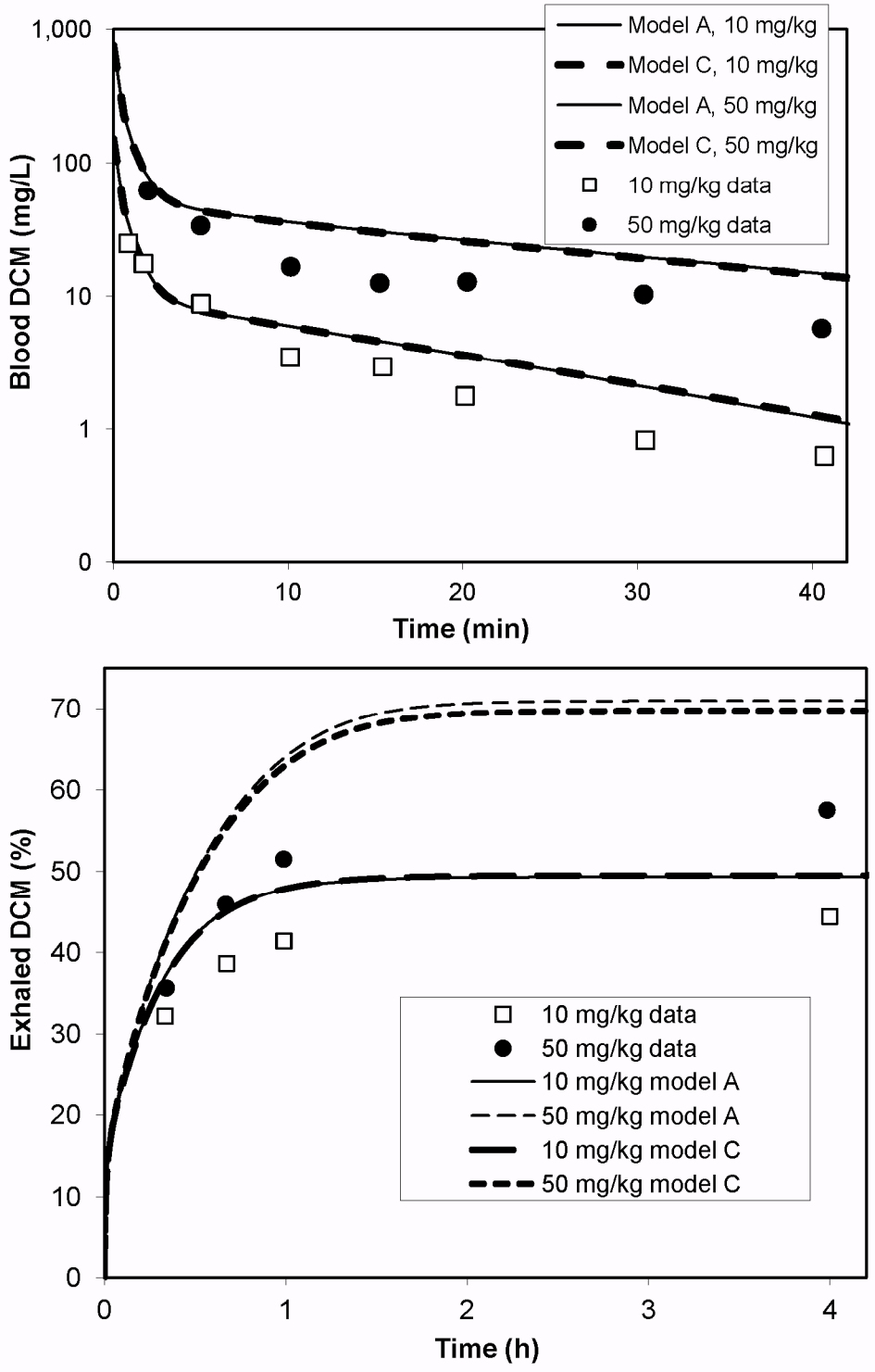


Figure C-4. Observations (data points) of Angelo et al. (1986b) and predictions for model Variations A and C (curves) of dichloromethane (DCM) blood concentrations (upper panel) and amount of dichloromethane exhaled (lower panel) following 10 and 50 mg/kg intravenous DCM injections in rats.

The most likely explanation for the overprediction of all four models versus the experimental observations in Figure C-4 is some shortcoming in model structure or parameter specification (e.g., the model lacks an explicit blood-volume compartment, which could significantly impact predictions for this particular route of administration). The discrepancy appears to exist primarily in the initial distribution phase, as the slope of the simulated clearance curve closely matches that of the data beyond 10 minutes (0.17 hours). When the simulations are adjusted downwards to match the observed level at 10 minutes after dosing, the fit to the remaining blood dichloromethane data was excellent (results not shown). Thus, it appears that the persistent error between model predictions and data, especially for the 50 mg/kg dose, beyond the initial distribution phase would be eliminated if a change in the model were introduced that only impacted that initial distribution phase. In contrast, Variation A matches the 3,000 ppm closed-chamber data almost exactly at 1.75 hours (Figure C-3), but then deviates from the data as time progresses.

There were also significant differences between model predictions of percent dichloromethane dose expired after intravenous injections and observed amounts (Figure C-4, lower panel). Variation C predictions are slightly closer to the data than Variation A at 50 mg/kg, while the two models were almost identical at 10 mg/kg. Like the blood concentration data, if simulation results are adjusted downward to match the initial measurements of percent exhaled dichloromethane, the subsequent predictions match the remaining data points much better (results not shown). Thus, the error again appears to come from the initial distribution phase.

Model fits to the open-chamber inhalation data of Andersen et al. (1987) are shown in Figure C-5. While both variations fit the dichloromethane blood concentrations reasonably well during the 4-hour inhalation phase at 1,000 ppm, neither fit the 1,000 ppm post-exposure clearance data well. Both variations also overpredict the initial uptake phase at 200 ppm (Figure C-5, lower panel); while the difference appears larger than occurred at 1,000 ppm (upper panel), this is only due to the difference in y-axis scale of the two plots and in fact is about the same as occurs at 1,000 ppm. In contrast to the 1,000 ppm results, the clearance-phase data at 200 ppm are fairly well predicted. The fit of Variation C to the 1,000 ppm data is only slightly better than Variation A, while the two are almost identical at 200 ppm.

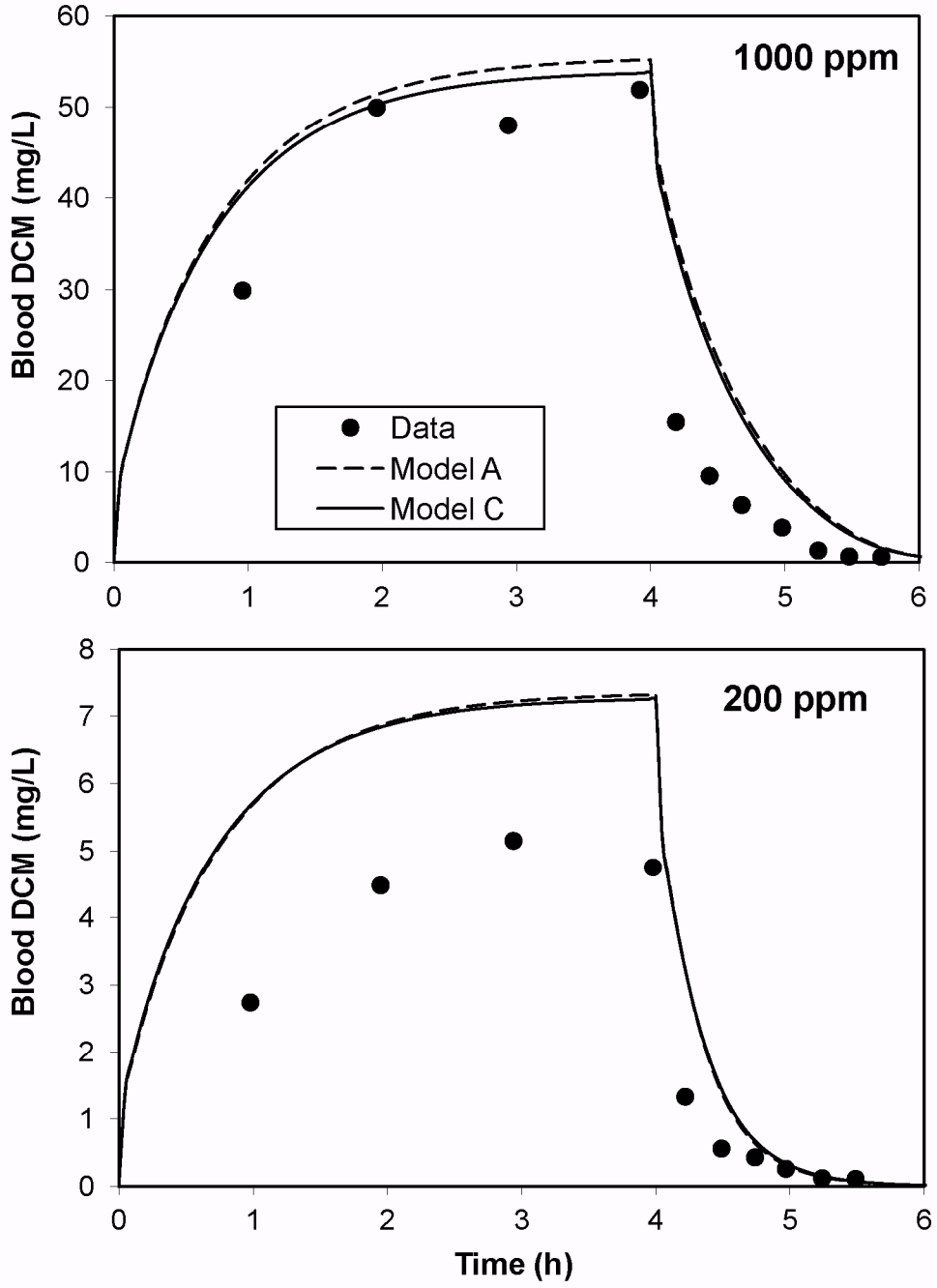


Figure C-5. Observations of Andersen et al. (1987) (data points) and simulations (curves) for models A and C for dichloromethane (DCM) in rat blood from inhalation of 200 and 1,000 ppm DCM for 4 hours.

The first clear difference between model predictions with Variations A and C are in the simulations of COHb after 200 and 1,014 ppm inhalation exposures, shown in Figure C-6, with data from Andersen et al. (1991). Variation A fits the peak COHb levels better than Variation B, and both tend to overpredict the 1-hour data (first time point) and the last two or three time

points. Here, Variation C fits the data better than Variation A. For example, at 1,014 ppm, Variation A overpredicts 16 of the 24 data points, while Variation C is less biased, overpredicting only 13 of the data points. Similarly at 200 ppm, Variation A overpredicts 17 of the 21 data points, while variation C only overpredicts 12 of the 21 measurements.

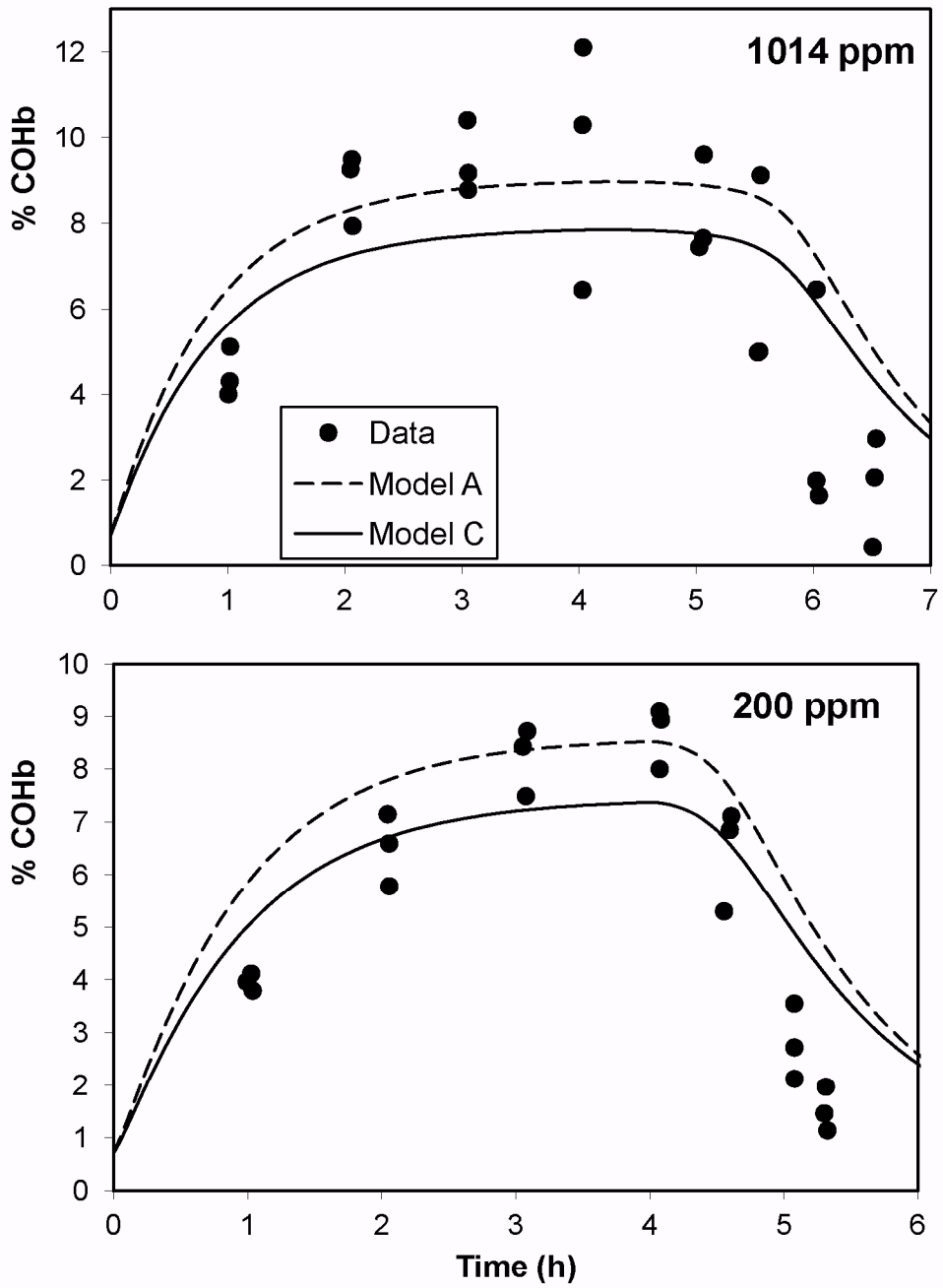


Figure C-6. Observations of Andersen et al. (1991) (data points) and simulations (curves) for models A and C for percent saturation of carboxyhemoglobin (percent COHb) in rat blood from inhalation of 200 and 1,014 ppm dichloromethane for 4 hours.

A quantitative comparison of Variations A and C is presented in Table C-2, showing data (Andersen et al., 1991; NTP, 1986) and model predictions for the fraction (percent) of 50 and 200 mg/kg gavage doses exhaled as dichloromethane or CO over 24 hours following exposure. Variation A overpredicts three of the four measurements by a factor of 1.03–1.04 (ratio of predicted value to mean measured value), and overpredicts the exhaled CO for a dose of 50 mg/kg by a factor of 1.20, indicating a slight underestimation of total dichloromethane metabolism and overestimation of CO yield from that metabolism. The ratio of Variation C-predicted values to the mean measured values was 1.00–1.01 for three of the four values and 0.86 for the fourth. Thus, the overall error from Variation C versus the exhalation data in Table C-2 is less than variation A although, like Variation A, there is one value that is much farther off. In this case, however, it is an underprediction of the amount of CO exhaled at 200 mg/kg. The difference between Variation C and the mean measured values was <1% of the dose, while Variation A differed from the measured values by up to 3% of the dose.

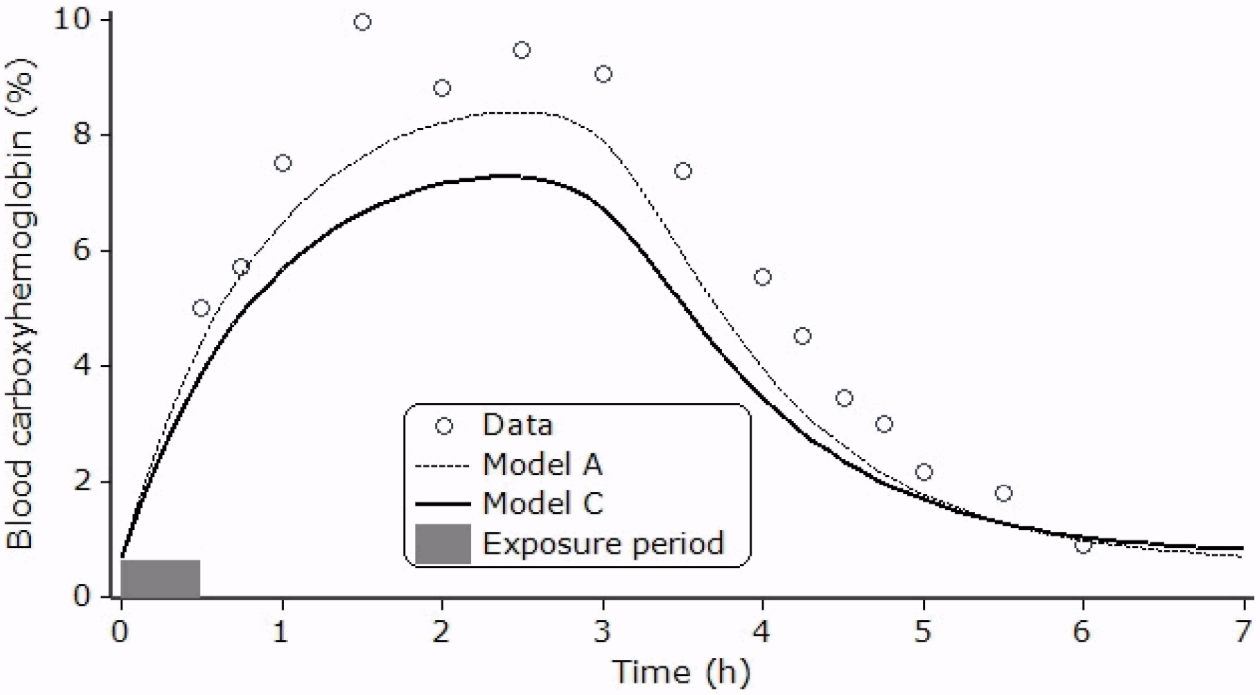
Table C-2. Comparison of PBPK model Variations A and C predictions of the amount of dichloromethane either exhaled unchanged or as CO

Dose metric	Dose (mg/kg)	Measured[a]	Variation A[a]	Variation C[a]	Variation A/ mean	Variation C/ mean
% Dichloromethane	50	64.2 ± 4.3	66.8	64.7	1.04	1.01
% Dichloromethane	200	77.6 ± 0.8	79.9	77.3	1.03	1.00
% CO	50	16.2 ± 1.2	19.4	16.3	1.20	1.01
% CO	200	7.2 ± 1.0	7.5	6.24	1.04	0.87

[a]Amounts are the cumulative percent of total dichloromethane dose collected in exhaled breath at 24 hours after gavage by Angelo et al. (1986b). Measured values are mean ± SD of reported mean values for three groups of six rats each; the first group was studied without prior exposure, while the second and third groups were studied on the 7th and 14th daily exposure, respectively. (Data sets were combined since no trend in dosimetry with repeated dosing over this period was detected at either 50 or 200 mg/kg.)

A final comparison of Variations A and C is shown in Figure C-7: predictions of blood COHb during and after a 30-minute inhalation exposure to 5,159 ppm dichloromethane, conducted by Andersen et al. (1991). It must first be noted that Andersen et al. (1991) changed several parameters from the primary set listed in Table 2 of their publication when depicting their model fit to these data (as noted in the legend of their Figure 4). In order to provide a relevant comparison of the two models, the parameters listed in their Table 2 (Variation A in Table C-1 here) were used. However, the V_{max} listed in Figure 4 of Andersen et al. (1991) is consistent with that value of V_{maxC}, given a rat BW = 0.24 kg. Therefore, this BW value was

used for both simulations shown. With the exception of that adjustment in BW, it can be said that these are data to which Variation C have not been fit.



The model Variation A simulation uses the parameters listed in Table 1 of Andersen et al. **(1991)** rather than those listed in Figure 4 of that publication to provide a fair comparison of the two variations (i.e., both are restricted to a single set of parameters). However, a value of V_{max} consistent with that listed by Andersen et al. **(1991)** could be obtained by setting the rat BW to 0.24 kg, so this was done for both simulations.

Figure C-7. Observations of Andersen et al. (1991) (data points) and simulations (curves) for models A and C for percent saturation of carboxyhemoglobin (percent COHb) in rat blood from inhalation of 5,159 ppm dichloromethane for 30 minutes.

Model Variation A clearly fits the data in Figure C-7 better than Variation C. As noted just above, these data were not used in estimating the parameters for Variation C. Given that Andersen et al. (1991) used different parameters in showing their model's ability to reproduce these data, and the methods do not state that these data were used in model fitting, it would appear that this is also the case for Variation A. Both models capture the overall shape of the time-course fairly well, specifically showing a continued rise in COHb levels until 2.3–2.5 hours, almost 2 hours after exposure ends. The difference between Variation C and the data is less than 30% of the measured values, except for the point at 1.5 hours, which appears to be an outlier. So for data to which a model has not been fit, this level of agreement would generally be considered good. As will be shown in the next section, Variation C actually predicts higher CYP

metabolism than Variation A. Since CO is assumed to be produced from CYP metabolism, the lower amount of COHb predicted here can be attributed to the lower CO yield estimated for Variation C (0.68) versus that used in Variation A (0.8). These results therefore suggest that this yield is not a constant but increases with concentration. Nevertheless, the possibility that Variation C underpredicts high-concentration CYP metabolism (and Variation A even more so), should be noted.

In summary, both model Variations A and C fit the data considered (Figures C-3 to C-7 and Table C-2) fairly well, although some sets (e.g., exhaled dichloromethane in Figure C-4) less well than others. In an absolute sense, there are no substantial differences between the goodness of fit to the fitted data for each model, and where differences occur versus the fitted data, Variation C is consistently better in describing the overall data set. For example, in Table C-2, the total discrepancy between Variation C and the data is less than that versus Variation A, although there is one measurement for which A is better. The resulting difference in the likelihood function computed by acslX, shown in Table C-1, is over 66 log-units, indicating a high statistical significance. The likelihood calculated by acslX is only approximate; in particular, the calculation assumes independence of each datum, which is clearly not the case for the gas uptake data shown in Figure C-3. Nevertheless, the difference in approximate likelihood values reflects a systematic reduction in the total relative error with Variation C versus Variation A.

Variation C does fit the COHb data in Figure C-7 less well than Variation A, although the difference is no more than that shown for the 50 mg/kg data in Figure C-4 and the 200 ppm data in Figure C-5. Given the shortcomings that appear in the CO/COHb submodel discussed elsewhere in this appendix and that Variation C otherwise predicts higher CYP metabolism than Variation A, Variation A's superior fit to this one data set was discounted relative to the quantitative improvement of Variation C to the fitted data. As shown in Section C.2.3, the two variations give rise to non-trivial differences in internal dose-metric predictions. These differences are considered sufficient justification to consider Variation C over Variation A.

C.2.3. Evaluation of Relative Flux of CYP and GST Metabolism of Dichloromethane

The estimated hepatic metabolism through the CYP or GST pathways during simulated medium- and high-level inhalation exposures of rats to dichloromethane for model Variations A and C is shown in Table C-3. Chronic exposures of 200, 1,000, 2,000, and 4,000 ppm, reflecting exposures used by Andersen et al. (1991) and NTP (1986) were simulated. Only a slight difference was seen in predicted CYP dosimetry between Variations A and C, but a larger difference in GST dosimetry. Variation C predicted 1.5–3% less CYP metabolite production in the liver than Variation A, depending on the exposure. However GST-mediated metabolism was 25–28% higher for Variation C than Variation A. At 200 ppm where the bulk of metabolism was via the CYP pathway, the result was almost identical total metabolism, but at 4,000 ppm

Variation C predicted 18% higher total metabolism. Thus, while model fits to experimental data shown in the previous section were mostly similar for Variations A and C, the predicted GST and total metabolism internal dose metrics under bioassay conditions show a greater difference.

Table C-3. Effect of PBPK model variation on predicted dichloromethane metabolite production in the liver of (male) rats from inhalation exposures[a]

Model variation	Exposure (ppm)	CYP-mediated metabolite production (mg/L liver/d)	GST-mediated metabolite production (mg/L liver/d)	Total metabolite production (mg/L liver/d)	GST:CYP metabolite production ratio
A	200	658	64.3	722	0.098
C	200	640	82.6	723	0.129
A	1,000	893	598	1491	0.67
C	1,000	877	747	1625	0.85
A	2,000	915	1384	2298	1.5
C	2,000	901	1725	2626	1.9
A	4,000	979	2873	3852	2.9
C	4,000	964	3581	4544	3.7

[a]Inhalation exposures of 200 or 1,000 ppm for 4 hours/day (Andersen et al., 1991) or 2,000 or 4,000 ppm dichloromethane for 6 hours/day, 5 days/week for 2 years (NTP, 1986).

To elucidate the exposure ranges where internal doses are linear and where CYP metabolism saturates, weekly average CYP and GST liver metabolic rates were simulated for Variations A and C over a wide span of inhalation concentrations, given a regimen of 6 hours/day, 5 days/week, with results shown in Figure C-8. Metabolite production is predicted to be approximately linear up to ~20 ppm for both models. From 20 to 100 ppm, CYP metabolism is still largely linear, but the small degree of saturation in that pathway that begins in that range leads to a larger relative increase, hence greater-than-proportional increase, in the GST pathway. This occurs because lower relative CYP metabolism leaves more dichloromethane available for the GST pathway. CYP metabolism becomes mostly saturated between 100 and 1,000 ppm but is not fully saturated even at 2,000 ppm. CYP metabolism is not predicted to be fully saturated by 2,000 ppm because exposures are only 6 hours/day, and blood concentrations fall quickly after each exposure. Since dichloromethane blood levels still increase with exposure level during that exposure-off period (because they are higher at the end of the exposure-on period), there is also increased metabolite production with exposure level during these exposure-off periods. However, the increase in metabolism per exposure-ppm is much less in this high-concentration range than occurs in the lower-concentration range because blood concentrations are falling throughout the exposure-off period. Depending on the model, GST metabolism is predicted to become dominant above 1,100–1,300 ppm.

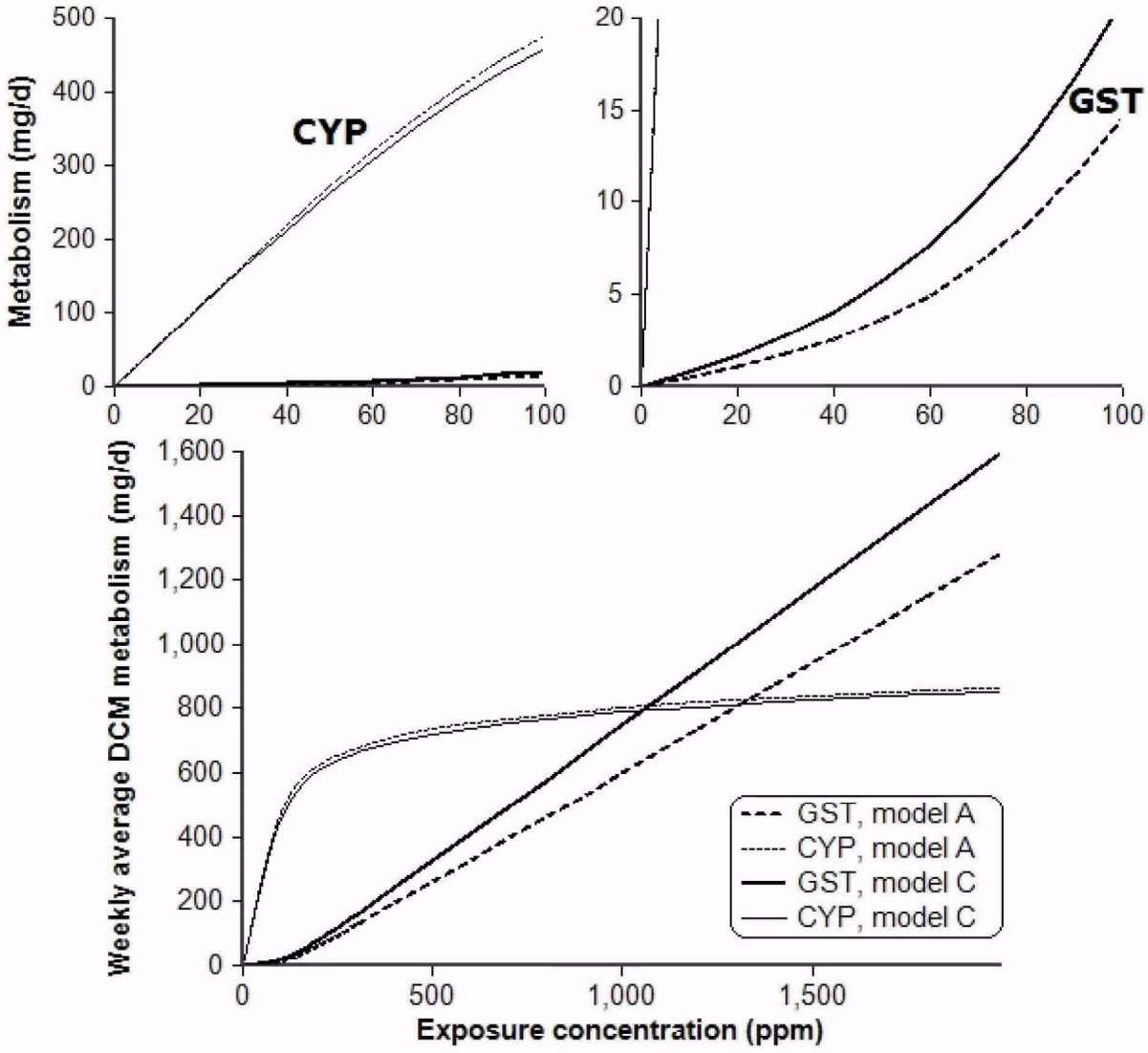


Figure C-8. Simulation results using model Variations A and C for weekly average metabolic rates by the GST and CYP pathways for 6 hours/day, 5 days/week inhalation exposures.

C.2.4. Evaluation of Model Predictions of Oral Absorption of Dichloromethane

For Variation C, the final set of parameters for which results are shown here was numerically fit by global optimization to: (1) the inhalation and intravenous exposure data shown previously; (2) data for blood and liver dichloromethane levels, and total expired dichloromethane and CO levels, measured by Angelo et al. (1986b) for rats exposed to gavage doses of 50 and 200 mg/kg dichloromethane; and (3) blood COHb levels observed after 526 mg/kg bolus doses by Pankow et al. (1991a). The parameter that specifically determines oral absorption identified through this global optimization is the gastrointestinal absorption constant, k_a, for which the optimized value was $k_a = 4.31$/hour. Resulting fits to the oral exposure data are shown in Figures C-9 and C-10. (Note that for the percent expired as CO,

panel C of Figure C-9, the percentage *declines* with increased concentration, balancing the increased percentage exhaled as dichloromethane.)

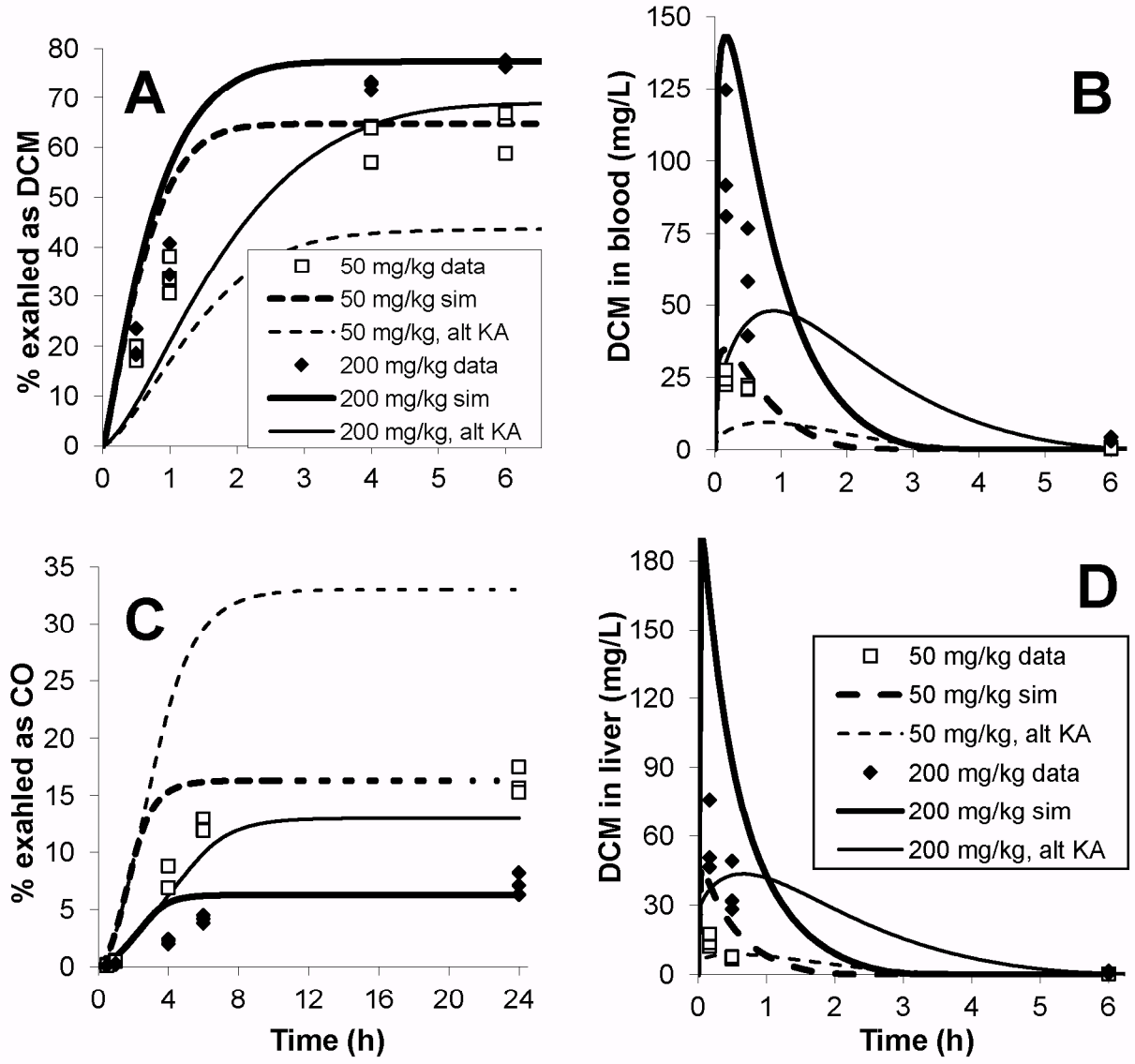
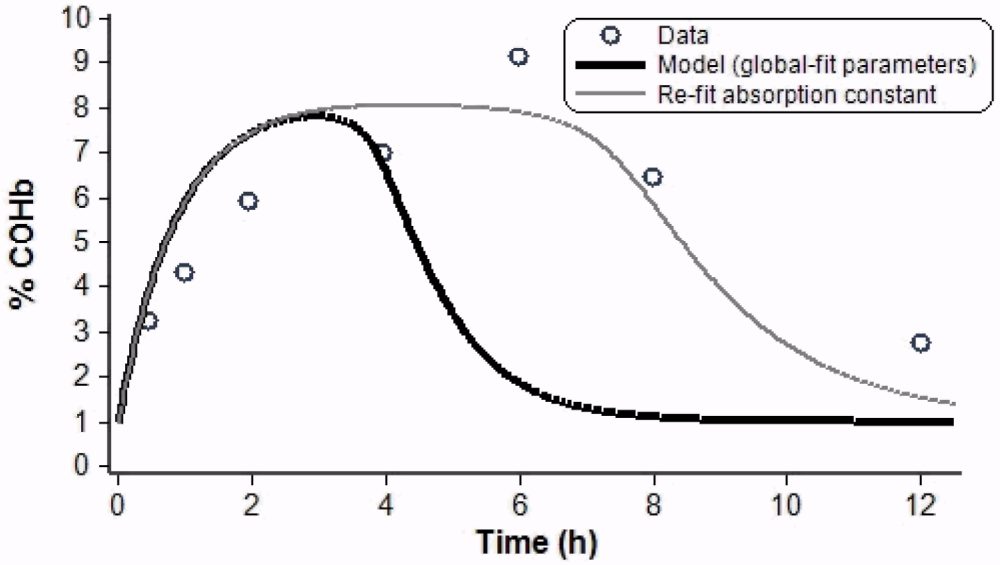


Figure C-9. Observations of Angelo et al. (1986b) (data) and model Variation C predictions for: (A) percent dose expired as dichloromethane (DCM); (B) blood DCM; (C) percent expired as CO; and (D) liver DCM in rats given a single dichloromethane gavage dose of 50 and 200 mg/kg, using a numerically fitted gastrointestinal absorption rate constant (k_a = 4.31/hour, heavy lines) and an alternate value of the constant (k_a = 0.62/hour, thin lines).



Model simulations performed with model C (heavy black line, k_a = 4.31/hour) or with an alternate value of k_a = 0.62/hour (thin grey line).

Figure C-10. Model predictions with of blood carboxyhemoglobin (COHb, percent of total Hb) from a single gavage dose of 526 mg/kg dichloromethane in rats, compared to the data of Pankow et al. (1991a).

The model fits to the oral PK data of Angelo et al. (1986b), shown in Figure C-9, are fair, although not as good as one might like. (In Figure C-9, the heavy lines are for the model with the global-fit k_a.) As discussed in Section C.2.1, the model was not expected to fit the shorter-time CO exhalation data (Figure C-9C) and indeed, the model predicts much more rapid exhalation than observed up to 6 hours, though it matches the total exhaled CO (cumulative amount) at 24 hours. In fact, a similar pattern occurs with exhaled dichloromethane in Figure C-9A, although the discrepancy is not as large as for CO. The dichloromethane levels measured in liver and blood are also overpredicted: only slightly so for blood (Figures C-9B), but by approximately threefold for liver. It should be noted that the timing of the concentration peak and subsequent clearance phase match the shape of the data reasonably well. Since the overprediction of liver and blood concentrations suggests that the absorption rate constant is too high (and as will be seen below, reducing k_a can improve the fit to other data), the change in predicted dosimetry from using a lower value of k_a is shown in Figure C-9 (thin lines). The result illustrates that reducing k_a alone not only reduces the predicted peak concentration but also alters the predicted fraction converted to CO (and overall metabolic efficiency) and the timing of the peak tissue levels. More specifically, reducing k_a with other parameters held constant: (1) reduces the fraction of dichloromethane expected to be exhaled (Figure C-9A); (2) increases the amount predicted to be converted to and exhaled as CO (Figure C-9B); and (3) shifts the predicted peak blood and liver concentrations from ~9 and 1 minutes, respectively, to ~45–55 minutes, much later than shown by the data.

Another possible explanation for the overprediction of the liver concentration data, in particular, is that with a delay of even a few minutes between exsanguination and tissue removal from the experimental animals used, metabolism is likely to continue and tissue concentrations could drop significantly. What the model predicts at 15 minutes after a bolus exposure, for example, the nominal time of data collection, does not account for this continued metabolism. The potential for such an effect to significantly alter internal dosimetry was illustrated by Sweeney et al. (1996) for butadiene.

Pankow et al. (1991a) measured blood COHb following a gavage dose of 525 mg/kg, data, which might also be used to estimate k_a. Model fits to these data are shown in Figure C-10. The heavy, black line is the fit obtained with the globally-fitted oral absorption rate constant. The thinner grey line is the result of re-fitting k_a to just these data while holding all other parameters constant. While the globally-fitted model matches the initial data, up to ~4 hours, fairly well, it then predicts a rapid decline in COHb levels, whereas the data show that blood levels remain elevated to ~8 hours, followed by a more gradual decline. The re-fitted value of k_a, 0.62/hour, describes this particular data set fairly well. Unfortunately, as shown above, this alternate absorption constant gives a much poorer fit to the other oral dosimetry data. Thus, while it is possible to improve the fit to specific data by choosing different values of k_a, doing so reduces the overall model quality. As discussed previously, the inability to describe the kinetics of COHb (with the globally-fit parameters) may also be due to the assumption that CO does not distribute into muscle and other tissues: the more gradual rise and fall shown by the data in Figure C-10 might be better predicted if diffusion of CO into and out of tissues, especially slowly-perfused tissues, was included.

Another factor in attempting to simulate the CO data along with other data sets is the difference in vehicle: Pankow et al. (1991a) used Oleum pedum tauri while Angelo et al. (1986b) used water with polyethylene glycol. Withey et al. (1983) compared oral uptake rates for four halogenated hydrocarbons, including dichloromethane, and found that dosing in corn oil versus water led to a significant decrease in the rate and apparent extent of uptake, with lower peak concentrations and a longer time-to-peak occurring with corn oil vehicle. The kinetics of orally administered dichloromethane with the water/polyethylene glycol vehicle (Figure C-9), clearly show the blood concentration already clearly falling from the peak at 30 minutes (panel B) and most of the dichloromethane exhalation being complete by 4 hours (panel A). Further, the difference in percent exhaled as dichloromethane and CO between the 50 and 200 mg/kg data (panels A and C) is explained by the model as resulting from saturation of the CYP pathway, which occurs because of the peak dichloromethane concentrations that result in turn because there is a rapid initial absorption rate. If the rate of absorption from the gastrointestinal tract is reduced to match the slower rise in COHb levels observed by Pankow et al. (1991a) over the first 4–6 hours (data in Figure C-10), the result is that most of the absorbed dose is predicted to be metabolized on first-pass, so blood dichloromethane levels remain below 5 mg/L, and the

predicted fraction exhaled as dichloromethane is much lower than observed, while the dose-dependence of the fraction exhaled as CO is lost (results not shown). Thus, while it is possible that the dichloromethane and exhaled CO kinetics of Pankow et al. (1991a) did differ that much from those observed by Angelo et al. (1986b), it is not possible to model both data sets with a single set of parameters, given the current model structure. Even if the apparent peak at 6 hours in Figure C-10 is due to experimental noise or variability (i.e., COHb concentrations plateau from ~4 to 8 hours), the slow rise in COHb from 0 to 4 hours requires either a significant change in model parameters or structure to describe.

One should also note that the CO exhalation data in Figure C-9C show continued elimination between 6 and 24 hours, a time-range when liver and blood dichloromethane levels have fallen to near zero, indicating that absorption is nearly complete. This suggests that the extended period of COHb elevation observed by Pankow et al. (1991a) in fact represents what occurred (but was not measured) in the experiments of Angelo et al. (1986b). If that is the case, then the discrepancy between the model simulation (with global-fit parameters) and data in Figure C-10 must occur because the model structure does not fully describe CO/COHb kinetics, rather than a simple difference in absorption kinetics.

Model Variation C simulations of total expired dichloromethane for oral doses with both globally fit parameters (values in Table C-1) and absorption constants re-fit to the Pankow et al. (1991a) data (values in legend of Figure C-10) are compared to the data of Kirschman et al. (1986) in Table C-4. While the Pankow-fit parameters give somewhat lower fraction exhaled (e.g., 70.9 versus 78.4% predicted with the global-fit parameters at 250 mg/kg), the difference is much less than that in the data, in the direction expected. In particular, with slower absorption one expects the liver concentration of dichloromethane to remain lower, in which case there is less metabolic saturation, allowing a higher fraction of the dose to be metabolized, thereby leaving a lower fraction to be exhaled. Since the two parameter sets give such similar results, however, only the global-fit parameter-based simulations will be specifically discussed. While the model-predicted exhalation is more than double that measured with the corn-oil vehicle at 250 mg/kg, the overprediction is only 27% versus the corn-oil observation at 2,000 mg/kg. More relevant to bioassay exposures, the model prediction is only 18% higher than the observed amount exhaled using the water vehicle at 250 mg/kg and only 4% higher than observed for a 500 mg/kg dose in water. The most obvious explanation for the difference in fraction exhaled with corn oil versus water vehicle is that absorption is slower with corn oil, allowing for more efficient (less saturated) metabolism, and hence, a lower fraction exhaled. The value of the first absorption constant, k_a, fit to the Pankow et al. (1991a) data is 14% of the global-fit value. These 250 mg/kg corn oil vehicle exhalation data can be matched if k_a is reduced to 0.087/hour: 2% of the globally-fitted value. It is possible that other factors are also involved; the balance between saturable and first-order metabolism in the model may be different in the animals used by Kirschman et al. (1986) versus the model, since the model fails to capture the full reduction in

percent exhaled for the water vehicle between 500 and 250 mg/kg. But neither is it implausible that absorption should be substantially slowed by the corn oil vehicle. Since the model does reproduce the water vehicle data fairly well, these results are otherwise a reasonable, if limited, validation of the model.

Table C-4. Observations and predictions of total expired dichloromethane resulting from gavage doses in rats[a]

Dose (mg/kg)	Dichloromethane exhaled (% of dose)			
	Observations		Predictions	
	Corn oil vehicle	Water vehicle	Global-fit	Pankow-fit
250	36.7	66.5	78.4	71.4
500	Not reported	78.3	81.2	77.2
1,000	45.7	Not reported	82.9	80.6
2,000	65.7	Not reported	83.8	82.6

[a]The y-axis of Figure 2 of Kirschman et al. (1986) appears to be mislabeled as mg instead of mg/kg; mg would result in unrealistic values. Assuming that this is supposed to be mg/kg, and assuming an average value of 250 g for a F344 rat, an expiration of 1,300 mg/kg given a dose of 2,000 mg/kg corresponds to 65% of the administered dose. This value is consistent with their observation in mice where 55% was observed expired as dichloromethane from a dose of 500 mg/kg, with the percentage expired increasing with dose.

Source: Kirschman et al. (1986).

A final comparison of model Variation C predictions to exhaled dichloromethane data is shown in Figure C-11. Model simulations for 1 and 50 mg/kg bolus oral doses are shown along with corresponding data from McKenna and Zempel (1981), as well as the data of Angelo et al. (1986b) at 50 mg/kg. The difference between the results of Angelo et al. (1986b) (used in model calibration) and McKenna and Zempel (1981) (not used for calibration) at 50 mg/kg dose shows the level of inter-laboratory variability. While the model matches the 50 mg/kg data of McKenna and Zempel (1981) quite well up to 1.5 hours, it then approaches a maximum around 65% versus the 72.5% observed by those investigators. The estimated fraction exhaled for a 1 mg/kg dose is likewise slightly below the observations for that exposure. However the discrepancies—less than 10% of the measured values—are well within what might result from experimental variability in both cases. Thus, these results are taken as confirmation of the model's ability to predict dosimetry at low exposure levels.

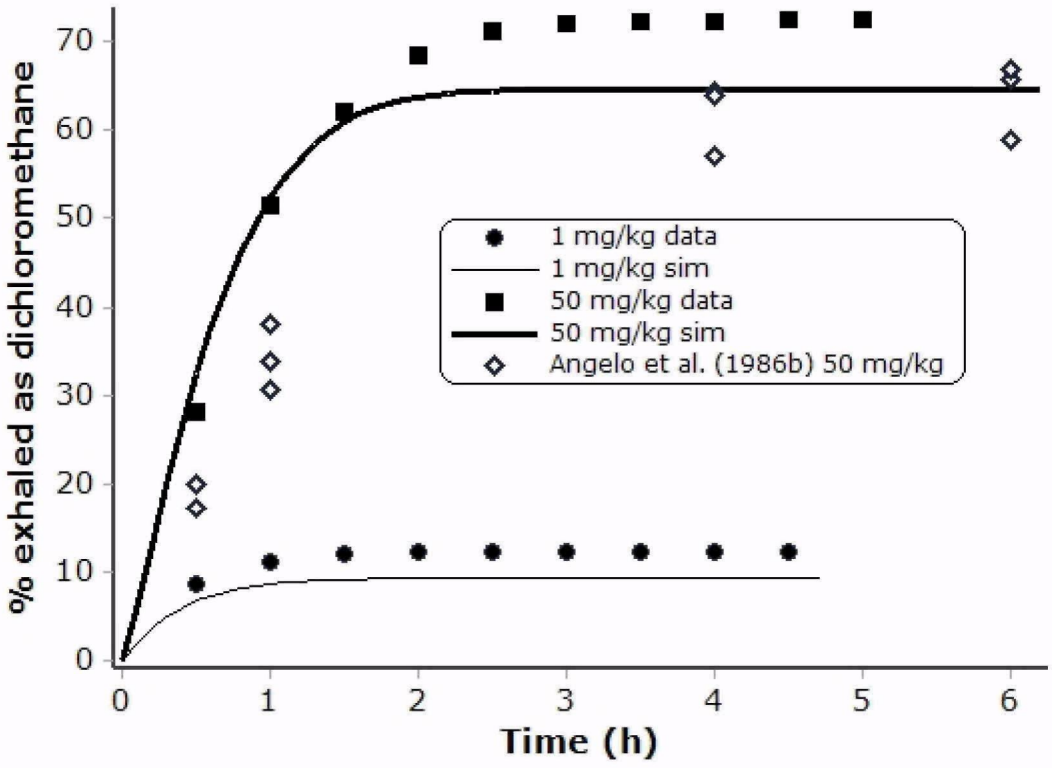


Figure C-11. Comparison of model Variation C predictions to dichloromethane exhalation data of McKenna and Zempel (1981) (data not used for model calibration) from bolus oral exposures to 1 and 50 mg/kg dichloromethane, along with 50 mg/kg bolus oral data of Angelo et al. (1986a, b) (data used for model calibration).

C.3. MODEL OPTION SUMMARY

The revised model presented here, Variation C, is a rat PBPK model represented by the basic model structure of Andersen et al. (1991) with the inclusion of lung dichloromethane metabolism via CYP (0.2% of liver) and GST (14.9% of liver) pathways [estimated from Reitz et al. (1989a)] and with liver metabolic parameters and CO yield factor re-calibrated against multiple data sets. Inclusion of lung metabolism in this model provides increased biological realism compared to the model of Andersen et al. (1991) and the re-calibration provides better overall model agreement with the available rat data sets, using a single set of parameters. While the fraction of CYP metabolism estimated in the rat lung is not quantitatively significant, all three metabolic parameters for the liver (V_{maxC}, K_m, and k_{fC}) were likely impacted to some extent during the re-optimization based on the presence of lung GST metabolism, since splitting GST metabolism into the lung compartment reduces the extent of flow-limitation on that pathway

Our evaluation also revealed some deficiencies of the available models. Oral exposure data show a slower rise to peak COHb levels and slower rate of exhalation of CO than predicted by the model, with the model-simulated rates for CO and COHb being largely determined by the kinetics of dichloromethane. Thus, it is not possible to fit those CO data using the existing CO

submodel and parameters, without significantly degrading the fit to the parent dichloromethane data. Cho et al. (2001) used a perfused rat hind-limb preparation to demonstrate that CO permeates muscle tissue (part of the slowly perfused tissue group in the PBPK model) to a large extent, slowing its wash-out from the hind-limb significantly versus radiolabeled albumin or sucrose. In fact, distribution of CO into muscle and other body tissues was incorporated into a human CO PBPK model by Bruce et al. (2008). The CO submodel, on the other hand, assumes that CO is not distributed beyond the blood compartment. That the CO submodel does not describe its distribution into other tissues could explain the discrepancy between the observed CO and COHb kinetics and model predictions.

Cho et al. (2001) also found that a significant fraction of CO was retained by the perfused tissue, estimating that the long-term fractional recovery to only be 45%. The yield factor fitted in the PBPK model here, P1, must then in part account for CO, which is, in fact, produced but not exhaled. That P1 was estimated to be 0.68, or 68%, for Variation C suggests that either <45% of CO is sequestered in the dichloromethane pharmacokinetics experiments or that its production is underestimated. Since the purpose of this analysis was to identify a model that best estimates dichloromethane kinetics in the rat with only moderate adjustments to the model structure, and elaboration of the CO submodel would result in additional parameters that need to be given values or identified with the data, the structure of the CO submodel was left intact and only the yield factor was adjusted.

Reitz et al. (1997) employed a k_a value of 5.0 $hours^{-1}$ for dichloromethane in deriving acute- and intermediate-duration oral minimal risk levels. These investigators cited previous work in which a k_a value of 5.0/hour resulted in reasonable kinetic predictions for bolus or drinking water exposures of 1,1,1-trichloroethane in rats (Reitz et al., 1989b), while a value of 5.4/hour provided good agreement between toxicokinetic observations and predictions for bolus doses of trichloroethylene in rats (Fisher et al., 1989). Pastino and Conolly (2000) fitted k_a = 4.15/hour for ethanol absorption in rats. Thus, the value of k_a obtained here, 4.31/hour, is consistent with other oral kinetic data. It must also be noted that since the model assumes 100% absorption of an oral dose, there is no other route of elimination except systemic circulation. However, at lower doses or dose-rates, a larger fraction of the dose will be eliminated on first-pass through the liver, and hence never reach systemic circulation, which can give the appearance of lower bioavailability. Finally, when long-term exposure patterns (comparable to chronic bioassays) are simulated, the average rate of absorption must equal the average (measured or estimated) rate of ingestion, independent of the values for these constants.

In summary, we have examined a common PBPK model structure with three parameter sets, Variations A–C, to describe dichloromethane dosimetry in rats as candidates for use in risk assessment where the purpose is to estimate internal doses of dichloromethane that occurred during various bioassays. In comparing model predictions to a variety of data, one can say that while all of the model variations do a fairly good job of fitting some of the data, none of them fit

all of the data very well, and there are some data for which none of the models provides a particularly good fit. With respect to the dichloromethane kinetics from inhalation and intravenous exposures shown in Figures C-3 to C-5, there was very little difference between predictions with the unadjusted parameters (Variation A) and the final revised parameters (Variation C). However, predictions of COHb levels after inhalation exposures were 10–20% lower with Variation C (Figures C-6 and C-7), while Variation C predicted a ~25% higher rate of GST-mediated metabolism (Figure C-8, Table C-3) and hence a lower fraction of dichloromethane exhaled unchanged after gavage (Table C-2) compared to Variation A. The rate of CYP metabolism is only slightly lower with Variation C versus Variation A. While some of these differences are small, the estimated likelihood (LLF in Table C-1) indicates that the re-fitted parameters provide a significantly better fit to the data used for calibration. This evaluation also indicates that the existing model structure for CO and COHb does not adequately describe the corresponding data for any of the parameter sets examined and that attempts to specifically use those data in setting key parameters would compromise the accuracy of the key dichloromethane dosimetry estimates. Therefore, Variation C is judged to be sufficiently better than the original model (Variation A) to support the use of Variation C instead of Variation A. Variation C was able to simulate exhaled dichloromethane data after oral dosing to which it had not been fit reasonably well (Figure C-11) and likewise provided fair agreement with water-vehicle data from another source (Table C-4, Global-fit predictions at 250 and 500 mg/kg). Hence, the model is expected to adequately predict rat internal dosimetry (dichloromethane blood concentrations or rates of metabolism) under bioassay exposure conditions.

APPENDIX D. SUMMARY OF EPIDEMIOLOGY STUDIES

D.1. OCCUPATIONAL COHORT STUDIES

D.1.1. Cellulose Triacetate Film Base Production Studies—Rochester, New York (Eastman Kodak)

Friedlander et al. (1978) reported a cohort mortality study of workers in an Eastman Kodak facility in Rochester, New York. This study was expanded and extended several times during the next 20 years (Hearne and Pifer, 1999; Hearne et al., 1990; Hearne et al., 1987). The latest analysis provided data on two overlapping cohorts. The first cohort (Cohort 1) consisted of 1,311 male workers employed in the roll coating division (n = 1,070) or the dope and distilling departments (n = 241) of the Eastman Kodak facility in Rochester, New York. Men who began working in these areas after 1945 and were employed in these areas for at least 1 year (including seasonal or part-time work that equaled 1 full-time year equivalent) from 1946 to 1970 were included. Follow-up time was calculated from the end of the first year of employment in the study area through December 31, 1994. The mean duration of work in Cohort 1 was 17 years. The total number of person-years of follow-up was 46,112, and the mean duration of follow-up was 35.2 years (range 25–49 years). The second cohort (Cohort 2) included 1,013 male workers in the roll coating division who were employed for at least 1 year in this division between 1964 and 1970. Follow-up time was calculated from January 1, 1964, for those who were employed there before 1964, or the date of first employment in the roll coating division for those who began in 1964 or later. Follow-up continued through December 31, 1994. The mean duration of work in Cohort 2 was 24 years. Total follow-up time was 26,251 person-years, and the mean duration of follow-up was 25.9 years (range 25–31 years). Cohort 2 was the focus of previous analyses by Friedlander et al. (1978) and Hearne et al. (1990; 1987).

For both cohorts, cause of death was based on the underlying cause of death recorded on the death certificates, which were routinely obtained by the company for the processing of life insurance claims. The expected number of deaths was calculated using appropriate age-, sex-, calendar time-, and cause-specific death rates for men in New York State (excluding New York City). In addition, another referent group was also used in the analysis of the second cohort. This other referent was based on the age-, sex-, calendar time-, and cause-specific death rates of other hourly male workers employed at the Eastman Kodak plant in Rochester, New York. (An internal referent group was also described for Cohort 1, but data for that analysis were not presented.)

Dichloromethane was first used in the film production process at the Eastman Kodak facility around 1944 (Hearne et al., 1987). Cellulose triacetate was dissolved in dichloromethane and then cast into a thin film onto revolving wheels. The film was then cured by circulating hot air in the coating machines, and the solvent was recovered and redistilled. 1,2-Dichloropropane

and 1,2-dichloroethane were also used as solvents from the 1930s to the 1960s, but dichloromethane was predominant (ratio 17:2:1 for dichloromethane:1,2-dichloropropane: 1,2-dichloroethane in general workplace air measurements) (Hearne et al., 1987).

The exposure assessment in the Rochester, New York Eastman Kodak cohort studies was based on employment records (start and stop dates for specific jobs in the relevant areas of the company) in combination with air monitoring data used to estimate the exposure level for a given job, location, and time period (Hearne et al., 1987). Air monitoring began in the 1940s, but few data are available before 1959. In the most recent update (Hearne and Pifer, 1999), more than 1,500 area and 2,500 breathing zone air samples were used in the exposure assessment process. Reductions in exposures in the dope department and the distilling department began after 1965. The highest exposure jobs were operator and maintenance workers (dope department) and filter washing and waste operator (distilling department), with estimated 8-hour TWA exposures of 100–520 ppm between 1946 and 1985. There was little change in estimated exposures for jobs in the roll coating division from the 1940s through 1985, but some reduction was seen from 1986 to 1994. The mean 8-hour TWA exposures were 39 ppm for Cohort 1 and 26 ppm for Cohort 2. These data were used to estimate a cumulative exposure index (i.e., the summation across all jobs held by an individual of the product of the average dichloromethane concentration as ppm and the duration of employment in that job). The authors refer to this as a "career exposure index." Additional adjustment in these estimates was made for respiratory protection, but the details of this adjustment were not described. For Cohort 1, the cumulative exposure categories used in exposure-effect analyses were <150, 150–349, 350–799, and ≥800 ppm. For Cohort 2, the cumulative exposure categories were <400, 401–799, 800–1,199, and ≥1,200 ppm. The cut points were chosen to produce an approximately equal number of expected total deaths in these categories.

There was no increased risk of mortality for all sites of cancer or for lung cancer in either cohort analysis (Table D-1). Data pertaining to smoking history, obtained from a survey of workers in the New York film production facility, indicate that smoking rates were similar in the exposed group, the internal comparison group, and the general population; therefore, it is unlikely that differences in smoking could be masking an effect of dichloromethane. The only specific sites for which the SMRs were >1.0 in both cohorts were brain and CNS cancer, Hodgkin lymphoma, and leukemia. Pancreatic cancer mortality risk was increased in Cohort 2 but not in Cohort 1. None of these associations were statistically significant. The Hodgkin lymphoma observations were based on a total of only four cases in both cohorts combined, and so were imprecise. Within Cohort 2, there was little difference in results for most sites using the different referent groups, but the point estimates for the SMRs for brain and CNS cancer, Hodgkin lymphoma, and leukemia were somewhat higher using the New York State referent group compared with the internal Eastman Kodak referent group. An attenuation of the dichloromethane association seen in the analyses using the internal Kodak referent group would

be expected if the risk of specific cancers was increased in this comparison group, possibly because of other workplace exposures.

The authors presented the exposure-effect analysis based on the estimated cumulative dichloromethane exposure groups for all sites of cancer, pancreatic cancer, lung cancer, brain cancer, and leukemia (Table D-2). There is no evidence of an exposure-effect for all site cancer mortality or lung cancer mortality risk. The relatively sparse number of deaths for the other specific cancer types makes it difficult to interpret the data. The patterns for pancreatic cancer differ between the two cohorts, with increased risk at the higher dose in Cohort 1 and a U-shaped curve seen in Cohort 2. For brain cancer mortality, a higher SMR was seen in the groups with cumulative exposure levels of ≥800 ppm-years compared with lower exposure groups. For leukemia in both cohorts, an increased mortality risk is seen in the highest exposure group (mean approximately 1,700 ppm-years).

Table D-1. Mortality risk in Eastman Kodak cellulose triacetate film base production workers, Rochester, New York

	Cohort 1: 1,311 men employed 1946–1970, followed through 1994				Cohort 2: 1,013 men employed 1964–1970, followed through 1994						
	New York referent group				New York referent group				Kodak referent group		
Cancer type	Obs	Exp	SMR	95% CI	Obs	Exp	SMR	95% CI	Exp	SMR	95% CI
Cancer, all sites	93	105.8	0.88	0.71–1.08	91	102.0	0.89	0.72–1.10	94.7	0.96	0.77–1.18
Liver[a]	1	2.4	0.42	0.01–2.36	1	2.4	0.42	0.01–2.33	1.8	0.55	0.01–3.07
Pancreas	5	5.5	0.92	0.30–2.14	8	5.3	1.51	0.65–2.98	5.1	1.55	0.67–3.06
Lung[a]	27	36.0	0.75	0.49–1.09	28	34.2	0.82	0.55–1.19	31.3	0.89	0.59–1.29
Brain[a]	6	2.8	2.16	0.79–4.69	4	2.1	1.88	0.51–4.81	2.7	1.46	0.39–3.75
Lymphatic system	5	6.6	0.75	0.24–1.78	6	5.7	1.06	0.39–2.30	5.7	1.05	0.38–2.28
Non-Hodgkin	2	4.1	0.49	0.06–1.76	3	3.5	0.85	0.17–2.50	3.6	0.84	0.17–2.46
Hodgkin	2	1.1	1.82	0.20–6.57	2	0.6	3.13	0.35–11.30	0.9	2.23	0.25–8.05
Multiple myeloma	1	1.5	0.68	0.01–3.79	1	1.5	0.65	0.01–3.62	1.3	0.79	0.01–4.39
Leukemia	8	3.9	2.04	0.88–4.03	6	3.5	1.73	0.63–3.76	4.4	1.37	0.50–2.98

[a]Liver includes liver and biliary duct; lung includes lung, trachea, and bronchus; brain includes brain and CNS.

Exp = number of expected deaths; Obs = number observed deaths

Source: Hearne and Pifer (1999).

Table D-2. Mortality risk by cumulative exposure in Eastman Kodak cellulose triacetate film base production workers, Rochester, New York

Cancer, referent group	SMRs (number of observed deaths)			
Cohort 1[a]				
Cumulative exposure (ppm yrs)	**<150**	**150–349**	**350–799**	**≥800**
Cancer, all sites				
Internal	0.81 (20)	1.02 (19)	1.10 (28)	1.07 (26)
New York	0.67	0.93	0.95	1.00
Pancreas				
Internal	0.74 (1)	0.00 (0)	0.77 (1)	2.34 (3)
New York	0.68	0.00	0.65	2.18
Lung[b]				
Internal	0.78 (5)	1.07 (6)	1.25 (9)	0.90 (7)
New York	0.52	0.90	0.86	0.77
Brain[b]				
Internal	0.58 (1)	0.78 (1)	1.65 (3)	0.85 (1)
New York	1.10	1.77	3.99	1.78
Leukemia				
Internal	0.83 (2)	0.00 (0)	0.48 (1)	2.73 (5)
New York	1.61	0.00	0.98	5.79
Cohort 2[c]				
Cumulative exposure (ppm yrs)	**<400**	**400–799**	**800–1,199**	**≥1,200**
Cancer, all sites				
Internal	0.89 (18)	0.96 (33)	1.11 (23)	1.08 (17)
New York	0.76	0.93	1.13	1.12
Pancreas				
Internal	2.58 (4)	0.00 (0)	0.95 (2)	1.43 (2)
New York	2.86	0.00	1.83	2.67
Lung[b]				
Internal	0.95 (6)	1.15 (12)	0.94 (6)	0.82 (4)
New York	0.80	1.00	0.89	0.79
Brain[b]				
Internal	0.00 (0)	1.13 (2)	1.37 (1)	1.49 (1)
New York	0.00	2.02	1.75	2.50
Leukemia				
Internal	0.00 (0)	0.84 (2)	0.75 (1)	2.70 (3)
New York	0.00	1.26	1.10	4.84

[a]Cohort 1: 1,311 men employed 1946–1970 in the roll coating division, dope department, or distilling department, followed through 1994; mean exposure (cumulative exposure yrs) 66, 244, 543, and 1,782 ppm-years in the four dose groups, respectively.
[b]Lung includes lung, trachea, and bronchus; brain includes brain and CNS.
[c]Cohort 2: 1,013 men employed 1964–1970 in the roll coating division, followed through 1994; mean exposure (cumulative exposure yrs) 168, 581, 981, and 1,670 ppm-years in the four dose groups, respectively.

Source: Hearne and Pifer (1999).

A strength of the Eastman Kodak cohort studies was the sampling data for dichloromethane that allowed an assessment of each worker's exposure using the monitoring data and the worker's job history, making exposure-effect analyses possible. Follow-up of the vital status of the cohort was >99% (Hearne and Pifer, 1999). There was also some information on smoking history for workers in the plant, based on a survey conducted in 1986 (Hearne et al., 1987). A difficulty in interpreting the data, however, is that there was some overlap between the cohorts: 707 of the men were included in both Cohort 1 and Cohort 2. Data are not presented in a way that would allow the reader to eliminate duplicate cases and person-years so that cases are only counted once when examining both cohorts. A strength of the Cohort 1 sampling strategy, compared with that of Cohort 2, is that Cohort 1 is limited to workers who began work at the plant after 1945. These workers would not have had workplace exposure to methanol and acetone, which were used at the plant in the film production process prior to that time. Because this is an inception cohort, follow-up began with the beginning of employment in the relevant area. In contrast, Cohort 2 was limited to workers who were employed from 1964 to 1970, so exposed workers who left or died before 1964 were not included. The relatively small number of cases with specific low incidence cancers (e.g., brain, leukemia) is also a limitation of the analyses of both of the cohorts in this study. In addition, the exposure levels in both cohorts (mean 8-hour TWA 39 and 26 ppm in Cohorts 1 and 2, respectively) are relatively low compared with values seen in other workplaces, including the cellulose triacetate fiber production cohorts described in Ott et al. (1983b) and Gibbs et al. (1996). Also, the outcome assessment is based on mortality (underlying cause from death certificates) rather than incidence data, and, because the Kodak studies were limited to men, there is no information on risk of breast cancer or other female reproductive cancers.

D.1.2. Cellulose Triacetate Film Base Production—Brantham, United Kingdom (Imperial Chemical Industries)

Tomenson et al. reported the results of a retrospective cohort mortality study of 1,473 men who worked at a film-base production facility in Brantham, England, anytime between 1946 and 1988 in jobs that were considered to have dichloromethane exposure. The start of the follow-up period was not specified by the authors but is likely to have been 1946 or the date of first employment at the plant. In the most recent analysis, follow-up of the cohort continued through December 31, 2006, and vital status was based on national records (United Kingdom National Health Service Central Register and the Department of Social Security). Cause of death was based on the underlying cause of death recorded on the death certificates. The expected number of deaths was calculated using age-, sex-, calendar time-, and cause-specific death rates for England and Wales. In addition, a comparison using mortality rates for the local areas (Tendring and Samford) for 1981–2006 and analyses limited to workers who had been employed for at least 3 months were also made. Total follow-up time was 51,966 person-

years, the median duration of work in the cohort was 5.3 years (interquartile range, 1.5, 16.8), and the median duration of follow-up was 36.8 years (interquartile range, 28.2, 43.1 years).

This facility produced cellulose diacetate film from 1950 to 1988, with other types of films also manufactured beginning in the 1960s (Tomenson et al., 1997). Dichloromethane was the solvent used in this process, and exposure occurred in the production of the triacetate film base and the casting of the film into rolls. The exposure assessment was based on >2,700 personal or air monitoring samples collected since 1975. An exposure matrix was constructed, assigning jobs to 1 of 20 work groups with similar exposure potential for each of four different time periods (before 1960, 1960–1969, 1970–1979, and 1980–1988). For the 1980–1988 period, exposure estimates for specific jobs were based on about 330 personal monitoring samples. For the earlier time periods, information about work tasks and location was used in combination with the information about the number of, use of, speed of, and problems with casting machines at different times from their initial introduction in 1950. The highest exposures were estimated to be in the casting machine operators and cleaners. Lifetime cumulative exposure to dichloromethane was calculated as the product of the mean level of exposure for the assigned work group and the duration of employment in that job summed across all jobs. Three categories of cumulative exposure were used for the analysis of ever-exposed workers: <400, 400–700, and ≥800 ppm-years. Approximately 30% of the workers in the cohort were classified as "unassigned" for the calculation of exposure group because sufficient information needed to determine exposures (i.e., the location and tasks assigned to laborers and maintenance workers) was not available. The mean 8-hour TWA exposure was estimated at 19 ppm for the cohort.

There was no increased risk of mortality for all sites of cancer (Table D-3), and the SMRs for most of the specific cancer sites examined (stomach, colon, rectum, liver, pancreas, lung, and prostate) were <1.0. The SMR for brain and CNS cancer was 1.83 (95% CI 0.79–3.60) (Table D-3). Tomenson et al. also present the exposure-effect analysis based on estimated cumulative dichloromethane exposure groups. The SMR for brain and CNS cancer was 1.24 (1 observed case) in the never exposed group, 1.56 (4 observed cases) for <400 ppm-years, 7.21 (2 observed cases) for 400–799 ppm-years, 0.2 (0 observed cases) for ≥800 ppm-years and 1.54 (2 observed cases) for the unassigned exposure groups. The authors interpret this pattern as indicating there was no relation with dichloromethane exposure.

Table D-3. Mortality risk in Imperial Chemical Industries cellulose triacetate film base production workers, Brantham, United Kingdom: 1,473 men employed 1946–1988, followed through 2006

Cancer type	Observed	Expected[a]	SMR	95% CI
Cancer, all sites	120	171.4	0.70	0.58–0.83
Liver and biliary duct	0	2.8	–	–
Pancreas	5	7.2	0.69	0.22–1.60
Lung, trachea, bronchus	27	56.3	0.48	0.31–0.69
Brain and CNS system	8	4.4	1.83	0.79–3.60
Lymphatic and hematopoietic	11	12.3	0.89	0.44–1.59
Leukemia	5	4.5	1.11	0.36–2.58

[a]Expected, calculated from observed and SMR data reported by the authors by using the following formula: expected = observed ÷ SMR; SMRs and CIs were not calculated for categories with zero observed cases.

Source: Tomenson et al.

A strength of this study was the monitoring data available that allowed assignment of cumulative exposure categories for use in exposure-effect analyses. However, 30% (439) of exposed workers had insufficient work histories to determine lifetime cumulative exposure. Air measurements were not available until 1975, and personal measures were not available until 1980. In addition, the duration of exposure was relatively low (median 5 years), the mean exposure level was relatively low (mean 8-hour TWA, 19 ppm), and there were very few deaths from specific types of cancer, which limit the statistical power of the study to examine associations among dichloromethane and specific cancers. Other limitations, as were also noted in the Kodak cohort studies, include the use of mortality rather than incidence to define risk, the reliance solely on underlying causes of death from death certificates to classify specific cancer types, and the lack of information on breast cancer risk.

D.1.3. Cellulose Triacetate Fiber Production—Rock Hill, South Carolina (Hoechst Celanese Corporation)

Two cohorts of cellulose triacetate fiber workers have been studied in Rock Hill, South Carolina (Lanes et al., 1993; Lanes et al., 1990; Ott et al., 1983b, d), and Cumberland, Maryland (Gibbs et al., 1996; Gibbs, 1992). Workers were exposed to dichloromethane, methanol, and acetone in both facilities.

Ott et al. (1983b, d) conducted a retrospective cohort mortality study of 1,271 acetate fiber production workers (551 men and 720 women) employed at least 3 months from 1954 to 1977 at Dow Chemical Company, Rock Hill, South Carolina. This analysis focused on ischemic heart disease mortality risk, and there was no presentation of cancer risk. The Rock Hill cohort study was updated through September 30, 1986 (Lanes et al., 1990) and December 31, 1990 (Lanes et al., 1993), and analyses of cancer mortality risks were included in these later

reports. Cause of death information was obtained from death certificates with coding based on the underlying and contributing causes (Ott et al., 1983b). The referent used in the updates was the general population of York County, South Carolina, and analyses were adjusted for age, race, gender, and calendar period. Because the results of the mortality risk analyses were similar for both updates, those from the 1993 paper are presented here. The mean duration of work in the cohort was not reported, but 56% worked for <5 years (calculated from Tables 3 and 4 of (Ott et al., 1983d). The mean duration of follow-up was 23.6 years in the analysis through 1986 (Lanes et al., 1990) but was not reported in the later paper (Lanes et al., 1993). The 1993 report added approximately 4.25 years of follow-up, which would result in an estimate of approximately 28 years of follow-up for this report.

The Rock Hill, South Carolina plant began producing cellulose triacetate fiber in 1954. Dichloromethane was used as the solvent for the initial mixing with cellulose triacetate flakes. This mixture was then filtered and transferred to the extrusion area for drying and winding. Air measurements taken in 1977–1978 were assumed to be representative of operations since dichloromethane use began in 1954, based on review of processing operations. The median 8-hour TWA exposures were estimated at 140, 280, and 475 ppm in the low, moderate, and high categories of exposure (Ott et al., 1983b). Employment records provided information on jobs held and dates employed, and this was used in conjunction with the exposure estimates for specific jobs and work areas to classify individual exposures. However, detailed work history information was only available for 475 (37%) of the workers (Lanes et al., 1990), and it is not clear how the exposure assessment was applied to workers with missing job history data.

Methanol was also used in the cellulose triacetate fiber production process, and methanol exposure was estimated as 1/10 that of dichloromethane. Acetone exposure was used in the production of acetate (cellulose diacetate) fiber at an adjacent part of the plant. The exposure to acetone was inversely related to that of dichloromethane, with estimated median 8-hour TWAs of 1,080 ppm acetone in the low dichloromethane exposure group and 110 ppm acetone in the moderate and high dichloromethane groups in the Rock Hill plant (Ott et al., 1983b).

In the latest follow-up (Lanes et al., 1993), there was no increase in mortality risk from cancer (all sites) or from cancer of the lung or pancreas (Table D-4). The SMR for liver and bile duct cancer, based on four observed cases, was 2.98 (95% CI 0.81–7.63). This was lower than the SMR of 5.75 (95% CI 1.82–13.8) that was reported in the 1990 analysis based on these same four cases but on a shorter follow-up period (and thus lower number of expected cases). Three of these cases were bile duct cancers. Biliary tract cancer is very rare; the authors estimated a total of 0.15 expected cases in the first of the follow-up studies (Lanes et al., 1990). This was the first cohort study that included women, and this study provided data on breast cancer risk. There were 3 observed breast cancer deaths compared with 5.59 expected, yielding an SMR of 0.54 (95% CI 0.11–1.57). No data were provided pertaining to reproductive risk factors (e.g., pregnancy history) for breast cancer among the women in this cohort, so it is difficult to assess

whether these potential confounders are likely to have been distributed differently in the cohort compared with the referent group (i.e., because of differences in pregnancy history that are related to employment status or socioeconomic status). Information about brain cancer, Hodgkin lymphoma, and leukemia (Table D-4) was not included in this report but was included in the report by Gibbs (1992) (see Table 11 of that report).

Table D-4. Mortality risk in Hoechst Celanese Corporation cellulose triacetate fiber production workers, Rock Hill, South Carolina: 1,271 men and women employed 1954–1977, followed through 1990

Cancer type	Observed	Expected	SMR[a]	95% CI[a,b]
Cancer, all sites	39	47.7	0.82	0.58–1.52
Liver and biliary duct	4	1.34	2.98	0.81–7.63
Pancreas	2	2.42	0.83	0.10–2.99
Lung, trachea, bronchus	13	16.21	0.80	0.43–1.37
Brain and CNS[c]	1	1.5	0.67	0.2–3.71
Hodgkin lymphoma[c]	0	0.24	–	–
Leukemia[c]	1	1.11	0.90	0.02–5.0
Breast cancer (women)	3	5.59	0.54	0.11–1.57

[a]SMRs and CIs were not calculated for categories with zero observed cases.
[b]CIs were calculated from Breslow and Day (1987) (Table 2.10).
[c]Data for brain and CNS cancer, Hodgkin lymphoma, and leukemia in the Rock Hill study were reported in Gibbs (1992).

Source: Lanes et al. (1993), except as noted in footnote c.

There are a number of limitations in this study including the small size of the cohort, small number of observed cancer deaths, availability of detailed work history information for only 37% of the workers, and use of mortality rather than incidence data. The exposure levels at this plant were high, but the duration of exposure for most of the cohort was relatively short (<5 years). It is the first cohort study, however, that included women and provided information on breast cancer risk.

D.1.4. Cellulose Triacetate Fiber Production—Cumberland, Maryland (Hoechst Celanese Corporation)

Gibbs et al. (1996) studied a cohort of 2,909 cellulose triacetate fiber production workers (1,931 men and 978 women) at a Hoechst Celanese plant in Cumberland, Maryland. This retrospective cohort mortality study included all workers who were employed on or after January 1, 1970, and who worked at least 3 months. This study also included a very small comparison group (256 men, 46 women) that was described as a "0" or "no" exposure group of workers at the plant who worked in jobs that were not considered to have had dichloromethane exposure;

totals for this study were 2,187 men and 1,024 women in the exposed and nonexposed groups combined.

The plant closed in 1981, and mortality was followed through 1989. Since 1955, employees of this plant were exposed to dichloromethane, methanol, acetone, and finishing oils used as lubricants. Before 1955, acetone was the only exposure. Industrial hygiene monitoring focusing on dichloromethane, acetone, and methanol began in the late 1960s. Exposure groupings (low = 50–100 ppm and high = 350–700 ppm) were assigned by area in which employees worked. The extrusion and spinning workers and jet wipers were among the high exposure group (300–1,250 ppm 8-hour TWA). The SMR analysis that was reported used Allegany County, Maryland as the comparison group. Cause of death information was obtained from death certificates, but the authors did not state whether they used underlying or underlying and contributing cause of death information. The mean duration of work in the cohort was not reported. The total follow-up period included 49,828 person-years (16,292 years in the high exposure group and 33,536 years in the low exposure group), and the mean duration of follow-up was 17.2 years (range 8–20 years). (These data were found in Hearne and Pifer (1999), Table 7).

There was little evidence of an increase in mortality risk from cancer (all sites) or from cancer of the liver and bile duct, pancreas, or brain in men or women (Table D-5). An increasing risk with increasing exposure level was seen for prostate cancer mortality in men. The *p*-value for the trend was not given, but the authors describe it as a "nonstatistically significant dose-response relationship." A statistically significant SMR for prostate cancer death was seen in the 350–700 ppm group when latency (at least 20 years since first exposure) was included in the analysis (SMR = 2.08, $p < 0.05$). Cervical cancer mortality risk was increased, but the small number of cases in the high exposure group did not allow a precise assessment of the pattern with respect to exposure level. There was no increased risk of breast cancer.

A primary limitation of this study is that workers who were exposed before 1970 but were not working at the plant in 1970 were not included in the cohort. The authors had attempted to create an inception cohort of all workers who were employed on or after January 1, 1954, but problems with the completeness of the personnel file made it impossible to use this study design. From what the author (Gibbs, 1992) was able to determine, the records of workers who had died, left the company, or retired before the mid to late 1960s (when a new personnel system was developed) were not available. Additional limitations include the small size of the cohort, small number of observed cancer deaths, and use of mortality (death certificate) data. This is particularly problematic for cancers with relatively high survival rates (such as prostate cancer and cervical cancer), since incidence rates are not estimated well by mortality rates in that situation.

Table D-5. Cancer mortality risk in Hoechst Celanese Corporation cellulose triacetate fiber production workers, Cumberland, Maryland: 2,909 men and women employed 1970–1981, followed through 1989

	Men (n = 1,931)				Women (n = 978)			
Cancer type, exposure level[a]	Obs	Exp	SMR	95% CI[c]	Obs	Exp	SMR	95% CI[c]
Cancer, all sites	121				42			
50–100 ppm	64	70.0	0.91	0.70–1.2	37	44.79	0.83	0.58–1.1
350–700 ppm	57	75.6	0.75	0.57–0.98	5	4.61	1.1	0.35–2.5
Liver	2				0			
50–100 ppm	1	1.33	0.75	0.02–4.2	0	1.04		–
350–700 ppm	1	1.24	0.81	0.02–4.5	0	0.10		–
Pancreas	3				1			
50–100 ppm	2	2.24	0.89	0.1–3.2	1	1.73	0.58	0.01–3.2
350–700 ppm	1	2.90	0.35	0.01–1.9	0	0.18		–
Lung	35				11			
50–100 ppm	20	25.7	0.78	0.48–1.2	9	8.24	1.1	0.50–2.1
350–700 ppm	15	27.3	0.55	0.31–0.91	2	0.87	2.3	0.28–8.3
Brain[a]	2				2			
50–100 ppm	1	1.88	0.53	0.01–2.96	2	0.66	3.1	0.37–10.9
350–700 ppm	1	1.94	0.52	0.01–2.87	0	0.07		
Hodgkin[a]								
50–100 ppm	1	0.4	2.5	0.06–13.9	0	0.23		
350–700 ppm	0	0.41			0	0.02		
Leukemia[a]								
50–100 ppm	4	2.14	1.9	0.51–4.8	0	1.25		
350–700 ppm	1	2.28	0.44	0.01–2.4	0	0.13		
Prostate	22				Not applicable			
50–100 ppm	9	6.41	1.4	0.64–2.7				
350–700 ppm	13	7.26	1.8	0.95–3.1				
Cervical	Not applicable				6			
50–100 ppm					5	1.69	3.0	0.96–6.9
350–700 ppm					1	0.19	5.4	0.13–30.1
Breast[a]	0				10			
50–100 ppm	0	0.03			9	9.8	0.92	0.42–1.7
350–700 ppm	0	0.02			1	1.07	0.93	0.02–5.2

[a]Data for brain and CNS cancer, Hodgkin lymphoma, leukemia, and breast cancer reported in Gibbs (1992).
[b]Referent group = Allegany County, Maryland. SMRs and CIs were not calculated for categories with zero observed cases.
[c]CIs were calculated from Breslow and Day (1987) (Table 2.10).

Exp = number of expected deaths; Obs = number of observed deaths

Sources: Gibbs et al. (1996); Gibbs (1992).

D.1.5. Solvent-Exposed Workers—Hill Air Force Base, Utah

Spirtas et al. (1991), Blair et al. (1998), and Radican et al. (2008) evaluated exposure to dichloromethane in relation to mortality risk in successive retrospective cohort studies of 14,457 civilian workers employed at Hill Air Force Base in Utah for at least 1 year from 1952 to 1956. The analysis was limited to the workers who were white or who had missing data on race, resulting in a sample size of 14,066 (10,461 men and 3,605 women). Spirtas et al. (1991) examined mortality through 1982 (3,832 deaths), Blair et al. (1998) updated mortality through 1990 (5,727 deaths), and Radican et al. (2008) extended the follow-up period through 2000 (8,580 deaths). The underlying and contributing causes of death information from death certificates was used to classify cause-specific mortality. SMRs were calculated by using mortality rates from the Utah population, and an internally standardized life table method was used to adjust for age at entry into the cohort and competing causes of death. In the Radican et al. (2008) analysis, adjusted relative risks (rate ratios) were estimated from a Poisson regression analysis with unexposed workers as the referent. The mean duration of work was not reported. In the analysis through 1982 (Spirtas et al., 1991), there were 22,770 person-years of follow-up in men and 3,091 person-years of follow-up in women who were classified as exposed to dichloromethane. The total number of workers classified as exposed to dichloromethane was 1,222 (Stewart et al., 1991), which would yield an estimated mean of approximately 21 years of follow-up through 1982. The additional follow-up of 18 years in Radican et al. (2008) would be expected to increase the mean follow-up time among those still alive to approximately 40 years.

Two industrial hygienists developed the exposure assessment based on walkthrough surveys, interviews with management and labor representatives, review of historical records, job descriptions, monitoring data and other information pertaining to chemicals used, and organization of the work site (Radican et al., 2008; Blair et al., 1998; Spirtas et al., 1991). Each worker was assigned exposure by using information on the worker's job history, which included job titles, department codes, and dates of employment. The most detailed exposure assessment was done for trichloroethylene, the primary focus of the study. Dichloromethane, one of 25 other exposures analyzed, was classified as a dichotomous exposure (ever exposed, never exposed).

Radican et al. (2008) presented the mortality risk for three specific cancers in relation to 15 of the 25 chemicals classified as dichotomized exposures. The rate ratios for non-Hodgkin lymphoma and multiple myeloma in relation to dichloromethane in men were 2.2 (95% CI 0.76–5.42, based on eight observed cases) and 2.58 (95% CI 0.86–7.72, based on seven observed cases), respectively. These rate ratios (particularly those for multiple myeloma) were considerably higher than the rate ratios for any of the other chemicals examined in which the next highest observed rate ratios were 2.1, 2.0, and 2.0 for *o*-dichlorobenzene, Freon, and the "other alcohols" category, respectively. No cases of either of these cancers were observed in women with dichloromethane exposure, but the rate ratio for breast cancer in these women was

2.35 (95% CI 0.98–5.65, based on six observed cases). Associations of similar magnitude (rate ratios of 2.3–2.8) were also seen among breast cancer and some other exposures (Freon, solder flux, isopropyl alcohol, and 1,1,1-trichloroethane). All of these risk estimates were slightly attenuated from the estimates in Blair et al. (1998).

This is the largest of the identified cohort studies that included women and specifically reported data pertaining to breast cancer risk. The major limitation of this study is that the exposure assessment for dichloromethane was based on a dichotomized classification. In addition, exposure to many different types of solvents was common; thus, in some analyses it can be difficult to assess the independent effects of individual exposures. Some aspects of reproductive history, such as age at first pregnancy, are known risk factors for breast cancer. Reproductive history was not included in this analysis, but Blair et al. (1998) noted that it is unlikely that these factors would confound the results of a few specific chemicals, since the referent group was an internal group within the cohort (and thus would be expected to be similar in terms of socioeconomic status) and there was no association overall between solvent exposures and breast cancer mortality.

D.2. CASE-CONTROL STUDIES OF SPECIFIC CANCERS

Twelve site-specific cancer case-control studies included dichloromethane as an exposure of interest. These studies involve six types of cancer: brain and CNS (Cocco et al., 1999; Heineman et al., 1994), breast (Cantor et al., 1995), kidney (Dosemeci et al., 1999), pancreas (Kernan et al., 1999), rectum (Dumas et al., 2000), and various forms of lymphoma and leukemia, including childhood leukemia (Gold et al., 2010; Wang et al., 2009; Costantini et al., 2008; Seidler et al., 2007; Miligi et al., 2006; Infante-Rivard et al., 2005).

D.2.1. Case-control Studies of Brain Cancer

Heineman et al. (1994) studied the association between astrocytic brain cancer (International Classification of Diseases 9th ed. [ICD-9] codes 191, 192, 225, and 239.7) and occupational exposure to chlorinated aliphatic hydrocarbons. Cases were identified by 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 the exclusion of 66 controls with a possible association between cause of death and occupational exposure to chlorinated aliphatic hydrocarbons (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. This exposure assessment procedure was quite detailed, in that additional coding of specific jobs within the 4-digit industry and occupation code categories was used, for example, to distinguish production of paint removers from production of paints, varnishes, lacquers, enamels, and allied products (Dosemeci et al., 1994). The investigators developed matrices linked to jobs with likely exposure to dichloromethane, five other chlorinated aliphatic hydrocarbons (carbon tetrachloride, chloroform, methyl chloroform, tetrachloroethylene, and trichloroethylene), and organic solvents (Dosemeci et al., 1994; Gómez et al., 1994). This assessment was done blinded to case-control status. Exposure was defined as the probability of exposure to a substance for a specific occupation and industry, 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.

Adjusting for age and study area, the OR for the association between any exposure to dichloromethane and risk of astrocytic brain cancer was 1.3 (95% CI 0.9–1.8). There was a statistically significant trend ($p < 0.05$) with increasing probability of exposure to dichloromethane with an OR = 1.0 (95% CI 0.7–1.6) for low probability, OR = 1.6 (95% CI 0.8–3.0) for medium probability, and OR = 2.4 (95% CI 1.0–5.9) for high probability compared with the referent group of unexposed men. An increased risk with higher duration of exposure was also observed with OR = 1.7 (95% CI 0.9–3.6) for $\geq$ 21 years of work in exposed jobs for all exposed workers and OR = 6.1 (95% CI 1.1–43.8) for the combination of $\geq$ 21 years of work in a high probability of exposure job. Similar results were seen in additional analyses controlling for age, study area, employment in electronics occupations and industries, and exposure to carbon tetrachloride, tetrachloroethylene, and trichloroethylene. In the analyses adjusting for these other exposures, only dichloromethane exhibited a trend with increasing probability of exposure. There was also evidence of an association between astrocytic brain cancer risk and dichloromethane exposure, based on the average intensity score, with an OR = 1.1 (95% CI 0.7–

1.7) for the low-medium intensity group and an OR = 2.2 (95% CI 1.1–4.1) for the high intensity group (trend p-value < 0.05). The combination of high intensity and high duration (≥21 years) was strongly associated with risk (OR = 6.1 [95% CI 1.5–28.3]), and a weaker association (OR = 1.4 [95% CI 0.6–3.2]) was seen for high intensity and shorter duration (2–20 years). The association between cumulative exposure score (low, medium, and high) and astrocytic brain cancer risk was nonlinear (ORs of 0.9, 1.9, and 1.2 in the low, medium, and high exposure categories, respectively).

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 limitations were the lack of direct exposure information and potential inaccuracy of the descriptions of work histories that were obtained from next-of-kin interviews. Heineman et al. (1994) acknowledge these limitations in the report, and in response to a letter by Norman and Boggs (1996) criticizing the methodology and interpretation of the study, Heineman et al. (1996) 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. Exposure to other solvents, including carbon tetrachloride, tetrachloroethylene and trichloroethylene were also common in cases and controls. These additional exposures provide additional evidence that recall bias was not distorting the observed effects, as the strong associations that were seen with the exposure measures for dichloromethane were not seen with the other solvents included in the analysis. In addition, adjustment for these other solvent exposures did not result in any attenuation in the observed association with dichloromethane. The relatively strong and statistically significant associations between dichloromethane and astrocytic brain tumors were seen along multiple measures of exposure, suggesting that the results were unlikely to be spurious. Nondifferential misclassification would, on average, attenuate true associations and would be unlikely to result in the types of exposure-response relationships that were observed in this study.

Norman and Boggs (1996) described an apparent inconsistency in the estimated trends in dichloromethane and carbon tetrachloride exposure based on the methodology used in this case-control study [described in more detail in Gómez et al. (1994)]. In response, Gómez (1996) noted that the apparent inconsistency was actually due to an error in the labeling of the lines on one of the figures in the report rather than an inconsistency with the estimated trends. Another point raised by Norman and Boggs (1996) was that the Heineman et al. (1994) findings were surprising in light of the lack of brain carcinogenesis in animals. In response, Heineman et al. (1996) pointed out that carcinogens commonly cause different cancers in animals and humans. It can also be noted that brain tumors are exceedingly rare in animal bioassays (Sills et al., 1999).

Norman and Boggs (1996) also suggested that the results of the Heineman et al. (1994) study be given no weight when compared with the results of the cohort studies. The authors responded by pointing out that the cohort studies had low statistical power and large CIs around their point estimates but were not inconsistent with an association between dichloromethane and brain cancer (Heineman et al., 1996). This point is strengthened further by the more recent results from the Rochester, New York Eastman Kodak cohort (Hearne and Pifer, 1999) described previously, since an approximately twofold increased SMR for brain and CNS cancers was seen in the longer follow-up period of this cohort. Dell et al. (1999) also noted the limitation of the lack of direct exposure measures in the Heineman et al. (1994) study, and the difficulty in categorizing jobs with occasional exposure to a specific solvent. As noted by Heineman et al. (1994), the "high probability" category for dichloromethane in this study included painting, paint or varnish manufacture, ship or boat building and repair, and electronics manufacture. The "high intensity" category included these jobs and jobs in roofing and the pharmaceutical industry. Dell et al. (1999) specifically questioned the inclusion of the roofing jobs as a job with potential for high intensity dichloromethane exposure. The number of individuals who were categorized based on this type of job, and more specific information pertaining to the more detailed coding within these job categories that may have contributed to the classification decisions, were not discussed in Dell et al. (1999), Heineman et al. (1994), Gómez et al. (1994), or Dosemici et al. (1994).

In another case-control study of brain cancer and dichloromethane exposure, Cocco et al. (1999) identified 12,980 female cases of cancer of the brain and CNS through the underlying cause of death listing (ICD-9 codes 191 and 192) on death certificates from 24 states from 1984 to 1992. This collection of death certificates is a data set created by the National Center for Health Statistics, NIOSH, and the National Cancer Institute to facilitate research on occupational exposures and mortality risk. The cases included 161 women with meningioma (ICD-9 codes 192.1, 192.3). Four women who died of nonmalignant diseases, excluding neurological disorders, were chosen as controls for each case. The controls were frequency matched to the cases by state, race, and 5-year age group. Occupation data were based on the occupation fields in the death certificates. This job was coded based on the three-digit industry and three-digit occupation (Department of Commerce) codes. The investigators developed job exposure matrices that were applied to these industry/occupation codes. The job exposure matrices included probability and intensity scores for 11 occupational hazards, one of which was dichloromethane, but also included other solvents, electromagnetic fields, chlorinated aliphatic hydrocarbons, benzene, lead, nitrosamines, insecticides, herbicides, and public contact. The investigators used logistic regression models to estimate ORs, adjusting for each workplace exposure, marital status, three levels of socioeconomic status (based on occupation), and age at death. For each chemical, four levels of intensity and probability were defined (unexposed, low, medium, and high).

A weak association between dichloromethane exposure and brain/CNS cancer was seen (OR 1.2 [95% CI 1.1–1.3]) (Cocco et al., 1999). There was no exposure-related trend in the association between probability or intensity of exposure and brain cancer. A similar but more imprecise association was seen with meningioma cancer (OR 1.2 [95% CI 0.7–2.2]). There were too few cases of meningioma to stratify by exposure probability and intensity.

The major limitations of this study are the use of mortality rather than incidence data and the reliance on occupation data from death certificates. The death certificate occupation data are based on "usual" occupation, which may be more prone to misclassification in studies of women because of gender-related differences in work patterns (i.e., shorter duration jobs for women compared with men). A relatively broad job exposure matrix was applied to the job information, and typically more generic job exposure matrices result in less sensitive assessment with limited ability to detect exposure-response trends (Teschke et al., 2002). Nondifferential misclassification of outcome and exposure would generally result in attenuated effect estimates.

D.2.2. Case-control Studies of Breast Cancer

Cantor et al. (1995) conducted a case-control study of occupational exposures and breast cancer using the 24-state (1984–1989) death certificate data described in the previous section. Cases were women with breast cancer coded as the underlying cause of death (ICD-9 code 174). Four female controls per case were selected from all noncancer deaths, frequency matched by age (5-year age groups) and ethnicity (black, white). The occupation listed on the death certificate was coded based on the three-digit industry and three-digit occupation (Department of Commerce) codes, and this was used with a job exposure matrix developed by the investigators to assess 31 workplace exposures, one of which was dichloromethane. Four exposure probability and three exposure level scores were assigned. ORs for probability and level were calculated for each ethnic group, adjusting for age at death and a measure of socioeconomic status (based on occupation). After excluding subjects whose death certificate occupations were listed as homemaker, there were 29,397 white cases and 4,112 black cases (total 33,509) and 102,955 white controls and 14,839 black controls (total 117,794).

There was little evidence of an association between exposure probability and breast cancer mortality using the probability exposure metric. The ORs were 1.05 (95% CI 0.97–1.1) and 0.76 (95% CI 0.3–2.0) in probability level 3 and level 4, respectively, for white women and 1.13 (95% CI 0.9–1.4) in probability level 3 for black women. (There were too few black women in exposure probability level 4 for analysis.) Weak associations were seen with exposure level. In white women, an OR of 1.17 (95% CI 1.1–1.3) was seen with the highest exposure level, and in black women the OR in this exposure group was 1.46 (95% CI 1.2–1.7). In the analysis that jointly considered exposure level and probability ratings but excluded the lowest probability of exposure, the OR for the highest category of exposure level was 1.28 in whites ($p < 0.05$) and 1.21 in blacks.

As with the Cocco et al. (1999) case-control study that used a similar methodology, the limitations of this study include the use of an outcome defined by mortality rather than incidence, use of usual occupation information as recorded in death certificates, and use of a very broad job exposure matrix to classify 31 different exposures. Although information on pregnancy and lactation history (known risk factors for breast cancer) was not available, the authors did adjust for socioeconomic status by using the occupation data, which may have corrected for some of the potential confounding due to reproductive history.

D.2.3. Case-control Studies of Pancreatic Cancer

Kernan et al. (1999) conducted a case-control study of 63,097 pancreatic cancer cases using the 24-state (1984–1993) death certificate data. The diagnosis of pancreatic cancer was based on underlying cause of death (ICD-9 code 157). Four controls who had died during the same time period of causes other than cancer were selected for each case, frequency-matched by state, race, gender, and 5-year age group (n = 252,386). Usual occupation and industry, based on the occupation data in the death certificate, were coded by using the three-digit (Department of Commerce) codes. A job-exposure matrix was used with the industry and occupation codes to evaluate exposure intensity and probability (each categorized as high, medium, or low) for formaldehyde, dichloromethane, 10 other solvents, and a combined "organic solvents" measure. Race- and gender-specific analyses were conducted by using logistic regression to estimate ORs and 95% CIs, adjusting for age, marital status (ever, never married), residential area (metropolitan, nonmetropolitan), and region (east, south central, south, and west).

The point estimates for the ORs in the low, medium, and high intensity categories in the four race-gender groups ranged from 0.8 to 1.3, with no exposure-effect trend seen in any group. The only statistically significant OR was for high exposure intensity in white females (OR 1.3 [95% CI 1.1–1.6]), with ORs of 1.0 (95% CI 0.9–1.1) for medium intensity and 1.1 (95% CI 1.0–1.2) for low intensity in this group. An elevated OR was seen with high exposure probability in black males (OR 2.2 [95% CI 1.0–4.8]) but not in white females (OR 1.0 [95% CI 0.8–1.4]) or white males (OR 1.0 [05% CI 0.8–1.3]), and the ORs were 0.9 for medium exposure probability in these three groups. There were relatively few black females in this study, resulting in imprecise estimates (OR 2.0 [95% CI 0.8–5.4] for medium exposure and OR 1.5 [95% CI 0.6–3.6] for high exposure).

The limitations of this study, as with the other case-control studies that used the 24-state death certificate data set, include the reliance on cause of death data from death certificates rather than medical-record validated incidence data and the use of death certificate occupation data. The job exposure matrix used with the occupation data was more focused than those used in Cocco et al. (1999) and Cantor et al. (1995). Although the analysis adjusted for some sociodemographic characteristics, it did not include measures of smoking history or diabetes, which are known risk factors for pancreatic cancer (Lowenfels and Maisonneuve, 2005).

D.2.4. Case-control Studies of Renal Cancer

Dosemeci et al. (1999) reported data from a population-based case-control study of the association between occupational exposures and renal cell cancer risk. The investigators identified newly diagnosed patients with histologically confirmed renal cell carcinoma 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 by 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 through 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- and part-time status. A job exposure matrix developed by the National Cancer Institute was used with the coded job data to estimate exposure status to dichloromethane and eight other chlorinated aliphatic hydrocarbons. ORs were adjusted for age, smoking, hypertension and use of drugs for hypertension, and body mass index. No association between renal cell carcinoma and exposure to dichloromethane was observed in men (OR 0.85 [95% CI 0.6–1.2]), women (OR 0.95 [95% CI 0.4–2.2]), or both sexes combined (OR 0.87 [95% CI 0.6–1.2]).

Strengths of this study include the use of incident cases of renal cancer from a defined population area and confirmation of the diagnosis using histology reports. The occupation history was based on usual and most recent job in combination with a relatively focused job exposure matrix. 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 measurements were not used, and a full lifetime job history was not obtained.

D.2.5. Case-control Studies of Rectal Cancer

Dumas et al. (2000) reported data from a case-control study of occupational exposures and rectal cancer conducted in Montreal, Quebec, Canada. The investigators identified 304 newly diagnosed cases of primary rectal cancer, confirmed on the basis of histology reports, between 1979 and 1985; 257 of these participated in the study interview. 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 by using electoral lists and random digit dialing. 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 from the interviews that could furnish clues about exposure. 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 subjects' 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. Logistic regression models adjusted for age, education, proxy versus subject responder status, cigarette smoking, beer drinking, and body mass index. Using the cancer control group, the OR for any exposure to dichloromethane was 1.2 (95% CI 0.5–2.8) and the OR for substantial exposure (confident that exposure occurred with ≥5 years of exposure at medium or high frequency and concentration) was 3.8 (95% CI 1.1–12.2). The results using the population-based control group for this exposure were not presented.

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 dichloromethane, resulting in relatively few exposed cases (or controls) and thus low statistical power for the analysis. The job exposure matrix applied to the job information was very broad since it was used to evaluate 294 chemicals.

D.2.6. Case-control Studies Lymphoma, Leukemia, and Multiple Myeloma

Several case-control studies examined risk of different types of hemato- and lymphopoietic cancer and occupational exposure to dichloromethane in Germany (Seidler et al., 2007), Italy (Costantini et al., 2008; Miligi et al., 2006), and the United States (Barry et al., 2011; Gold et al., 2010; Wang et al., 2009). Another study examined maternal occupational exposure in relation to risk of childhood acute lymphoblastic leukemia in Quebec (Infante-Rivard et al., 2005). Each of these population-based studies used a fairly extensive exposure assessment protocol, as described below.

Seidler et al. (2007) examined occupational solvent exposure in relation to lymphoma risk in a population-based case-control study in 6 areas of Germany. Incident cases (ages 18 to 80 years) were identified through hospitals and physician practices. The sample (n = 710; 87% of the identified cases) was primarily made up of non-Hodgkin lymphoma (n = 554 B cell and n = 35 T cell) patients. Controls drawn from population registries were individually matched to cases by age (within 1 year), gender, and area. The participation rate among identified controls was 44% (total n = 710). Data were collected through a structured in-person interview covering demographics, medical history, alcohol and tobacco use, and a work history including details of all jobs lasting at least 1 year. This information included job and task information, and

14 specific supplementary questionnaires for jobs with likely exposure to solvents and other chemicals. An industrial hygienist, blinded to case-control status, reviewed the work history data and developed measures of intensity (low = 1–10 ppm, medium = 10–100 ppm, and high = >200 ppm) and frequency of exposure (low = 1–5% of working time, medium = >5–30% of working time, high = >30% of working time) to dichloromethane. Measures of three other chlorinated solvents and four aromatic hydrocarbons were also developed in this manner. Cumulative exposure was calculated as the sum, across all jobs, of the product of the intensity and frequency and job duration measure for each solvent. Conditional logistic regression, adjusting for smoking (pack years) and alcohol use, was used to examine associations between exposure measures and all lymphoma. Unconditional logistic regression, with additional adjustment for the matching factors, was used to examine associations within specific subsets of disease (e.g., B-non-Hodgkin lymphoma), using all controls as the comparison group. The adjusted ORs for all lymphoma and dichloromethane exposure was 0.4 (95% CI 0.2–1.0), 0.8 (95% CI 0.3–1.9), and 2.2 (95% CI 0.4–11.6) for cumulative exposures of >0–≤26.3, >26.3–≤175, and >175 ppm-years, respectively, compared to 0 ppm-years (trend $p = 0.40$). A similar pattern was seen in the analysis limited to B-non-Hodgkin lymphoma cases: OR = 0.4 (95% CI 0.2–1.1), 0.9 (95% 0.3–2.3), and 2.7 (95% CI 0.5–14.5) for cumulative exposures of >0–≤ 26.3, >26.3–≤175, and >175 ppm-years, respectively, compared to 0 ppm-years (trend $p = 0.29$).

Another population-based case-control study focusing on solvent exposure and non-Hodgkin lymphoma risk was conducted in Connecticut (Barry et al., 2011; Wang et al., 2009). This study was limited to women, ages 21–84 years, identified through the Yale University Rapid Case Ascertainment system, with diagnosis between 1996 and 2000. A total of 832 eligible cases were identified, and 601 (72%) participated in the study. Controls were identified through random digit dialing (ages <65 years) and Medicare file (ages≥65 years). The participation rate was 69% among controls identified through random digit dialing and 47% among controls selected through the Medicare files. The final sample included 717 controls. Barry et al. (2011) is limited to 518 cases and 597 controls with blood or buccal cell samples available for genotyping. Six variants in four genes involved in the metabolism of benzene (or other solvents, including dichloromethane) were selected for analysis. These genes were CYP2E1 (rs2070673 and rs2031920), EPHX1 (rs2234922 and rs1051740), NQO1 (rs1800566), and MPO (rs2333227). Data pertaining to work history were collected through structured interviews, focusing on all jobs held for at least 1 year. The information collected included job title, company name, and duties. A job exposure matrix was used to link the job data to measures of intensity and probability (a four-category scale used for each: none, low, medium, and high) for general classes of solvents and for dichloromethane and seven other specific solvents.

The association seen with ever exposure to dichloromethane and non-Hodgkin lymphoma was OR = 1.5 (95% CI 1.0–2.3) (Wang et al., 2009). There was little difference in risk by

probability or intensity score. In the subset of patients and controls used in the genetic analysis, the association with ever exposure to dichloromethane (OR 1.69, 95% CI 1.06–2.69) was similar to that seen in the full sample (Barry et al., 2011). The increased risk was seen among carriers of the CYP2E1 rs20760673 TT genotype (OR 4.42, 95% CI 2.03–9.62), but not among carriers of the TA or AA genotype (OR 0.80, 95% CI 0.36–1.75) (interaction $p < 0.01$). A similar interaction was seen between this genotype and exposure to carbon tetrachloride and methyl chloride. The functional significance of this variant, which occurs in the promoter region, is not known. No association was seen between any of the solvents and CYP2E1 rs2031920, EPHX1 rs1051740, NQO1 rs1800566, or MPO rs2333227.

The studies in Italy were part of a large study of hemato-lymphopoietic cancers conducted in 11 geographic areas chosen based on the historical presence of solvent-based industries or farming activities (Costantini et al., 2001). The number of areas varied depending on the specific disease in the analysis: eight for non-Hodgkin lymphoma (Miligi et al., 2006), seven for leukemia, and six for multiple myeloma (Costantini et al., 2008). Incident cases, ages 20–70 years, were identified through hospital and hematology centers, with classification and confirmation of non-Hodgkin lymphoma diagnosis based on pathology review and consideration of cell type of origin. The participation rate among identified cases was 83% for non-Hodgkin lymphoma (Miligi et al., 2006), 85% for leukemia, and 83% for multiple myeloma (Costantini et al., 2008). Controls, frequency matched by area, gender, and 5-year age groups to the cases were identified through computerized demographic files or National Health Service files covering each of the study areas. The participation rates among the randomly selected controls were 73, 72, and 76% for those included in the non-Hodgkin lymphoma, leukemia, and multiple myeloma analyses, respectively (Costantini et al., 2008; Miligi et al., 2006). Data were collected through a structured in-person interview covering demographics, medical history, family medical history, and alcohol and tobacco use (mean length, approximately 60 minutes). The interview also included a detailed work history with the inclusion of job- and industry-specific modules focusing on solvents and agricultural exposures; jobs lasting ≥5 years were included in this assessment (Costantini et al., 2001). These data were reviewed by industrial hygienists, blinded to case-control status, and used to develop measures of exposure duration, probability (low, medium, and high), and intensity (very low, low, medium, and high) for five general classes of hydrocarbon solvents (aromatic, chlorinated, technical, aliphatic, and derivative oxygenate) and eight specific solvents (including dichloromethane). Analyses were conducted adjusting for gender, age, education, and geographic area, and the reference group consisted of individuals with no occupational exposure to any solvent.

The analysis of non-Hodgkin lymphoma included 1,428 cases and 1,530 controls (Miligi et al., 2006). No association was seen with the general exposure category of "any" occupational solvent exposure using the intensity measure (adjusted OR 1.0 and 1.1, respectively, for very low/low and medium/high intensity, compared with unexposed) or with duration of medium-high

exposure (adjusted OR 1.0 and 1.0, respectively, for ≤15 and >15 years, compared with unexposed). A more pronounced pattern was seen between dichloromethane exposure intensity and non-Hodgkin lymphoma risk, with adjusted OR = 0.9 (95% CI 0.5–1.6; 23 exposed cases) for the very low/low intensity and adjusted OR = 1.7 (95% CI 0.7–4.3; 13 exposed cases) for the medium/high intensity groups (trend *p*-value = 0.46). The sparse number of exposed cases precluded analysis by duration. In analysis by disease subtype, medium/high dichloromethane exposure intensity was associated with small lymphocytic non-Hodgkin lymphoma (adjusted OR 3.2 [95% CI 1.0–10.1]; 6 exposed cases; total n = 285).

The leukemia analysis included 586 cases and 1,278 controls (Costantini et al., 2008). No association was seen with dichloromethane exposure intensity for very low/low intensity (7 exposed cases), adjusted OR = 0.7 (95% CI 0.3–1.7) or for medium/high intensity (2 exposed cases), adjusted OR = 0.5 (95% CI 0.1–2.3). There were too few exposed cases of multiple myeloma (4 exposed to dichloromethane; total n = 263) for analysis.

Gold et al. (2010) conducted a population-based case-control study of multiple myeloma in Seattle, Washington and Detroit, Michigan using the Surveillance, Epidemiology and End Results (SEER) cancer registries to identify newly diagnosed cases. Eligible cases were ages 35–74 years, diagnosed between January 1, 2000 and March 31, 2002; 365 cases were identified who met these criteria. Of these, 64 (18%) died before being contacted, 28 (8%) could not be located, and 18 (5%) were not contacted because consent for the recruitment process was not obtained from their physician. The remaining 255 patients were contacted, and 181 (71% of those contacted and confirmed eligible) participated in the study. Controls were identified through random digit dialing (ages 35–64 years) and Medicare file (ages 65–74 years). The participation rate among the 1,133 potential controls identified was 52%, for a total of 481 controls. Data were collected through a computer-assisted personal interview that included a detailed work history covering all jobs held since age 18. Job-specific modules for 20 solvent-related jobs were also included, with details on tasks performed and chemicals used. A job exposure matrix was developed to classify exposure to each of six chlorinated solvents (including dichloromethane), with measures of probability, frequency, intensity, and confidence. Ratings were conducted by two reviewers, blinded to case-control status. Measures of duration of exposure and cumulative exposure were also developed. Analyses were conducted adjusting for gender, age, race, education, and SEER site. One set of analyses classified any possible exposure among the exposed, and a second set of analyses included the low-confidence exposure jobs with the unexposed group. In general, somewhat stronger associations or patterns were seen in the second analyses. For example, the OR for ever-exposed was 1.5 (95% CI 0.9–2.3) in the first analysis and 2.0 (95% CI 1.2–3.2) with the reclassification of the low confidence jobs. In the second set of analyses, a non-monotonic increasing trend was seen with duration of exposure (OR 1.0, 2.0, 1.1, 2.7, and 2.1, respectively, in unexposed, 1–4 years, 5–7 years, 8–24 years, and

25–47 years, trend $p = 0.01$). Similar patterns were seen with cumulative exposure (trend $p = 0.08$) and cumulative exposure lagged by 10 years (trend $p = 0.06$).

Infante-Rivard et al. (2005) examined the association between maternal occupational exposures, before and during pregnancy, and risk of childhood acute lymphoblastic leukemia (ICD-9 code 204.0) using data from a population-based case-control study in Quebec, Canada. Incident cases diagnosed from 1980 to 2000 were identified from the cancer hospitals in the province, and diagnosis was confirmed based on clinical records from an oncologist or hematologist. Between 1980 and 1993, cases ages 0–9 years at diagnosis were included, and from 1994 to 2000 the age range was expanded to 14 years. The number of eligible cases identified was 848 and of these, 790 parents (93%) participated in the study. Population-based controls, individually matched to the sex and age at diagnosis of the cases, were identified from government registries of all children in the province (1980–1993) and the universal health insurance files (1994–2000). The parents of 790 (86%) of the 916 eligible controls who were identified participated in the study. Data were collected by using a structured telephone interview. Some information (i.e., job title, dates, type of industry, industry name and address) was obtained for all jobs held since age 18, and additional information (e.g., materials and machines used, typical activities) was obtained for jobs held by the mother from 2 years before the pregnancy through the birth of the child. Specialized exposure modules were also used to collect information about specific jobs (e.g., nurse, waitress, hair dresser, textile dry cleaner). All of this information was reviewed by chemists and industrial hygienists, blinded to case-control status, to classify exposure to over 300 chemicals, although the primary focus of the study was on solvents (21 individual substances, including dichloromethane, and six mixtures). The exposure assessment included ratings of confidence (possible, probable, and definite), frequency of exposure during a normal workweek (<5, 5–30, or >30% of the time), and level of concentration (low = slightly above background, high = highest possible exposure in the study population, and medium for in-between levels). A weak association was seen between any dichloromethane exposure during the 2 years before pregnancy up to the birth and risk of leukemia in the child (OR 1.34 [95% CI 0.54–3.34]), and results were similar when limited to exposures during pregnancy. Stronger associations were seen with probable or definite exposure (OR 3.22 [95% CI 0.88–11.7]) compared with possible or no exposure. The estimates for categories based on concentration and frequency were similar but there was no evidence for an increasing risk with increasing exposure level.

D.3. CONTROLLED EXPERIMENTS EXAMINING ACUTE EFFECTS

With the exception of Putz et al. (1979), there is no description in the published reports of the informed consent and other human subjects research ethics procedures undertaken in these studies, but there is no evidence that the conduct of the research was fundamentally unethical or

significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

Stewart et al. (1972a, b) reported results from four experiments that were initiated based on the chance observation of an elevation in COHb saturation levels by one of the investigators the morning after he had spent 2 hours working with varnish remover. Participants were medical students and faculty (including at least one of the coauthors). A total of 11 healthy nonsmoking volunteers were placed in an exposure chamber with mean concentrations of dichloromethane of 213 ppm for 1 hour (n = 1), 514 ppm for 1 hour and then 869 ppm for 1 hour (n = 3), 514 ppm for 1 hour (n = 8), or 986 ppm for 1 hour (n = 3). The COHb saturation postexposure peaked at 2.4% in the one subject exposed to 213 ppm, 4–8.5% in the 3 subjects exposed to 514 and 869 ppm, 3.4% (mean) in the 8 subjects expose to 514 ppm, and 10% (mean) in the 3 subjects exposed to 986 ppm. Stewart et al. (1972a, b) noted that these experiments indicated that dichloromethane exposure above 500 ppm resulted in COHb saturation levels that exceeded and were more prolonged than those seen with the threshold limit value exposures to CO. The exposures also resulted in symptoms of CNS depression indicated by visual evoked response changes and reports of light-headedness. Although return of COHb levels to background levels could take >24 hours, all of the other symptoms were reversible within a few hours after exposure ceased. Because only one subject participated in the lower exposure (213 ppm) protocol, this study provides little information regarding effects at levels below 500 ppm.

Winneke (1974) measured auditory vigilance, visual flicker fusion frequency, and 14 psychomotor tasks in a total of 38 women exposed to dichloromethane at levels of 300–800 ppm for 4 hours in an exposure chamber. A comparison group (nine females, nine males) exposed to 100 ppm CO for 5 hours was also included. Exposure to 800 ppm dichloromethane resulted in a statistically significant decrease in the performance of 10 of the 14 psychomotor tasks. In tests of auditory vigilance and visual flicker fusion, depressed response was seen at 300 ppm and was further depressed at 800 ppm. These effects were not seen with CO exposure.

Forster et al. (1974) exposed four healthy young men to dichloromethane levels ranging from 0 to 500 ppm for 7.5 hours/day for a total of 26 days over a 6-week period to investigate alterations in hemoglobin affinity for oxygen and altered pulmonary function. While no changes were observed in pulmonary function, the percent oxyhemoglobin saturation was increased, suggesting an increased hemoglobin affinity for oxygen (as occurs with increasing CO levels), with no indication of adaptation to restore this affinity for oxygen to normal.

Putz et al. (1979) examined the behavioral effects seen after exposure to dichloromethane and to CO. Twelve healthy volunteers (six men and six women) each acted as his/her own control in separate 4-hour exposures to 70 ppm CO and 200 ppm dichloromethane. These levels were chosen so that the COHb level would reach 5% from each of these exposures. These were conducted as double-blind experiments so that neither the investigators nor the participant knew the exposure condition under study. Informed consent was obtained, and the study was reviewed

by the National Institute of Occupational Safety and Health (NIOSH) Human Subject Review Board. The performance tests were dual tasks (an eye-hand coordination task in conjunction with a tracking task), with five measures of performance assessed at six time points over the 4-hour test period and an auditory vigilance task. Two levels of difficulty were assessed for each task to allow assessment of whether the exposure effect was similar in low and high difficulty tasks. The tests of eye-hand coordination, tracking tasks, and auditory vigilance revealed significant impairment with both exposures under the more difficult task conditions. Effects were similar or stronger in magnitude for dichloromethane compared with CO.

D.4. WORKPLACE MEDICAL PROGAM AND CLINICAL EXAMINATION STUDIES

Kolodner et al. (1990) investigated the effect of occupational exposure to dichloromethane among male workers at least 19 years old at two General Electric plastic polymer plants where dichloromethane was one of the chemicals used. Four dichloromethane exposure categories were established based on full-shift personal air monitoring data (8-hour TWA) collected in 1979–1985, job titles, and industrial hygienists' knowledge of plant operations. The mean 8-hour TWA and number of workers in each of the four exposure groups were 49.0 ppm for the 19 workers in the highest, 10.9 ppm for the 49 workers in the intermediate, 3.3 ppm for the 56 workers in the low, and <1.0 ppm for the 772 workers in the minimal/no exposure group.

Data from 1984 annual medical exams and 1985 absence data from payroll records were evaluated. Only 5 of the 896 workers eligible for inclusion in the study refused the exam completely in 1984. Six hypotheses were specifically tested: absence due to illness, hepatotoxicity (manifested by nausea, weakness and fatigue, palpable liver, abdominal tenderness, jaundice, hepatomegaly, abnormal serum γ-glutamyl transferase, ALT, AST, or bilirubin), diabetes mellitus (manifested by weight loss, weakness and fatigue, polydypsia, polyuria, impaired vision, excessive weight loss, elevated fasting blood sugar, and abnormal urinary glucose or urinary acetone), CNS toxicity (manifested by headache, lightheadedness, dizziness and vertigo, ataxia, weakness and fatigue, and abnormalities detected in the central motor, central sensory, cranial nerve, gait, neurocoordination, or Bibinski reflex examinations), cardiovascular abnormalities (manifested by fatigue, dyspnea, chest pain with exertion, palpitations, or abnormalities detected in the point maximum impulse exam, blood pressure measurements, or electrocardiogram), and neoplastic breast changes (154 women were included in this portion of the study—manifested by painful breast, breast swelling, lump, nipple discharge, or abnormalities detected in the breast examination).

Workers were placed in exposure categories based on their current jobs. In addition, exposure to high noise levels occurred in both plants, and workers in each plant had exposure to another chemical, either phenol or phosgene. The workers tended to move from entry-level jobs with high dichloromethane exposure to supervisory jobs with lower dichloromethane exposure,

based on the seniority system in place at both plants. Thus, current exposure levels reported did not necessarily reflect cumulative exposure and age was inversely related to exposure. Age was controlled in the analysis of some of the continuous variables using analysis of covariance, but age adjustment was not employed in the analysis of dichotomous variables. The mean age was 35.3, 39.7, 37.1, and 29.5 years in the minimal/no, low, medium[12], and high exposure groups, respectively. The small number of workers in the exposed groups limited the ability to evaluate the effects of dichloromethane exposure on health outcomes related to age, since age had to be adjusted in these analyses. The racial distribution did not differ among the exposure groups.

The authors indicated the only null hypothesis that could not be accepted based on the data was the hypothesis of CNS symptoms. However, it should be noted that the small size and younger age distribution in the high exposure group and the lack of adjustment for age in most of the analyses make it difficult to interpret the statistical testing that was performed; age is similar among the three other groups, so there is less of an issue with respect to potential confounding when comparing the prevalence among the minimal/no, low and medium exposure groups. Among these three groups, there was some indication of an increase in two of the liver enzymes (serum gamma glutamyl transferase and serum AST), but not in serum ALT. Data pertaining to neurological, hepatic, and cardiac function are shown in Table D-6. Among the six neurological symptoms evaluated, a statistically significant positive exposure-effect relationship between dizziness/vertigo and dichloromethane exposure was identified. This trend was driven most strongly by the low frequency of this reported symptom in the minimal/no exposure group (1.2%), but there was no linear trend across the higher levels of exposure (7.5, 2.1, and 5.3% in the low, medium, and high exposure groups, respectively).

[12]The "medium" exposure group is also referred to as the "intermediate" exposure group in Kolodner et al. (1990).

Table D-6. Clinical findings in male plastic polymer workers[a]

	Exposure group			
	Minimal or no (n = 772)	**Low (n = 56)**	**Medium (n = 49)**	**High (n = 19)**
Mean 8-hr TWA exposure	<1.0	3.3	10.9	49.0
Mean age	35.3	39.7	37.1	29.5
Neurological				
Headache	8.7	7.5	10.4	5.3
Lightheadedness	2.9	3.8	4.2	5.3
Dizziness/vertigo	1.2	7.5	2.1	5.3
Ataxia	0.0	1.9	0.0	0.0
Babinski	0.0	0.0	0.0	0.0
Gait	0.0	0.0	0.0	0.0
Faintness/syncope[b]	0.1	0.0	2.1	0.0
Seizures[b]	0.4	0.0	2.1	0.0
Paresis/paralysis[b]	0.7	0.0	0.0	0.0
Parasthesis[b]	4.0	7.5	14.6	0.0
Head trauma/concussion[b]	0.8	1.9	0.0	0.0
Peripheral motor exam[b,c]	0.5	0.0	0.0	0.0
Peripheral sensory exam[b,c]	1.1	2.4	5.1	0.0
Rhomberg exam[b,c]	0.0	0.0	2.6	0.0
Hepatic				
Serum gamma glutamyl transferase	8.0	16.1	12.2	5.3
Serum total bilirubin	3.0	1.8	2.0	10.0
Serum AST	1.8	3.6	4.1	0.0
Serum ALT	9.1	10.7	8.2	5.3
Cardiac[d]				
Palpitations: percent abnormal	1.2	9.1	2.1	0.0
Electrocardiogram				
Borderline/abnormal	18.5	16.7	19.1	8.3
Bradycardia/tachycardia abnormalities	20.2	16.7	25.5	0.0
General rhythm abnormalities	12.0	11.1	17.0	8.3
Atrial, atrioventricular, or sinus abnormalities	0.8	0.0	0.0	0.0
Bundle blocks or ventricular abnormalities	3.9	5.6	10.6	8.3
Axis deviations	2.6	1.9	2.1	8.3
Wave abnormalities	4.0	3.7	10.6	0.0
Hypertrophy	3.8	3.7	6.4	0.0
Evidence of infarction	2.3	5.6	2.1	0.0

[a] Percent reporting neurologic symptoms or displaying abnormal values in measures of neurological function, hepatic function, and cardiac function

[b] The authors considered these to be screening variables rather than hypothesis-testing variables.

[c] n = 629, 42, 39, and 14 in minimal, low, medium, and high groups, respectively.

[d] n = 728 (727 for bradycardia/tachycardia), 54, 47, and 12 in minimal, low, medium, and high groups, respectively.

Source: Kolodner et al. (1990).

Soden (1993) compared health-monitoring data from dichloromethane-exposed workers in the Rock Hill triacetate fiber production plant to workers from another plant making polyester fibers owned by the same company in the same geographic area. Exposed and control workers were chosen from among workers who had worked at least 10 years in their respective areas and who participated in the company's health-monitoring program between 1984 and 1986 and were still employed on December 31, 1986. Controls were matched by race, age, and gender to each Rock Hill worker for a sample size of 150 and 260 in the exposed and control groups, respectively. (The aim of the study had been 1:2 matching.) The 8-hour TWAs among the Rock Hill workers were those reported by Lanes et al. (1990), namely 475 ppm for dichloromethane, 900 ppm for acetone, and 100 ppm for methanol. None of these exposures occurred at the polyester plant. There was a 90% participation rate in the health-monitoring program. Six questions in the health history portion of the health-monitoring program concerned cardiac and neurological symptoms (chest discomfort with exercise; racing, skipping, or irregular heartbeat; recurring severe headaches; numbness/tingling in hands or feet; loss of memory; dizziness). Part of this program included blood samples used for standard clinical hepatic and hematologic parameters: serum ALT, AST, total bilirubin, and hematocrit. The clinical measures were available for 90 (60%) of the exposed and 120 (46%) of the control group; some participants declined this part of the health-monitoring program because similar tests had been part of recent personal medical care. There was little difference in the frequency of reported symptoms between exposed workers and controls: chest discomfort reported by 2.0% of exposed and 4.0% of the controls, irregular heartbeat reported by 5.5% of exposed and 6.0% of the controls, recurring severe headaches reported by 3.5% of exposed and 5.5% of the controls, numbness/tingling in hands and feet reported by 6.4% of exposed and 8.1% of the controls, loss of memory reported by 1.3% of exposed and 0.4% of the controls, and dizziness reported by 2.7% of exposed and 4.8% of the controls (Soden, 1993). The levels of the blood values were similar in the exposed and control groups, except for a 3.1 IU/L decrease in serum AST activity ($p = 0.06$). The authors concluded that this difference was not clinically significant. They did not discuss the potential bias introduced by the selective participation in this part of the study.

Ott et al. (1983a, b, e) evaluated several parameters of hepatic and hematopoietic function in workers exposed to dichloromethane in a triacetate fiber production plant in Rock Hill, South Carolina. Two hundred sixty-six Rock Hill workers and a comparison group of 251 workers in an acetate fiber production plant in Narrows, Virginia were included in the examination of urinary and blood measures. These groups included men and women, blacks and whites, and smokers and nonsmokers. The median 8-hour TWA exposure for dichloromethane ranged from 60 to 475 ppm in Rock Hill. Acetone at levels up to >1,000 ppm was present in both plants, but dichloromethane and acetone exposures were inversely related.

There were differences in blood collection procedures between the two plants and in the age, sex, race, and smoking history distribution of the study groups. The demographic and

smoking differences were accounted for in the analysis by stratification. Statistically significant differences were seen between the workers in the two plants for COHb, serum alanine aminotransferase (ALT), total bilirubin, and mean corpuscular hemoglobin concentration (MCHC) (although the direction and magnitude of these differences were not reported, and the authors stated that the difference in serum ALT could be due to the differences in blood collection procedures, which involved a sitting versus recumbent position of the subjects at the exposed and nonexposed plants, respectively) (Ott et al., 1983a). Within the Rock Hill plant, analyses were also conducted to examine associations between dichloromethane exposure and the clinical parameters within specific race-sex groups by using multiple regression to control for smoking status, age, and time of venipuncture. Positive associations were seen with COHb in all race-sex groups (increases of 0.7–2.1% per 100 ppm increase in dichloromethane) and with total bilirubin (increases of 0.05–0.08 mg/dL per 100 ppm increase in dichloromethane) in all groups except nonwhite men (which was a much smaller group, n = 20, than the other groups). Red cell count, hematocrit, hemoglobin, and aspartate aminotransferase (AST) were also positively associated with dichloromethane exposure in white females. The increase in total bilirubin level was not supported by parallel changes in other measures of liver function or red blood cell turnover, suggesting that this measure was not reflecting liver damage or hemolysis.

The increased red cell count, hemoglobin, and hematocrit in women exposed to high levels of dichloromethane (up to 475 ppm, 8-hour TWA) may indicate a compensatory hematopoietic effect. The fact that these changes were not significant among men may be due to higher baseline hemoglobin, which was observed when comparisons were made between nonsmoking men and women. No such difference in baseline values was observed among the smoking men and women, suggesting that a compensatory advantage may be lost in smokers.

D.5. STUDIES OF REPRODUCTIVE OUTCOMES

Pregnancy outcomes in women exposed to dichloromethane have been investigated in two studies. Taskinen et al. (1986) studied spontaneous abortions among women employed in eight pharmaceutical factories between 1973 and 1980. Data on pregnancy outcomes were collected from a national hospital and clinic discharge registry in Finland from 1973 to 1981 by matching the worker rosters to the registry. Exposure to dichloromethane was one of eight solvents or classes of solvents included in the study. The study consisted of two parts. The first investigated the rate of spontaneous abortions (number of spontaneous abortions divided by the sum of spontaneous abortions and births) during, before, or after employment in the pharmaceutical industry. One hundred and forty-two spontaneous abortions and 1,179 births were identified among the female workers at the eight plants. Employment hire and termination dates were obtained from plant records. The spontaneous abortion rate was 10.9% during employment compared with 10.6% before and after employment. These results compared to a rate of 8.5% in the general population in the geographic area where the factories were located.

The rate of spontaneous abortions among workers declined over the period of the study, with a 3-year moving average of 15% at the beginning declining to 9.5% at the end of the study. Over the same period, the industrial hygiene was said to have improved in the plants. Ten congenital malformations of different types were identified among the women (five among those who were employed in the pharmaceutical industry during the pregnancy and five among those whose pregnancies occurred before or after this employment).

The second part of the study by Taskinen et al. (1986) was a case-control study of the risk of spontaneous abortions in relation to workplace exposures during pregnancy. The source population consisted of women who were employed in one of the eight Finnish pharmaceutical factories during at least 1 week of the first trimester of pregnancy during the study period. Cases (n = 44) were selected from this population based on hospital or clinic records indicating a spontaneous abortion, and 130 controls (women who had given birth) were age-matched (3:1 matching; age within 2.5 years) to each case. Occupational exposure data were obtained by questionnaires completed by the plant physician or the nursing staff, blinded to the case status of the study member, in consultation with labor protection chiefs and department foremen. The questionnaire requested information about job history and job tasks, exposure to eight specific solvents or classes of solvents (aliphatic solvents, alicyclic solvents, toluene, xylene, benzene, chloroform, dichloromethane, and other solvents), antineoplastic agents, carcinogens, hormones, antibiotics, heavy lifting, known chronic diseases, acute diseases during pregnancy, smoking status, and previous pregnancies. Exposure frequency to each solvent was based on the cumulative weighted sum of the number of days/week the woman was exposed to the solvent. While overall response to the questionnaire was 93%, less than half the questionnaires contained information about smoking or previous pregnancies, precluding inclusion of these variables in the analysis. The distribution of broad categories of occupations (i.e., pharmaceutical workers and packers, laboratory assistants) was similar in both groups. However, exposure to each of the solvents was higher in the cases compared with controls, and the results for dichloromethane were relatively strong. For dichloromethane, the prevalence of exposure was 28.9 and 14.3% in cases and controls, respectively, resulting in an odds ratio (OR) of 2.3 (95% CI 1.0–5.7). There was also evidence of an increasing risk with higher exposure frequency, with an OR of 2.0 (95% CI 0.6–6.6) with exposures of less than once a week and 2.8 (95% CI 0.8–9.5) with exposures of once a week or more. An association was also seen with exposure to four or more solvents (OR 3.4, [95% CI 1.0–12.5]). Weaker associations were seen with other specific solvents (e.g., chloroform, toluene).

Bell et al. (1991) investigated the relation between birth weight and maternal exposure to airborne dichloromethane as a result of living around the triacetate film facility in Rochester, New York. For this population-based cross-sectional study, birth certificates were obtained for all births in 1976–1987 in Monroe County, where the triacetate film facility is located. Multiple births and births of infants weighing <750 grams were excluded. Data abstracted from the

certificate included date of birth, census tract of residence, age, race, educational level of the mother and father, sex, gestational age, multiple births, month of the pregnancy that prenatal care began, total previous births, total previous live births, and conditions present during the pregnancy. An air dispersion modeling system for 250 air emissions, including dichloromethane and predicting average annual ground level concentrations in the surrounding community, was used to assign dichloromethane exposure levels to each birth mother. One of four levels of exposure was assigned to each census tract based on the isopleth of exposure in which more than half of the census tract population resided. Because of the few births among nonwhites that occurred in areas of higher exposure, the study was restricted to whites (n = 91,302). The number of births that occurred in each of the four exposure levels was n = 1,085 in the high-exposure group (50 $\mu g/m^3$ [0.014 ppm]), n = 1,795 in the moderate-exposure group (25 $\mu g/m^3$ [0.007 ppm]), n = 6,044 in the low-exposure group (10 $\mu g/m^3$ [0.003 ppm]), and n = 82,076 in the no-exposure group. At the levels of dichloromethane exposure in this population, no significant adverse effect on birth weight was found. There was an 18.7 g decrease in birthweight (95% CI –51.6, 14.2) in the high- compared with the no-exposure group, adjusting for maternal age, maternal education, parity, previous pregnancy loss, late start of prenatal care, sex of the child, and pregnancy complications. No significant association was found between any combination of exposure levels and birth weight. There was no association between exposure group and risk of a low birthweight infant (i.e., <2,500 g, OR 1.0 [95% CI 0.81–1.2] in the high- compared with the no-exposure group). The authors point out a number of problems with assignment of dichloromethane exposure. It is possible that the dichloromethane exposure was overestimated using the model. Comparisons to ambient air sampling levels collected 6 times/year resulted in the dichloromethane exposure derived from the model being twice as high as the ambient air samples. There was also inaccuracy in the assignment of dichloromethane exposure level to each birth because the exposure assignment was made using the predominant value of the isopleth for a census tract.

Two studies investigated the occurrence of oligospermia among men occupationally exposed to dichloromethane exposure. Kelly (1988) studied 34 men employed in an automotive plant as bonders, finishers, and press operators. These men were self-referred to a health center for a variety of complaints, including neurological symptoms, musculoskeletal symptoms, and shortness of breath. Twenty-six of the men were bonders and eight were finishers or press operators. The job as bonder consisted of dipping hands into an open bucket of dichloromethane and splashing it onto plastic automobile parts. The dichloromethane exposure for bonders averaged 68 ppm with a range of 3.3–154.4 ppm. Eight men, all of whom were bonders, reported symptoms of testicular and epididymal tenderness, with confirmation on medical exam. They ranged in age from 20 to 47 years old and had been bonders for up to 2.9 years. The COHb levels for the eight workers with genital symptoms ranged from 1.2 to 17.3%, with an average of 6.9% anywhere from 4 to 90 hours postexposure. The COHb levels for the two men who

smoked were among the highest, namely 7.3 and 17.3%. Four of the eight workers agreed to provide semen samples; their sperm counts were 2–26 × 10^6/cm^3. The authors stated that men with sperm counts as low as 25 × 10^6/cm^3 may still be fertile, but none of these men had had any children since working with dichloromethane despite not using contraceptives. There was one miscarriage. All four men reported dipping their hands into open buckets of dichloromethane without any protective equipment, and two men reported feeling dizzy, giddy, and high at work.

Based on the results of the Kelly (1988) report, Wells et al. (1989) planned to do a study of oligospermia among 20 exposed workers and 20 unexposed workers to dichloromethane. The exposed workers were nonvasectomized men who had worked for the 3 months prior to recruitment in furniture stripping shops. Eleven men were recruited from among 14 eligible workers at six different shops where dichloromethane was utilized. Names of acquaintances of the exposed were solicited as potential referents. Only one exposed man provided any names. Therefore, the study was redirected as a report on the 11 exposed men. The mean TWA dichloromethane exposure was 122 ppm (range 15–366 ppm) with a mean COHb of 5.8% (range 2.2–13.5%). The mean COHb for smokers, 10.2% (range 8.1–13.5), was higher than for nonsmokers, 3.9% (range 2.2–5.9), and the nonsmoker levels were higher than the 2% level considered to be the upper limit of normal in nonsmoking populations. The mean sperm count was 54 × 10^6/cm^3 (range 23–128 × 10^6/cm^3) compared to a population value of 47 × 10^6/cm^3 for the same geographic area based on samples analyzed at the same laboratory. Using the standard definition for oligospermia of 20 × 10^6/cm^3, none of the 11 workers had oligospermia.

APPENDIX E. SUMMARY OF OTHER (NONCHRONIC) DURATION TOXICITY STUDIES AND MECHANISM STUDIES IN ANIMALS

E.1. SHORT-TERM (2-WEEK) AND SUBCHRONIC STUDIES OF GENERAL AND HEPATIC EFFECTS

E.1.1. Oral and Gavage Studies

Two short-term (2-week) studies examined hepatic and renal effects of dichloromethane exposure in F344 rats (Berman et al., 1995) and CD-1 mice (Condie et al., 1983). Berman et al. (1995) administered dichloromethane by gavage in corn oil for up to 14 days to groups of eight female F344 rats at dose levels of 0, 34, 101, 337, or 1,012 mg/kg-day. Starting at day 4, deaths occurred in the 1,012 mg/kg-day exposure group, with 7 of 8 rats dying before the end of the 14-day exposure period. In the dose groups that did not experience this high mortality, incidences of increased necrotic hepatocytes were 0/8, 0/8, 0/8, and 3/8 for the 0, 34, 101, and 337 mg/kg-day groups, respectively. The increase in liver lesions was not accompanied by increases in serum activities of ALT or AST. Kidneys, spleen, and thymus were also histopathologically examined in this study, but none showed exposure-related lesions. The results indicate that 101 mg/kg-day was a NOAEL and 337 mg/kg-day was a LOAEL for increased incidence of degenerative lesions in female rats exposed for 14 days. In a companion study with groups of eight female F344 rats that were given single doses of 0, 101, 337, 1,012, or 1,889 mg/kg-day, incidences of rats with increased necrotic hepatocytes were 1/8, 0/8, 8/8, 7/8, and 8/8, respectively (Berman et al., 1995).

Condie et al. (1983) detected exposure-related liver lesions in a 14-day gavage study in which dichloromethane in corn oil was administered to male CD-1 mice at dose levels of 0, 133, 333, or 665 mg/kg-day. Incidences of mice with minimal or slight cytoplasmic vacuolation were 1/16, 0/5, 3/5, and 4/5 for the control through high-dose groups, respectively. The kidneys were also examined histopathologically in this study but showed no exposure-related lesions. No other tissues were prepared for histologic examination. Blood urea nitrogen, serum creatinine, and serum ALT activities were not significantly altered by exposure. All dose levels significantly reduced to the same extent the active transport of *p*-aminohippurate into renal cortical slices in vitro, a measure of proximal tubule function. The results most clearly identify 133 mg/kg-day as a NOAEL and 333 mg/kg-day as a LOAEL for increased incidence of hepatocyte vacuolation in male mice.

Kirschman et al. (1986) examined the toxicity of dichloromethane in a 90-day drinking water study in F344 rats (20/sex/dose level). The nominal concentration of dichloromethane in the water was 0.15, 0.45, or 1.5%. Based on BW and water consumption data, average intakes were reported to be 0, 166, 420, or 1,200 mg/kg-day for males and 0, 209, 607, or 1,469 mg/kg-day for females. Clinical chemistry tests (hematological and chemical variables in samples of

blood and urine) and tissue histopathology were evaluated in groups of five rats/sex/dose level after 1 month of treatment. These endpoints were also evaluated in the remaining rats sacrificed after 90 days of exposure.

Exposure to dichloromethane did not affect mortality or cause adverse clinical signs of toxicity. Gross necropsy was also unremarkable. Reported changes in mean values for clinical chemistry variables compared with controls included elevated serum ALT activities for all treated males at 1 month and for the high-dose females at 3 months, elevated serum AST activity in high-dose females at 3 months, elevated serum lactate dehydrogenase activities in mid- and high-dose females at 3 months, and decreases in serum concentrations of fasting glucose, cholesterol, and triglycerides in all exposed groups of both sexes at 1 and 3 months. Actual values for clinical chemistry variables or the magnitude of the changes, however, were not presented in the report.

No histopathologic alterations were seen in tissues after 1 month of treatment (a detailed description of tissues examined was not presented). In rats exposed for 3 months, exposure-related histopathologic changes were restricted to the liver. Elevated, statistically significant incidences of hepatocytic vacuolation were observed in all exposed male and female groups (see Table E-1). The most frequently observed vacuolation was described as generalized and occurring throughout the lobule, and Oil Red-O-staining indicated that most were lipid-containing vacuoles. The incidences of generalized vacuolation scored as mild or moderate were higher in all of the female dose groups compared with the controls. The authors stated that the no-observed-adverse-effect level (NOAEL) based on this study is <200 mg/kg-day and the lowest-observed-adverse-effect level (LOAEL) for males was 166 mg/kg-day. The authors did not explicitly provide a LOAEL for females. The results indicate that 166 mg/kg-day and 209 mg/kg-day were the LOAELs for liver effects in male and female rats, respectively.

Table E-1. Incidences of histopathologic changes in livers of male and female F344 rats exposed to dichloromethane in drinking water for 90 days

Lesion, by sex	Controls	Low dose	Mid dose	High dose
Males—n per group[a]	15	15	15	15
Estimated mean intake (mg/kg-d)	0	166	420	1,200
Number (%) with: Hepatocyte vacuolation (generalized, centrilobular, or periportal)	1 (7)	10[b] (67)	9[b] (60)	7[b] (47)
Generalized vacuolation severity: minimal mild moderate	0 (0) 0 0 0	5[b] (33) 4 0 1	8[b] (53) 7 1 0	6[b] (40) 6 0 0
Centrilobular severity: minimal mild moderate	0 (0) 0 0 0	1 (7) 1 0 0	0 (0) 0 0 0	2 (13) 0 2 0
Hepatocyte degeneration	0 (0)	0 (0)	0 (0)	2 (13)
Focal granuloma	1 (7)	0 (0)	0 (0)	1 (7)
Females—n per group[a]	15	15	15	15
Estimated mean intake (mg/kg-d)	0	209	607	1,469
Number (%) with: Hepatocyte vacuolation (generalized, centrilobular, or periportal)	6 (40)	13[b] (87)	15[b] (100)	15[b] (100)
Generalized vacuolation severity: minimal mild moderate	5 (33) 5 0 0	13[b] (87) 8 4 1	15[b] (100) 6 5 4	15[b] (100) 8 6 1
Centrilobular severity: minimal mild moderate marked	0 (0) 0 0 0 0	0 (0) 0 0 0 0	1 (7) 0 1 0 0	11[b] (28) 2 4 3 2
Hepatocyte degeneration	0 (0)	0 (0)	0 (0)	12[b] (80)
Focal granuloma	0 (0)	0 (0)	4 (27)[c]	6[b] (40)

[a]20 per group; 5 sacrificed at 1 month; these endpoints for the remaining 15 per group.
[b]Statistical significance testing not reported by authors; Fisher's exact test for comparison with control p-value < 0.05 (two-sided).
[c]Statistical significance testing not reported by authors; Fisher's exact test for comparison with control p-value < 0.10 (two-sided). Authors stated LOAEL = 166 mg/kg-day in males but did not explicitly provide LOAEL for females; NOAEL is <200 mg/kg-day.

Source: Kirschman et al. (1986).

Kirschman et al. (1986) conducted a similar 90-day study in B6C3F$_1$ mice (20/sex/dose level). The estimated average intakes were 0, 226, 587, or 1,911 mg/kg-day for males and 231, 586, or 2,030 mg/kg-day for females. Six mice (two controls, two low dose, and two mid dose) died during the study from unknown causes. Administration of dichloromethane did not cause

adverse clinical signs of toxicity or affect food consumption, ophthalmology, or serum ALT activity. Gross necropsy examinations were also unremarkable.

Histopathologic evaluation of tissues from mice killed after 1 month of treatment did not reveal any compound-related effects. Evaluation at 3 months showed subtle generalized or centrilobular changes in the liver (characterized as increased vacuolation with fat deposition), which was evident in all exposed groups and most prominent in mid- and high-dose female groups (Table E-2). The most frequently detected change was characterized as a generalized vacuolation. Some evidence was found for an increase in severity of the generalized vacuolation with dichloromethane exposure, but the incidence of this lesion in the control mice was substantial, especially in females (Table E-2). Incidences for centrilobular vacuolation were significantly increased only for the mid-dose female group. No other changes were found.

Using the results from this study to select doses for a chronic study, Kirschman et al. (1986) expressed the opinion that the mid-dose level (587 mg/kg-day) was the LOAEL in this study. Although incidences for generalized vacuolation were increased in the low- and mid-dose male groups, the incidences in the high-dose groups were not significantly increased compared with controls (Table E-2). The study authors identified a LOAEL of 587 mg/kg-day for centrilobular vacuolation in male B6C3F_1 mice. The NOAEL for males was considered by the investigators to be between 226 and 587 mg/kg-day.

Table E-2. Incidences of histopathologic changes in livers of male and female B6C3F$_1$ mice exposed to dichloromethane in drinking water for 90 days

Lesion, by sex	Controls	Low dose	Mid dose	High dose
Males—n per group[a]	14	14	14	15
Estimated mean intake (mg/kg-d)	0	226	587	1,911
Number (%) with: Hepatocyte vacuolation (generalized, centrilobular, or periportal)	9 (64)	12 (86)	13 (93)	12 (80)
Generalized vacuolation, severity: minimal mild moderate marked	7 (50) 4 2 1 0	12[b] (86) 3 7 2 0	13[b] (93) 9 5 0 0	10 (67) 7 3 0 0
Centrilobular severity: minimal mild moderate	2 (14) 2 0 0	0 (0) 0 0 0	1 (7) 0 0 1	5 (33) 1 3 1
Females—n per group[a]	14	11	13	15
Estimated mean intake (mg/kg-d)	0	231	586	2,030
Number (%) with: Hepatocyte vacuolation (generalized, centrilobular, or periportal)	13 (93)	11 (100)	13 (100)	13 (87)
Generalized vacuolation severity: minimal mild moderate marked	13 (93) 1 8 4 0	11 (100) 3 7 1 0	13 (100) 5 6 2 0	13 (87) 3 6 1 3
Centrilobular severity: minimal mild moderate marked	0 (0) 0 0 0 0	0 (0) 0 0 0 0	5[c] (39) 0 2 3 0	1 (7) 0 1 0 0

[a]20 per group; 5 sacrificed at 1 mo.
[b]Statistical significance testing not reported by authors; Fisher's exact test for comparison with control p-value = 0.10 for low dose group and p = 0.032 for mid-dose group (two-sided).
[c]Statistical significance testing not reported by authors; Fisher's exact test for comparison with control p-value = 0.016 (two-sided). Authors say LOAEL = 587 mg/kg-day; NOAEL between 226 and 587 mg/kg-day for males; not explicitly stated for females.

Source: Kirschman et al. (1986).

E.1.2. Inhalation Studies

Data pertaining to general (e.g., BW, mortality), hepatic, and renal effects from several inhalation exposure studies in various species with exposure periods of 3–6 months are described below. (Studies providing detailed neurological data are described separately in Section 4.4.2 and Appendix E, Section E.6.) One identified study (Heppel et al., 1944) is not discussed in further detail because key details (e.g., sex, strain of animals) were not presented and subsequent studies provide more informative data. Two 14-week studies in dogs, monkeys, rats, and mice were conducted with exposures at 0, 1,000, and 5,000 ppm (Haun et al., 1972; Weinstein et al., 1972; Haun et al., 1971) and at 0, 25, and 100 ppm (Haun et al., 1972). Neurological effects and hepatic degeneration were seen at the 1,000 ppm dose. In the lower-dose portion of the Haun et al. (1972) study in mice, decreased CYP levels in liver microsomes and some histopathologic liver changes (fat stains and cytoplasmic vacuolation) were seen at 100 ppm, but more obvious adverse effects were not observed. Leuschner et al. (1984) reported data from a high exposure (10,000 ppm) 90-day study of rats; beagle dogs were also included in this study at an exposure level of 5,000 ppm. No evidence of toxicity was reported by the authors of this study. In a 13-week exposure study conducted by NTP (1986), decreased BWs and increased incidence of foreign body pneumonia were seen at 8,400 ppm in F344 rats, and histologic changes in the liver in B6C3F$_1$ mice were seen at 4,200 ppm.

Haun et al. (1972; 1971) and Weinstein et al. (1972) reported results from studies in which groups of 8 female beagle dogs, 4 female rhesus monkeys, 20 male Sprague-Dawley rats, and 380 female ICR mice were continuously exposed to 0, 1,000, or 5,000 ppm dichloromethane for up to 14 weeks in whole-body exposure chambers. Gross and histopathologic examinations were scheduled to be made on animals that died or were sacrificed during or at termination of the study. At 5,000 ppm, obvious nervous system effects (e.g., incoordination, lethargy) were observed in dogs, monkeys, and mice. At 1,000 ppm, these effects were most apparent in dogs and monkeys (Haun et al., 1971). Food consumption was reduced in all species at 5,000 ppm and in dogs and monkeys at 1,000 ppm. All exposed animals either lost weight or showed markedly decreased BW gains compared with controls. For example, rats exposed to 1,000 or 5,000 ppm for 14 weeks showed average BWs that were roughly 10 and 20% lower than control values. Significant numbers of dogs (4) and mice (123), as well as 1 monkey, died within the first 3 weeks of exposure to 5,000 ppm. Because of this high mortality, all surviving 5,000 ppm animals were sacrificed at 4 weeks of exposure, except for half (10) of the rats that went on to survive the 14-week exposure period. At 1,000 ppm, 6/8 dogs died by 7 weeks, at which time the remaining two were sacrificed. Monkeys, rats, and all but a few mice survived exposure to 1,000 ppm for 14 weeks.

Gross examination of tissues showed yellow, fatty livers in dogs that died during exposure to 1,000 or 5,000 ppm, "borderline" liver changes in 3 monkeys exposed to 5,000 ppm, and mottled liver changes in 4/10 rats exposed to 5,000 ppm for 14 weeks (Haun et al., 1971).

Comprehensive reporting of the histologic findings from this study were not available, but Haun et al. (1972) reported that the primary target organ was the liver and that in some exposed animals, the kidney was also affected. Light and electron microscopy of liver sections from groups of 4–10 mice sacrificed after 1, 4, 8, and 12 hours and 1, 2, 3, 4, 6, and 7 days of exposure to 5,000 ppm showed hepatocytes with balloon degeneration (dissociation of polyribosomes and swelling of rough endoplasmic reticulum) as early as 12 hours of exposure (Weinstein et al., 1972). The degeneration peaked in severity after 2 days of exposure and subsequently partially reversed in severity. Information on possible histopathologic changes in mice exposed to 1,000 ppm was not provided.

The results from this study demonstrate that dogs and mice were more sensitive than rats and monkeys to lethal effects, nervous system depression, and possibly liver effects from continuous exposure to 1,000 or 5,000 ppm. The results indicate that continuous exposure to 1,000 ppm was an adverse effect level for mortality and effects on the nervous system and liver in dogs (exposed for up to 4 weeks) and for BW changes in rats (exposed for 14 weeks). The 5,000 ppm level induced mortality in beagle dogs, ICR mice, and rhesus monkeys (but not in Sprague-Dawley rats); obvious nervous system effects in dogs, mice, monkeys, and rats; and gross liver changes in dogs, mice, monkeys, and rats.

Haun et al. (1972) also conducted studies with groups of 20 mice, 20 rats, 16 dogs, and 4 monkeys exposed continuously to 0, 25, or 100 ppm dichloromethane for 100 days (14 weeks). The animals presumably were of the same strains and sexes as those used in the studies involving exposure to 1,000 or 5,000 ppm dichloromethane (Haun et al., 1972; Weinstein et al., 1972; Haun et al., 1971). All animals underwent necropsy and histopathologic evaluation at termination of the exposure, but a list of the tissues examined and incidence or severity data were not presented in the report. Hematology and clinical chemistry variables (including COHb levels) were measured in blood samples collected at biweekly or monthly intervals during exposure. COHb levels were elevated in a dose-related manner in monkeys and peaked at about 5% (approximately 0.8% preexposure) after 6 weeks of exposure. COHb levels in dogs were unaffected by the 25 ppm exposure level and rose to about 2% (from about 0.6%) from week 4 in high-dose dogs. Additional groups of mice were included for assessment of hexobarbital sleep times at monthly intervals levels of cytochromes P-450, P-420, and b_5 in liver microsomes at monthly intervals and spontaneous physical activity at several intervals during the study.

No clinical signs of toxicity or alterations in weight gain were seen in any of the species examined. In dogs and monkeys, hematology and clinical chemistry results throughout the study and at termination were unremarkable, as were the results of the gross and histopathologic examinations. In mice exposed to 100 ppm, CYP levels in liver microsomes were significantly decreased (compared with control values) after 30, 60, and 90 days of exposure to 100 ppm, whereas levels of cytochrome b_5 and P-420 decreased after 30 days and increased after 90 days of exposure. At 25 ppm, no significant differences from controls were seen in mouse liver levels

of cytochromes. Mice exposed to 25 ppm showed no histopathologic changes, while histologic changes in mice at 100 ppm were restricted to positive fat stains and some cytoplasmic vacuolation in the liver. In rats at both exposure levels, the livers showed positive staining for increased fat, and the kidneys showed evidence of nonspecific tubular degenerative and regenerative changes. Haun et al. (1972) indicate that no distinctively adverse effects were found in monkeys, dogs, rats, or mice continuously exposed to 25 or 100 ppm for up to 14 weeks. Decreased CYP levels in liver microsomes and some histopathologic liver changes (fat stains and cytoplasmic vacuolation) were seen at the 100 ppm dose.

Leuschner et al. (1984) exposed Sprague-Dawley rats (20/sex/dose level) to 0 or 10,000 ppm and beagle dogs (3/sex/dose level) to 0 or 5,000 ppm dichloromethane in whole-body exposure chambers. Exposure periods were 6 hours/day for 90 consecutive days. Endpoints evaluated in both species included clinical signs, food and water consumption, BW, hematology, clinical chemistry, urinalysis, and gross and microscopic evaluation of 27 organs at termination. Electrocardiography and blood pressure measurements were also done in dogs. The only significant effect observed in rats was a slight redness of the conjunctiva 1–10 hours after each exposure. In dogs, compound-related effects were restricted to slight sedation throughout the exposure period and slight erythema lasting up to 10 hours after exposure. In this 90-day study involving daily 6-hour exposures, 10,000 and 5,000 ppm were NOAELs for behavioral, clinical chemistry, hematologic, and histologic signs of toxicity in Sprague-Dawley rats and beagle dogs, respectively.

NTP (1986) exposed groups of F344 rats and B6C3F$_1$ mice (10/sex/dose level) to target concentrations of 0, 525, 1,050, 2,100, 4,200, or 8,400 ppm dichloromethane 6 hours/day, 5 days/week for 13 weeks in whole-body exposure chambers. Endpoints monitored included clinical signs, BW, and necropsy at termination. Comprehensive sets of tissues and organs in control and high-dose animals were histologically examined; tissues from the lower dose groups were examined to determine the no-observed-effect level. One male and one female rat from the 8,400 ppm exposure group died before the end of the study, but the cause of death was not discussed. The final mean BWs of 8,400 ppm male and female rats were reduced by 23 and 11%, respectively, relative to controls. Foreign-body pneumonia was present in 4/10 male and 6/10 female rats exposed to 8,400 ppm and in 1/10 female rats from the 4,200 ppm exposure group. The liver lipid/liver weight ratios for 8,400 ppm rats of both sexes and 4,200 ppm female rats were significantly lower than in controls. In mice, 4/10 males and 2/10 females exposed to 8,400 ppm died before the end of the study, and these deaths were considered treatment-related. Histologic changes in exposed mice consisted of hepatic centrilobular hydropic degeneration (of minimal to mild severity) in 3/10 males and 8/10 females at 8,400 ppm and in 9/10 females from the 4,200 ppm exposure group. Histologic changes in the 2,100 ppm mouse group were not mentioned. The liver lipid/liver weight ratio for the high-dose female mice was significantly lower than in controls. In this 13-week study involving 6-hour exposure periods for

5 days/week, 4,200 ppm was a NOAEL and 8,400 ppm was a LOAEL for decreased BWs and increased incidence of foreign-body pneumonia in F344 rats. In $B6C3F_1$ mice, 2,100 ppm was a NOAEL and 4,200 ppm was a LOAEL for histologic changes in the liver.

E.2. REPRODUCTIVE TOXICITY STUDIES

E.2.1. Gavage and Subcutaneous Injection Studies

In a study sponsored by the General Electric Company (1976), Charles River CD rats (10/sex/dose level) were administered 0, 25, 75, or 225 mg/kg-day dichloromethane by gavage in water for 90 days. The test material was dichloromethane (of unspecified purity) purchased from Dow Chemical Company. At approximately 100 days of age, the rats were mated 1 to 1 to produce the F1 generation. F1 rats (15/sex/dose level) received the same treatment as F0 for 90 days, at which time they were sacrificed and necropsied. Comprehensive sets of 24 tissues from 10 male and 10 female F1 rats from the control and 225 mg/kg-day groups were examined microscopically after embedding, sectioning, and staining. F1 rats were monitored for clinical signs, BW effects, and food consumption. Reproductive parameters examined were fertility index, number of pups per litter, and pup survival. F1 rats also underwent hematology and clinical chemistry tests and urinalysis at 1, 2, and 3 months of the study and ophthalmoscopic examination at 3 months. There were no significant compound-related alterations in any of the endpoints monitored.

Raje et al. (1988) administered dichloromethane (250 or 500 mg/kg) by subcutaneous injection 3 times/week for 4 weeks to male Swiss-Webster mice (20/group). Mating with unexposed females started 1 week after the last exposure and continued for 2 weeks. After the mating period, the males were sacrificed and the testes were examined microscopically. On GD 17, the females were sacrificed and the uterine horns examined for live, dead, or resorbed fetuses. The authors reported that exposure to dichloromethane had no statistically significant effects on number of litters, implants/litter, live fetuses/litter, percent dead/litter, percent resorbed/litter, or fertility index. Examination of the testes showed no significant alterations compared with controls.

E.2.2. Inhalation Studies

Nitschke et al. (1988b) conducted a two-generation reproductive toxicity study in rats. Groups of F344 rats (30/sex/dose level) were exposed by inhalation in whole-body chambers to 0, 100, 500, or 1,500 ppm dichloromethane (99.86% pure) 6 hours/day, 5 days/week for 14 weeks and then mated to produce the F1 generation. Exposure of dams continued after mating on GDs 0–21 but was interrupted until PND 4. After weaning, 30 randomly selected F1 pups/sex/dose level were exposed as the parental generation for 17 weeks and subsequently mated to produce the F2 generation. The results showed no statistically significant exposure-related changes in reproductive performance indices (fertility, litter size), neonatal survival,

growth rates, or histopathologic lesions in F1 (Table E-3) or F2 weanlings sacrificed at time of weaning. None of the values in Table E-3 were significantly different from control values using a $p = 0.05$ level of statistical significance, supporting a NOAEL of 1,500 ppm for the effects examined in this study.

Table E-3. Reproductive outcomes in F344 rats exposed to dichloromethane by inhalation for 14 weeks prior to mating and from GDs 0–21

	Exposure (ppm)[a]			
	0	**100**	**500**	**1,500**
Fertility index[b]	77%	77%	63%	87%
Gestation index[c]	100%	100%	100%	100%
Gestation survival index[d]	99.6%	100%	100%	96.6%
4-d survival index[e]	91.0%	95.2%	98.5%	98.6%
28-d survival index[f]	99.4%	99.4%	100%	99.5%
Sex ratio on d 1 (M:F)	48:52	50:50	50:50	52:48
Litter size				
D 0	11 ± 2	10 ± 2	10 ± 3	11 ± 2
D 28	7 ± 2	7 ± 2	7 ± 2	8 ± 2
Pup BWs, g				
D 1	5.2 ± 0.4	5.3 ± 0.5	5.3 ± 0.4	5.2 ± 0.4
D 4	7.4 ± 0.7	7.5 ± 1.1	7.7 ± 0.7	7.3 ± 0.7
D 28, male	44.6 ± 5.8	45.9 ± 5.0	47.0 ± 5.4	45.0 ± 5.9
D 28, female	43.2 ± 4.3	43.8 ± 4.5	44.4 ± 5.7	43.0 ± 4.8

[a]100 ppm = 347 mg/m^3, 500 ppm = 1,737 mg/m^3, 1,500 ppm = 5,210 mg/m^3.
[b]Number of females delivering a litter expressed as a percentage of females placed with a male.
[c]Number of females delivering a live litter expressed as a percentage of the number of females delivering a litter.
[d]Percentage of newborn pups that were alive at birth.
[e]Percentage of pups surviving to day 4.
[f]Percentage of pups alive on day 4 and surviving to day 28.

Source: Nitschke et al. (1988b).

Raje et al. (1988) exposed groups of male Swiss-Webster mice (20/group) to 0, 100, 150, or 200 ppm dichloromethane (HPLC grade, JT Baker Chemical Co.) in inhalation chambers 2 hours/day, 5 days/week for 6 weeks. Mating with unexposed females started 2 days after the last exposure. As in the subcutaneous injection protocol described in the previous section, after the 2-week mating period, the males were sacrificed and the females were sacrificed on GD 17. Exposure of the male mice to dichloromethane had no statistically significant effects on number of litters, implants/litter, live fetuses/litter, percent dead/litter, or percent resorbed/litter, and no significant alterations in the testes were noted. The fertility index was 95, 95, 80, and 80% in the control, 100, 150, and 200 ppm groups, respectively. This decrease was not statistically significant as reported by the authors. Details of the statistical analyses were not provided. The

overall χ^2 p-value was 0.27. Using a Cochran-Armitage exact trend test on these data, EPA calculated a one-sided p-value of 0.059. Individual p-values for the comparison of each group with the control group were 0.97, 0.17, and 0.17 for the 100, 150, and 200 ppm groups, respectively. The results for the combined 150 and 200 ppm groups were statistically different from the combined controls and 100 ppm group (Fisher's exact test, one-sided p-value = 0.048), suggesting a NOAEL of 100 ppm and LOAEL of 150 ppm.

E.3. DEVELOPMENTAL TOXICITY STUDIES

E.3.1. Gavage Studies and Culture Studies

Narotsky and Kavlock (1995) evaluated developmental effects of dichloromethane (99.9% pure) in F344 rats (17–21/dose group) treated with 0, 337.5, or 450 mg/kg-day dichloromethane by gavage in corn oil on GDs 6–19. Dams were weighed on GDs 6, 8, 10, 13, 16, and 20 and allowed to deliver naturally. They were sacrificed on PND 6 to count uterine implantation sites. Pups were grossly examined for developmental abnormalities and weighed on PNDs 1, 3, and 6. Dead pups or pups with no gross abnormalities were sacrificed and examined for soft tissue abnormalities. Maternal weight gain during pregnancy was significantly reduced in high-dose dams (by 33%, as estimated from Figure 5 of the paper); this group also exhibited rales and nasal congestion. Treatment with dichloromethane did not induce resorptions or alter the number of live litters on PND 1 or 6, the number of implants, the number of live pups on PND 1 or 6, or pup weight per litter. No gross or soft tissue abnormalities were observed.

Rat embryos in culture medium were exposed to 0, 3.46, 6.54, 9.79, or 11.88 μmol/mL dichloromethane for 40 hours. At the end of the exposure, embryos were observed for development of yolk sac vasculature, crown-rump length, total embryonic protein content, and number of somite pairs. A concentration of dichloromethane of 6.54 μmol/mL of culture medium resulted in decreased crown-rump length, decreased somite number, and decreased amount of protein per embryo, whereas no effects were seen at 3.46 μmol/mL (Brown-Woodman et al., 1998). A time-course experiment conducted with a dichloromethane concentration of 9.22 μmol/mL showed that marked differences in growth and development from controls were not significant until about 8 hours of culture. Brown-Woodman et al. (1998) noted that the concentrations that caused embryotoxicity in this study were much higher than those found in individuals studied under controlled exposure conditions and comparable to those found in postmortem blood after fatal inhalation.

E.3.2. Inhalation Studies

Schwetz et al. (1975) exposed pregnant Swiss-Webster mice (30–40/group) and Sprague-Dawley rats (20–35/group) by inhalation in whole-body chambers to 0 or 1,250 ppm dichloromethane (97.86% pure) 7 hours/day on GDs 6–15. Maternal BWs were recorded on GDs 6, 10, and 16 and on the day of sacrifice (GD 18 for mice, GD 21 for rats). At sacrifice,

uterine horns were excised and examined for fetal position and number of live, dead, or absorbed fetuses. Fetuses were observed for gross, soft tissue, and skeletal abnormalities. The only effects seen on developing fetuses were changes in the incidence of minor skeletal variants. In rats, the incidence of lumbar ribs or spurs was significantly decreased compared with controls, whereas the incidence of delayed ossification of sternebrae was significantly greater than in controls. In mice, a significant number of litters contained pups with a single extra center of ossification in the sternum. Exposure to dichloromethane produced significantly elevated blood COHb content in dams of both species (approximately 9–10% after 10 exposures versus 1–2% in controls). BWs in exposed mouse dams were significantly increased (11–15%) compared with those in controls but were not affected in exposed rat dams. Mean absolute liver weights of exposed dams of both species were significantly elevated compared with controls, but mean relative liver weights were not affected. The results indicate that 1,250 ppm was a LOAEL for minimal maternal effects (increased COHb and increased absolute liver weight) and adverse effects on the fetuses.

Hardin and Manson (1980) conducted a study in female Long-Evans rats to determine whether exposure before and during gestation is more detrimental to reproductive outcome than exposure either before or during gestation alone. Four groups of 16–21 rats were formed in which the rats were exposed by inhalation in whole-body chambers to either filtered air or to 4,500 ppm dichloromethane (technical grade, >97% pure) 6 hours/day for 12–14 days before breeding and/or on GDs 1–17. Maternal BWs were measured every 4 days. Dams were euthanized on GD 21, and livers and uteri were removed. Livers were weighed, and uterine horns were examined for fetal position and number of live, dead, or absorbed fetuses. Fetuses were observed for gross, soft-tissue, and skeletal abnormalities. Exposure during gestation (with or without pregestation exposure) significantly increased maternal absolute and relative liver weights by about 10–12 and 9–12%, respectively, and decreased fetal BW by about 9–10% relative to those exposed to filtered air during gestation. None of the groups showed significant alterations in the incidence of gross, external, skeletal, or soft-tissue anomalies.

Using the same study design and exposure level, Bornschein et al. (1980) observed behavioral activities at various ages in the offspring. Assessed activities included head movement/pivoting when placed in a novel environment (4 days of age), limited crawling (10 days), movement in a photocell cage (15 days), use of running wheel (45–108 days), and shock avoidance (4 months). Exposure during gestation (with or without pregestation exposure) caused altered rates of behavioral habituation to novel environments in the pups tested as early as 10 days of age; these altered rates were still present at 150 days of age. Growth, food and water consumption, wheel running activity, and avoidance learning were not significantly affected by exposure to dichloromethane. The results indicate that 4,500 ppm was a LOAEL for maternal effects (10% increased absolute and relative liver weight) and for effects on the fetuses (10% decreased fetal BW and altered behavioral habituation to novel environments).

In a study of early-life (including gestational) exposures, Maltoni et al. (1988) exposed 54 pregnant Sprague-Dawley rats to 100 ppm dichloromethane via inhalation 4 hours/day, 5 days/week for 7 weeks, followed by 7 hours/day, 5 days/week for 97 weeks. Exposure apparently started on GD 12. Groups of 60 male and 69 female newborns continued to be exposed after birth to 60 ppm dichloromethane 4 hours/day, 5 days/week for 7 weeks, followed by exposure 7 hours/day, 5 days/week for 97 weeks. Additional groups of 60 male and 70 female newborn were exposed after birth to 60 ppm dichloromethane 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 8 weeks. BWs were measured every 2 weeks during exposure and every 8 weeks thereafter. At the end of exposure, animals were sacrificed and histologic examinations were performed on 20 tissue types.

Early life exposures of Sprague-Dawley rats to dichloromethane (Maltoni et al., 1988) did not affect mortality or BW in any group. Also, there was no significant effect of exposure to dichloromethane on the percentage of animals with benign and malignant tumors and malignant tumors, the number of malignant tumors per 100 animals, or the percentage of animals with benign mammary tumors, malignant mammary tumors, leukemias, pheochromocytomas, and pheochromoblastomas. The results provide no evidence that gestational exposure to 100 ppm dichloromethane during early life stages of development increases the susceptibility of Sprague-Dawley rats to the potential carcinogenicity of dichloromethane, but further conclusions from these results are precluded because the study included only one exposure level that was below the maximum tolerated dose for adult Sprague-Dawley rats. Experiments comparing cancer responses from early-life exposures with adult exposures are not available for F344 rats or $B6C3F_1$ mice, the strains of animals in which carcinogenic responses to dichloromethane have been observed.

E.4. NEUROTOXICOLOGY STUDIES

E.4.1. Oral Exposures

Moser et al. (1995) conducted neurobehavioral evaluations in female F344 rats following an acute or 14-day oral administration of dichloromethane. A FOB protocol was utilized to determine changes in autonomic parameters (lacrimation, salivation, pupil response, urination, defecation), neuromuscular parameters (gait, righting reflex, forelimb and hind-limb grip strength, landing foot splay), sensorimotor parameters (tail pinch, click response, touch response), excitability measures (handling reactivity, arousal, clonic, and/or tonic movements), and activity (rearing, motor activity). A baseline FOB was performed on all rats prior to initial dichloromethane administration. After dichloromethane administration, a FOB was conducted at selected time points followed by a motor activity test in a maze. In the acute study, rats were dosed with 0, 101, 337, 1,012, or 1,889 mg/kg dichloromethane (8 rats per group). At 4 and 24 hours after the administered dose, rats were tested for the neurological parameters. Significant changes in the neuromuscular and sensorimotor parameters were observed and occurred mostly

in rats administered with the highest dose. These significant changes were only observed at the 4-hour time point and not when measured at 24 hours. The NOAEL identified by the authors for this study was 337 mg/kg based on no observable changes in the FOB. In the 14-day study, rats were administered 0, 34, 101, 337, or 1,012 mg/kg-day. FOB testing was conducted on days 4 and 9 (before the daily dose) and approximately 24 hours after the last (14^{th}) dose. With the exception of the activity measurements, all other neurobehavioral parameters (neuromuscular, sensorimotor, autonomic, excitability) were significantly affected from the 4^{th} day through the entire 14-day exposure cycle. The NOAEL identified for the 14-day study was 101 mg/kg-day based on FOB changes associated with the dichloromethane exposure.

A single dose acute neurophysiology study by Herr and Boyes (1997) evaluated the effect of dichloromethane on FEPs in adult male Long-Evans rats. Rats were implanted with epidural electrodes over the visual cortex area. After placement in an enclosed rectangular mirror chamber, FEPs were stimulated with a 10 μsec flash. Baseline FEPs were collected and rats were injected intraperitoneally with 0 (corn oil, n = 16), 57.5 (n = 15), 115 (n = 15), 230 (n = 14), or 460 (n = 15) mg/kg dichloromethane. Animals were retested at 15 minutes, 1 hour, and 5 hours after injection. Amplitude decreases in the early FEP components were observed. The FEP amplitude changes were time- and dose-dependent with maximal effects at 15 minutes after dichloromethane dosage. All of the waveform amplitudes returned to control levels when measured at the 1-hour time point for all doses tested. Response latencies were still different from controls when measured 5 hours after dosing, but the effect was less pronounced than at the 15-minute and 1-hour time points. In this study, 57.5 mg/kg did not produce any significant changes in the FEP measures as compared to controls and was considered this study's NOAEL. The LOAEL was 115 mg/kg based on changes in the FEP amplitudes.

Kanada et al. (1994) examined the effect of dichloromethane on acetylcholine and catecholamines (dopamine, norepinephrine, serotonin) and their metabolites in the midbrain, hypothalamus, hippocampus, and medulla from male Sprague-Dawley rats (4–5/group) in a neurochemical/neuropathology study. The rats were sacrificed 2 hours after a single gavage dose of 0 or 534 mg/kg of undiluted dichloromethane. Administration of dichloromethane significantly increased the concentration of acetylcholine in the hippocampus by approximately 10% and increased dopamine and serotonin levels in the medulla by approximately 75%. Dichloromethane decreased norepinephrine levels in the midbrain and hypothalamus by 12–15%, and serotonin levels were decreased in the hypothalamus by approximately 30%. There was a trend toward decreased dopamine in the hypothalamus, but the variability between the animals was so high that the effect was not significant. (These values for the percent changes were estimated by EPA from the figures presented in the paper.) The authors speculated that increased acetylcholine release associated with exposure to dichloromethane and other solvents may originate from the nerve terminals.

E.4.2. Inhalation Exposures

Neurobehavioral: spontaneous motor activity—acute and short-term studies. Heppel and Neal (1944) evaluated the neurological effects of 5,000 ppm dichloromethane in five male rats by measuring changes in spontaneous activity during and after exposure. The five rats were not randomly selected, since the investigators indicated they picked the most active animals in the litter. During the 1-hour testing runs, rats were placed in a rotating drum. Spontaneous activity was reported as the number of drum revolutions/hour. Twenty control test runs (1 run/day) were conducted prior to dichloromethane exposure runs. After the preexposure period, rats were exposed to 5,000 ppm dichloromethane every other day for 1 hour, and activity was measured in the same manner as in the control runs. Once dichloromethane exposure was stopped, the animals were allowed to recover for 30 minutes and a second 1-hour test run was performed to evaluate spontaneous activity during recovery. On nonexposure days, spontaneous activity was also measured in 1-hour intervals to compare to the preexposure period. A total of five dichloromethane exposures, five postexposure, and five nonexposure trials were conducted over 10 days. Spontaneous activity significantly declined during exposure, from an average number of revolutions of 576 on nonexposure days to 59 revolutions during dichloromethane exposure ($p < 0.01$, Fisher's t-test)

Weinstein et al. (1972) continuously exposed female ICR mice to 5,000 ppm dichloromethane for up to 7 days. Clinical behavioral observations of the mice were made during dichloromethane exposure. Within the first few hours of exposure, spontaneous activity increased in comparison to control animals. After 24 hours of continuous exposure, there was a considerable decrease in spontaneous activity as noted by observation only. The mice also appeared to be very lethargic and had a hunched posture and a rough hair coat, which are all signs of CNS depressive effects in rodents. These effects became progressively worse until after 96 hours of exposure, where many mice resumed normal activity. After the 7-day exposure, mice were nearly as active as the control animals but had a rougher coat and were judged to be emaciated and dehydrated.

Male Wistar rats exposed to 500 ppm dichloromethane 6 hours/day for 6 days exhibited an increase in preening frequency and time 1 hour after the last exposure relative to controls (Savolainen et al., 1977). However, there were no significant changes in other types of spontaneous activity.

In the study by Kjellstrand et al. (1985), male NMRI mice were exposed to dichloromethane concentrations ranging from 400 to 2,500 ppm. At concentrations $\geq$ 600 ppm, exposures for 1 hour produced a biphasic pattern of activity characterized by an initial increase in activity [as high as 200% of preexposure motor activity at 2,200 ppm, as estimated from Figure 6 in Kjellstrand et al. (1985)], during exposure, followed by a decrease that reached the lowest point 1–2 hours after the end of exposure (as low as 40% motor activity at 2,200 ppm in comparison to preexposure). Motor activity returned to normal levels after the decreased activity

observed 1–2 hours after exposure was stopped, indicating that the effect was reversible in this study design.

Neurobehavioral: spontaneous motor activity—subchronic (14 week) studies. Haun et al. (1971) reported results from studies in which female beagle dogs, female rhesus monkeys, male Sprague-Dawley rats, and female ICR mice were continuously exposed to 0, 1,000, or 5,000 ppm dichloromethane for up to 14 weeks in whole-body exposure chambers. Gross and histopathologic examinations were made on animals that died or were sacrificed during or at termination of the study. At 5,000 ppm, obvious nervous system effects (e.g., incoordination, lethargy) were most apparent in dogs and were also observed in monkeys and mice. Rats did not demonstrate any of these sedative effects. At 1,000 ppm, these effects were observed to a lesser extent in monkeys and mice, but dogs still displayed prominent CNS depressive behavior. Histopathologic analysis revealed edema of the brain in three dogs that died during exposure to 5,000 ppm dichloromethane. No other gross brain-related changes were reported. The results indicate that continuous exposure to 1,000 ppm was an adverse effect level for mortality and effects on the nervous system and liver in dogs (exposed for up to 4 weeks) and for BW changes in rats (exposed for 14 weeks). The 5,000 ppm level induced mortality in beagle dogs, ICR mice, and rhesus monkeys (but not Sprague-Dawley rats); obvious nervous system effects in dogs, mice, monkeys, and rats; and gross liver changes in dogs, mice, monkeys, and rats.

In the study by Thomas et al. (1972), female ICR mice (10/group) were exposed continuously to 0, 25, or 100 ppm dichloromethane for 14 weeks. Spontaneous activity of mice was evaluated by using closed circuit television for monitoring. Mice were evaluated in daily 2-hour testing sessions. The 25 and 100 ppm exposure groups were tested for 2 weeks prior to the onset of dichloromethane exposure. Starting at week 9, mice exposed to 25 ppm dichloromethane exhibited increases in spontaneous activity, but no quantitative measurements or statistical analyses were reported. The authors stated that no significant effect was observed in the group exposed to 100 ppm.

Neurobehavioral: FOB—subchronic (13 week) study. Only one study, a 13-week inhalation study in F344 rats (Mattsson et al., 1990), has conducted an FOB testing paradigm following a subchronic exposure to dichloromethane. Groups of rats (12/sex/exposure level) were exposed to 0, 50, 200, or 2,000 ppm dichloromethane 6 hours/day, 5 days/week for 13 weeks. An additional group of rats was exposed to 135 ppm CO to induce approximately 10% COHb, approximately the level produced by saturation of oxidative metabolism of dichloromethane. After the 13 weeks of exposure (beginning 65 hours after the last exposure), rats were subjected to an FOB to evaluate any neurobehavioral changes from the dichloromethane exposure. Autonomic parameters were first characterized. Then, the rat was placed in a clear plastic box to evaluate locomotor activity and then responsiveness to touch,

sharp noise, and tail pinch. Hind-limb grip strength was also measured by using a strain gauge. All animals were examined clinically at weekly intervals and were tested at the end of the exposure period by FOB, grip strength, BW, temperature, and sensory-evoked potentials. No exposure-related effects were observed on the FOB, grip strength, or sensory-evoked potentials. No histopathologic changes were noted in brains, spinal cords, or peripheral nerves from the high-dose dichloromethane group (or the CO exposure group) compared with control animals. In the absence of changes, lower concentrations were not examined.

Neurobehavioral: learning and memory—acute study. In a study by Alexeef and Kilgore (1983), a learning and memory evaluation was conducted following acute exposure to dichloromethane. Mice were exposed to 168 mg/L (~47,000 ppm) dichloromethane and were tested for learning ability by using a passive-avoidance conditioning task. Male Swiss-Webster mice (3, 5, and 8 weeks old) were used in this study. In the passive avoidance task, mice were placed on a metal platform that extended into a hole. If the mouse went into the hole (a darkened area, which would be the preferred area for the mouse), it received a foot shock. Prior to the training session, mice were exposed to either air or ~47,000 ppm dichloromethane. Animals were exposed to dichloromethane until there was a loss of the righting reflex, which would take about 20 seconds on average, and then placed back in their home cage. One hour after exposure, animals were trained to learn the passive avoidance task. A mouse was considered to have learned the task once it remained on the platform for at least 30 seconds without entering the hole. Mice were then tested for recollection of the task at either 1, 2, or 4 days after the initial training session. In the learning phase of the task, 74% of the control mice retained the task in comparison to 59% of the dichloromethane-exposed group, indicating the significant effect of dichloromethane on learning. There was also an age-related effect since exposed 3-week-old mice were less likely to recall the task than 5- or 8-week-old mice. There was no difference in task recall between the 5- and 8-week-old mice. Dichloromethane at the exposure used in the study was demonstrated to be nonanalgesic, since pain-response times were comparable to those in air-exposed animals in the hot-plate pain test, and therefore, the results of the passive avoidance test were not confounded by potential analgesic effects. As a result, it is demonstrated that exposure to an acute and high concentration of dichloromethane alters learning ability in mice.

Neurophysiological studies. The effect of dichloromethane on sensory stimuli was evaluated by measuring sensory-evoked responses during an acute exposure (Rebert et al., 1989) and following a subchronic (13-week) exposure (Mattsson et al., 1990). Rebert et al. (1989) evaluated the effects of dichloromethane on sensory-evoked potentials (auditory, visual, and somatosensory) in F344 rats exposed to 0, 5,000, 10,000, and 15,000 ppm dichloromethane for 1 hour in a head-only exposure chamber. Twelve adult male rats were implanted with chronic

epidural electrodes placed over the visual and somatosensory cortices. Each rat served as its own control, with a 1-week recovery period between testing sessions. During each testing session, spontaneous electroencephalograms were recorded. Additionally, brainstem-auditory-evoked responses (BAERs) (tone stimulus), cortical-auditory-evoked potentials (CAEPs) (click stimulus), FEPs (flash stimulus), and somatosensory-evoked potentials (SEPs) (tail current stimulus) were measured in response to the stimuli. Dichloromethane decreased the SEP response to the tail current stimulus, and earlier components of the FEP response were attenuated and eventually eliminated with increasing exposures. The BAER response profile was also significantly altered. Dichloromethane completely abolished the CAEP at all concentrations tested. Slight recovery of this response was noted approximately 1 hour after exposure. The collective results strongly suggest a CNS depressive profile for dichloromethane and indicate that this chemical affects the auditory, visual, and somatosensory regions of the brain.

In a subchronic exposure study, male and female F344 rats were exposed to dichloromethane 6 hours/day, 5 days/week for 13 weeks beginning at 16 weeks of age (Mattsson et al., 1990). Twelve animals of each sex were selected for exposure to 0, 50, 200, or 2,000 ppm dichloromethane or 135 ppm CO. For electrophysiological measures, rats were surgically implanted with epidural electrodes 10 weeks after the onset of exposure. Electrodes were placed over the somatosensory, visual, and cerebellar region. Electrophysiological measures that were recorded included FEP measurements, cortical flick fusion responses, CAEPs, BAERs, and SEPS recorded from the sensory and cerebellar regions. None of these measures were significantly altered by any dichloromethane or CO treatment in this study. However, it should be noted that all of the electrophysiological measures were conducted at least 65 hours after the last dichloromethane exposure. As a result, it can be concluded that a subchronic exposure to dichloromethane in adult rats did not result in persistent changes in any of the neurophysiological measures that were evaluated in this study. The potential for differential effects based on age (either in the young or at more advanced ages) was not examined in this study. It is not known if any neurological compensation occurred, since SEP measurements were not taken during actual dichloromethane exposure in this subchronic study.

Neurochemistry and neuropathology studies. The studies evaluating specific neurochemical changes in relation to dichloromethane exposure include studies of effects of short-term (3-day to 2-week) exposures (Fuxe et al., 1984; Savolainen et al., 1981) and subchronic (3-month) exposures (Karlsson et al., 1987; Briving et al., 1986; Rosengren et al., 1986).

Savolainen et al. (1981) examined three different exposure schemes in male Wistar rats. The rats (15 per group) were exposed to 500, 1,000, or 1,000 ppm TWA dichloromethane 6 hours/day, 5 days/week for 2 weeks. (Note: The abstract of this paper describes the exposures as 500, 1,000, and 100 ppm TWA, but, based on information in the body of the paper, the abstract

appears to be incorrect.) The 1,000 ppm TWA exposure consisted of a basal 100 ppm exposure with two 2,800 ppm 1-hour peak concentrations (at 1 and 4 hours) resulting in a time-weighted exposure of 1,000 ppm. Brains were removed from rats at the end of study and analyzed. The 1,000 ppm TWA group displayed increases in cerebral RNA. Other changes noted for this group in the cerebrum included significant increases in nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase and succinate dehydrogenase activity. In the 1,000 ppm constant exposure group, acid proteinase activity was below the levels observed in control animals in the first week but increased to levels above control animals in the second week. In the cerebellum, there were no changes in RNA concentration, and there was a decrease in succinate dehydrogenase activity in both the 1,000 and 1,000 ppm TWA groups. After a 7-day withdrawal, RNA levels in the cerebrum were significantly lower in the 1,000 ppm group. Succinate dehydrogenase levels remained lowered in the 1,000 ppm TWA group after the 7-day exposure-free period. No significant effects were seen at 500 ppm.

Fuxe et al. (1984) evaluated changes in brain catecholamine levels after a 3-day exposure to dichloromethane using male Sprague-Dawley rats. Rats were exposed to 70, 300, and 1,000 ppm dichloromethane 6 hours/day for 3 consecutive days. Additional groups of rats were exposed to the same levels of dichloromethane and given intraperitoneal injections of the tyrosine hydroxylase inhibitor, α-methyl-dl-*p*-tyrosine methyl ester (H44/68), 2 hours prior to sacrifice. Brains were removed, stained, and evaluated for catecholamine changes 16–18 hours after the last exposure. Catecholamine levels were measured in the hypothalamus, frontal cortex, and caudate nucleus among other brain regions. At all exposures, there was a significant decrease by approximately 10–15% of catecholamine concentrations in the posterior periventricular region of the hypothalamus. In the medial part of the caudate nucleus, which is involved in memory processes, catecholamine levels were significantly higher (12%) in the 70 ppm group but significantly lower in the 300 ppm (1%) and 1,000 ppm (8%) groups compared with controls. The impact of dichloromethane was also evaluated on the hypothalamic-pituitary gonadal axis. The hypothalamus regulates secretion of reproductive hormones, such as follicle-stimulating hormone and luteinizing hormone. The levels of the hormone release were not significantly changed with dichloromethane exposure. However, when rats were dosed concurrently with H44/68 and dichloromethane, statistically significant, inversely dose-related increases in luteinizing hormone levels were observed (330, 233, and 172% higher than controls in the 70, 300, and 1,000 ppm groups, respectively). Overall, the study demonstrates significant changes in catecholamine levels in the hypothalamus and caudate nucleus. No significant changes in catecholamine levels in the frontal cortex were reported. Catecholamine level changes in the hypothalamus did not appear to significantly affect hormone release; however, decreased catecholamine levels in the caudate nucleus at higher exposures may lead to memory and learning impairment.

A series of studies were conducted in male and female Mongolian gerbils exposed continuously to 210 (Karlsson et al., 1987; Briving et al., 1986), 350, or 700 ppm (Rosengren et al., 1986) dichloromethane for 3 months, followed by a 4-month exposure-free period. High mortality rates occurred at 350 (6/10 males and 3/10 females by 71 days) and 700 ppm (10/10 males and 9/10 females by 52 days). Rosengren et al. (1986) monitored two astroglial proteins, S-100 and GFA, as well as DNA concentrations in the brain. Decreased DNA concentrations were noted in the hippocampus at both the 210 and 350 ppm exposures. At 350 ppm, there was also decreased DNA concentration in the cerebellar hemispheres, indicating a decreased cell density in these regions, probably due to cell loss. Increased astroglial proteins were found in the frontal and sensory motor cerebral cortex, which directly correlated to the astrogliosis that was observed in those areas. Up-regulation of these astroglial proteins is a good indicator of neuronal injury (Rosengren et al., 1986).

Karlsson et al. (1987) measured DNA concentrations in different regions of the gerbil brain. After the solvent-free exposure period, brains were removed and the olfactory bulbs and cerebral cortices were dissected. Brain weights and weights of the dissected brain regions were the same between control and dichloromethane-exposed animals. The total protein concentration per wet weight was not significantly different between dichloromethane-exposed and control animals. However, DNA concentrations per wet weight were significantly decreased in the hippocampus after dichloromethane exposure. No other examined regions demonstrated significant changes in DNA concentrations after dichloromethane exposure. This selective DNA concentration decrease observed in the hippocampus is a sign of neurotoxicity and may possibly explain why some studies have noted memory and learning deficits with dichloromethane exposure. In a companion paper, in which only the 210 ppm level was tested, it was found that exposure to dichloromethane decreased the levels of glutamate, γ-aminobutyric acid, and phosphoethanolamine in the frontal cortex, while glutamine and γ-aminobutyric acid were increased in the posterior cerebellar vermis (Briving et al., 1986). Increased levels of glutamate in the posterior cerebellar vermis could reflect an activation of astrocytic glia, since glutamine synthetase is localized exclusively in astrocytes. The gerbils did not have a solvent-free exposure period as in the other two studies (Karlsson et al., 1987; Rosengren et al., 1986). The exposure regime in these studies did not affect BW or brain weight. Furthermore, the neurochemical changes observed in these studies were not attributed to formation of CO.

Neurological changes have been investigated by measuring changes in neurotransmitter levels and changes in neurotransmitter localization. Changes in catecholamine levels in the caudate nucleus after an acute exposure (Fuxe et al., 1984), as well as decreased DNA content in the hippocampus after a subchronic dichloromethane exposure (Rosengren et al., 1986), suggest that memory functions are altered since both brain regions are associated with learning and memory. The results from Fuxe et al. (1984) directly correlated with the finding that learning and memory were impaired in mice after an acute (single) and very high exposure (47,000 ppm)

to dichloromethane (Alexeeff and Kilgore, 1983). Additionally, changes in the hippocampus also suggest memory effects after a long-term, continual exposure to dichloromethane, although no conclusive evidence has been presented to date. In another subchronic, continuous exposure to 350 ppm dichloromethane for 3 months, decreased DNA concentration was observed in the cerebellar hemispheres of Mongolian gerbils and is suggestive of cell loss (Rosengren et al., 1986). However, in a 2-week exposure study in male Wistar rats, RNA changes were not noted in the cerebellum, although enzyme activity was significantly decreased in this region (but was increased in the cerebrum) (Savolainen et al., 1981). These results suggest that the cerebellum is a target for dichloromethane. Noted neurobehavioral effects that may be linked to impaired cerebellar function include changes in motor activity and impaired neuromuscular function (Moser et al., 1995).

E.5. MECHANISTIC STUDIES OF LIVER EFFECTS

E.5.1. Liver Tumor Characterization Studies

Several studies have examined the time course of appearance of liver tumors in B6C3F$_1$ mice exposed to 2,000 or 4,000 ppm and possible links between hepatic nonneoplastic cytotoxicity, enhanced hepatic cell proliferation, and the development of liver tumors (Casanova et al., 1996; Maronpot et al., 1995; Foley et al., 1993; Kari et al., 1993). The studies provide no clear evidence for a sustained liver cell proliferation response to dichloromethane that can be linked to the development of dichloromethane-induced liver tumors. Additionally, a few studies have examined if dichloromethane-induced liver tumors are the result of proto-oncogene activation and tumor suppressor gene inactivation (Maronpot et al., 1995; Devereux et al., 1993; Hegi et al., 1993).

Kari et al. (1993) [also summarized by Maronpot et al. (1995)] reported data from six groups of 68 female B6C3F$_1$ mice exposed to six "stop-exposure" protocols of differing durations and sequences, with each exposure concentration standardized at 2,000 ppm for 6 hours/day, 5 days/week. The six stop-exposure protocols were 26 weeks of exposure followed by 78 weeks without exposure, 78 weeks without exposure followed by 26 weeks of exposure, 52 weeks without exposure followed by 52 weeks with exposure, 52 weeks of exposure followed by 52 weeks without exposure, 78 weeks of exposure followed by 26 weeks without exposure, and 26 weeks without exposure followed by 78 weeks of exposure. A control group (no exposure, 104 weeks duration) and a maximum exposure (104 weeks duration) group were also included. Exposure for 26 weeks did not result in an increased incidence of liver tumors (adenomas or carcinomas). Respective percentages of animals with liver tumors were 27 (18/67), 40 (27/67), and 34% (23/67) for the controls, early 26-week exposure, and late 26-week exposure groups, respectively. Exposure to 2,000 ppm for 52 weeks (followed by no exposure until 104 weeks), 78 weeks (either early or late exposure periods), or 104 weeks produced increased incidences of mice with liver tumors ($p < 0.05$), but this increase was not

seen in the 52-week late exposure group. Respective percentages of animals with liver tumors (adenomas and carcinomas combined) were 44 (28/64), 31 (21/67), 62 (42/68), 48 (32/67), and 69% (47/68) for the 52-(early exposure), 52-(late exposure), 78-(early exposure), 78-(late exposure), and 104-week exposure periods, respectively. With the 78-week exposures, the difference in the liver tumor incidence between the early and late exposure periods was statistically significant ($p < 0.01$).

Histopathologic examination of liver tissue at interim killings at eight time periods (13, 26, 52, 68, 75, 78, 83, or 91 weeks) of exposure to 2,000 ppm (n = 20 mice per killing) found no evidence of nonneoplastic cytotoxicity that preceded the appearance of proliferative neoplastic liver lesions. Incidences of mice with liver adenomas or carcinomas were elevated (between 40 and 60%) at five of the six interim killings after 52 weeks. The incidence rates at each time period were 0/20 (0%) at 13 weeks, 1/20 (5%) at 26 weeks, 8/20 (40%) at 52 weeks, 4/26 (15%) at 68 weeks, 13/20 (65%) at 75 weeks, 12/19 (63%) at 78 weeks, 8/20 (40%) at 83 weeks, and 20/30 (66%) at 91 weeks. The collected liver lesion data identify no exposure-related increased incidence of nonneoplastic liver lesions that could be temporally linked to liver tumor development. Liver tumors first appeared at about the same time in control and exposed animals (52 weeks).

Foley et al. (1993) examined indices of cell proliferation in livers of female B6C3F$_1$ mice exposed to 1,000, 2,000, 4,000, or 8,000 ppm dichloromethane (6 hours/day, 5 days/week) for 1, 2, 3, or 4 weeks or to 2,000 ppm for 13, 26, 52, or 78 weeks but found no evidence for sustained cell proliferation with prolonged exposure to dichloromethane. To label liver cells in S-phase, tritiated thymidine (1- to 4-week exposure protocols) or bromodeoxyuridine (13- to 78-week protocols) was administered subcutaneously via an osmotic mini-pump for 6 days prior to killing. Labeled hepatocytes in liver sections (from 10 mice in each exposure/duration group) were counted to assess the number of cells in S-phase per 1,000 cells. S-phase labeling indices in livers of exposed mice at most killings were equivalent to or less than those in control mice. A transient increase in S-phase labeling index of about two- to fivefold over controls was observed at the 2-week killing of mice exposed to 1,000, 4,000, or 8,000 ppm. Because of the transient nature and small magnitude of the response, it is not expected to be of significance to the promotion of liver tumors in chronically exposed mice. Foley et al. (1993) also compared cell proliferation labeling indices in foci of cellular alteration and nonaffected liver regions in control and exposed mice but found no significant difference between control and exposed mice. S-phase labeling was accomplished by immunohistologic staining for proliferating cell nuclear antigen in liver sections from 24 control mice and 15 exposed mice, with livers showing foci of cellular alteration. In both control and exposed livers, the labeling index was about four- to fivefold higher in foci of cellular alteration than in surrounding unaffected liver tissue.

In mice exposed to 2,000 ppm for 13–78 weeks, relative liver weights were statistically significantly elevated compared with controls; about 10% increased at 13 and 26 weeks and

about 30–40% increased at 52 and 78 weeks. Histologic changes in liver sections of 2,000 ppm mice exposed for 13–78 weeks were restricted to hepatocellular hypertrophy (observed at all killing intervals) and preneoplastic (foci of cellular alteration) and neoplastic (adenoma and carcinoma) lesions. No signs of liver tissue degeneration were found. Adenoma and focus of alteration were first detected at 26 weeks (2/10 versus 0/10 in controls). At 52 weeks, 4/10 exposed mice had proliferative lesions (one focus, one adenoma, and two carcinomas), compared with 1/10 in controls (one adenoma). At 78 weeks, 7/10 exposed mice had proliferative lesions (two foci, three adenomas, six carcinomas) compared with 1/10 in controls (one adenoma). In summary, the results indicate that inhalation exposure to 2,000 ppm dichloromethane produced an increased incidence of liver tumors in female $B6C3F_1$ mice. No evidence was found for sustained cell proliferation or liver tissue degeneration in response to dichloromethane exposure, but exposure was associated with relative liver weight increases and hepatocellular hypertrophy.

Casanova et al. (1996) found no clear evidence of increased cell proliferation in the livers of male $B6C3F_1$ mice exposed to dichloromethane concentrations >1,500 ppm 6 hours/day for 3 days. Three or four groups of three mice were exposed to 146, 498, 1,553, or 3,923 ppm unlabeled dichloromethane for 2 days and then exposed to [^{14}C]-labeled dichloromethane for 6 hours on the third day. Radiolabel incorporated into liver DNA deoxyribonucleosides was measured as an index of cell proliferation. Radiolabel incorporated into liver DNA deoxyribonucleosides increased approximately fivefold from 146 to 1,553 ppm, but further increases were not apparent at 3,923 ppm. (In contrast, as described in Appendix E, Section E.8.1, radiolabel incorporation into lung DNA deoxyribonucleosides displayed a 27-fold increase over this concentration range.) The small magnitude of the increase in radiolabel incorporation into liver DNA deoxyribonucleosides with increasing exposure concentration suggests that little if any enhanced cell proliferation occurred in the liver in response to dichloromethane exposure.

E.5.2. Liver Metabolic Studies

As described in detail in Section 3.3, GST-T1 enzymatic activity and distribution is variable among species, and there is also considerable intraspecies variability among humans. In summary, Reitz et al. (1989a) demonstrated a greater metabolic activity with respect to dichloromethane in livers of $B6C3F_1$ mice compared with F344 rats, Syrian golden hamsters, and humans. The rates of in vitro metabolism by the GST pathway were about 4-, 12-, and 20-fold greater in $B6C3F_1$ mouse liver samples than in F344 rat, human, and Syrian golden hamster samples, respectively (Reitz et al., 1989a). A more recent study characterized the dichloromethane metabolic capacity specifically of hepatic GST-T1 (Thier et al., 1998a). Enzymatic activities of GST-T1 in liver from F344 rats, $B6C3F_1$ mice, Syrian golden hamsters, and humans with three different GST-T1 phenotypes (nonconjugators, low conjugators, high

conjugators) showed the following order with dichloromethane as a substrate: mouse >> rat > human high conjugators > human low conjugators > hamster > human nonconjugators.

E.6. MECHANISTIC STUDIES OF LUNG EFFECTS

E.6.1. Lung Tumor Characterization Studies

Kari et al. (1993) (also summarized in Maronpot et al. (1995)) demonstrated that only 26 weeks of exposure to 2,000 ppm was necessary to produce significantly increased incidences of lung tumors in female $B6C3F_1$ mice. In the six "stop-exposure" protocol experiments (described in the previous discussion of liver tumor characterization studies), early but not late exposure for 26 or 52 weeks resulted in an increased incidence of animals with lung tumors (adenoma or carcinomas). Respective percentages of animals with lung tumors were 7.5 (5/67), 31 (21/68), 4 (3/67), 63 (40/63), and 15% (10/67) for the controls, early 26-, late 26-, early 52-, and late 52-week exposure groups, respectively. With the 78-week exposures, both the early and late exposure regimens produced an increased incidence of lung tumors compared with controls (56 [38/68] and 19% [13/68], respectively), compared with the incidence of 63% (42/67) seen in the group exposed for the full 104 weeks. Thus, a plateauing of risk was seen, with similar incidence rates seen with the early 52-week, early 78-week, and 104-week exposure periods. The difference in the lung tumor incidence between the early and late exposure periods of similar duration was statistically significant ($p < 0.01$) for the 26-, 52-, and 78-week duration protocols. A greater increase in multiplicity of lung tumors was also seen with the early 78-week exposure period. As with the liver tumor data from the same series of experiments, these data suggest that early exposure was more effective than late exposure and that the increased risk continued after cessation of exposure.

Histopathologic examination of lung tissue from mice killed at 13, 26, 52, 68, 75, 78, 83, or 91 weeks of exposure to 2,000 ppm (n = 20 mice per killing) found no evidence of nonneoplastic cytotoxicity that preceded the appearance of proliferative neoplastic lung lesions. In contrast, incidences of mice with lung adenomas or carcinomas (combined) were elevated at interim killings ≥52 weeks; incidences for the interim killings of mice exposed to 2,000 ppm (6 hours/day, 5 days/week) between 13 and 91 weeks were 0/20 (0%) at 13 weeks, 0/20 (0%) at 26 weeks, 6/20 (30%) at 52 weeks, 6/26 (23%) at 68 weeks, 8/20 (40%) at 75 weeks, 9/19 (47%) at 78 weeks, 10/20 (50%) at 83 weeks, and 14/30 (47%) at 91 weeks. Lung hyperplasia was found at an increased incidence only at 91 weeks, well after the 26- and 52-week periods that induced increased incidences of mice with lung tumors.

Kanno et al. (1993) found no evidence for histologic changes or increased cell proliferation in lung tissue of female $B6C3F_1$ mice exposed to 2,000 or 8,000 ppm dichloromethane for 1, 2, 3, or 4 weeks compared with control mice, or in mice exposed to 2,000 ppm for 13 or 26 weeks. Osmotic mini-pumps were used to deliver tritiated thymidine and label cells undergoing replicative DNA synthesis over 6-day periods before killing. Labeled

cells undergoing rapid DNA synthesis and cell proliferation were assessed in sections of proximal and terminal bronchioles and alveoli of lungs from groups of 5 mice exposed for 1–4 weeks or 10 mice exposed for 13 or 26 weeks. There were no exposure-related histopathologic or labeling index changes in the alveoli, but lower labeling indices were found in the bronchiolar epithelium of exposed mice compared with controls.

The combined results from the Kari et al. (1993) and Kanno et al. (1993) studies are consistent with the hypothesis that dichloromethane-induced lung tumors in $B6C3F_1$ mice are not preceded by overt cytotoxicity, enhanced and sustained cell proliferation, or hyperplasia in the lung. Two other studies (Casanova et al., 1996; Foster et al., 1992), however, have reported evidence for enhanced cell proliferation in lungs of $B6C3F_1$ mice exposed for acute durations to airborne dichloromethane. Only one of these studies (Foster et al., 1992), however, looked for sustained cell proliferation in the lung with prolonged exposure. In agreement with the results from Kanno et al. (1993), no evidence was found for sustained cell proliferation in lungs with prolonged exposure to dichloromethane at concentrations demonstrated to induce lung tumors in mice.

Casanova et al. (1996) detected evidence of increased cell proliferation in the lungs of male $B6C3F_1$ mice exposed to dichloromethane concentrations >1,500 ppm 6 hours/day for 3 days. Three or four groups of three mice were exposed to 146, 498, 1,553, or 3,923 ppm unlabeled dichloromethane for 2 days and then exposed to [^{14}C]-labeled dichloromethane for 6 hours on the third day. Radiolabel incorporated into lung DNA deoxyribonucleosides (after removal of DNA-protein cross-links containing radiolabeled formaldehyde) was measured as an index of cell proliferation. Radiolabel incorporation into lung DNA deoxyribonucleosides increased with increasing exposure concentration, with the amount increasing by about 27-fold between 146 and 3,923 ppm. In hamsters that did not develop tumors in response to chronic inhalation exposure to 3,500 ppm dichloromethane (Burek et al., 1984), no evidence for enhanced radiolabel incorporation into lung DNA deoxyribonucleosides was found following acute exposure (Casanova et al., 1996).

E.6.2. Clara Cell Studies

Foster et al. (1992) found enhanced cell proliferation in bronchiolar cells and, to a lesser degree, alveolar cells in the lungs of male $B6C3F_1$ mice exposed for acute durations (2, 5, 8, or 9 days) to 4,000 ppm dichloromethane (6 hours/day, 5 days/week), but the response was less distinct after subchronic durations of exposure (89, 92, or 93 days). To measure cell proliferation, mice (n = 5 per exposure-duration group) were given subcutaneous doses of tritiated thymidine for 5 consecutive days prior to killing. Labeled cells in bronchiolar or alveolar epithelium in lung sections were counted to assess the number of cells in S-phase per 1,000 cells. Counts of bronchiolar epithelium cells in S-phase in exposed mice sacrificed on days 2, 5, 8, and 9 were approximately 2-, 15-, 3-, and 5-fold higher, respectively, than those of

unexposed mice at day 0 of the experiment. In exposed mice sacrificed on days 89, 92, and 93, less than twofold increases in bronchiolar epithelium cell labeling were observed. Increased cell labeling was found in alveolar epithelium only on day 8 (about seven- to eightfold increase) and day 9 (about fourfold increase). Vacuolation of the Clara cells of the bronchiolar epithelium was observed on day 2 (scored as ++, majority of cells affected), day 9 (+++, virtually all the cells affected), and day 44 (+, moderate effect to cells) but was not apparent on days 5, 8, 40, 43, 89, 92, or 93. No hyperplasia of the bronchiolar epithelium or changes to Type II alveolar epithelial cells were observed in the lungs of any of the exposed mice at any time point. The appearance and disappearance of the Clara cell vacuolation was generally correlated with the appearance and disappearance of enhanced cell proliferation in the bronchiolar epithelium; enhanced cell proliferation was observed on days 2, 5, 8, and 9 (along with appearance of Clara cell vacuolation on days 2 and 9) but was not observed on days 89, 92, and 93 when Clara cell lesions also were not observed. This suggests that cell proliferation was enhanced in response to Clara cell damage but was not sustained with repeated exposure to dichloromethane.

Currently, a mechanistic connection has not been established between the acute effects of dichloromethane on Clara cells in the bronchiolar epithelium and the development of lung tumors in $B6C3F_1$ mice exposed by inhalation to concentrations ≥2,000 ppm dichloromethane for 2 years (NTP, 1986) or for 26 weeks followed by no exposure through 2 years (Maronpot et al., 1995; Kari et al., 1993). There is a concordance between exposure concentrations inducing acute Clara cell vacuolation (≥2,000 ppm) and those inducing lung tumors (≥2,000 ppm). However, transient acute Clara cell vacuolation does not appear to progress to necrosis or lead to sustained cell proliferation (which could promote the growth of tumor-initiated cells) and appears to be dependent on CYP metabolism of dichloromethane [see the following paragraphs discussing pertinent findings reported by Foster et al. (1994; 1992)]. In contrast, there is consistent and specific evidence for an association between the formation of DNA-reactive GST-pathway metabolites and the formation of lung and liver tumors from inhalation exposure (see Sections 4.5 and 4.7.3).

Foster et al. (1992) noted that the appearance and disappearance of Clara cell vacuolation in mouse lungs showed concordance with temporal patterns for immunologic staining for CYP2B1 and CYP2B2 levels in lung sections. A similar temporal pattern was reported for CYP2B1 and CYP2B2 monooxygenase activities (ethoxycoumarin O-dealkylation or aldrin epoxidation) assayed in lung microsomes. When there was a marked decrease in CYP2B1 and CYP2B2 staining (e.g., on day 5) or monooxygenase activities, the lesion was not present. Similarly, the appearance of the lesion was preceded (the day before) by the recovery of monooxygenase activities or immunologic staining close to control levels. These patterns suggested to Foster et al. (1992) that Clara cells may have developed tolerance to dichloromethane due to inactivation of a CYP isozyme.

In subsequent studies, increased percentages of vacuolated bronchiolar epithelium cells were noted in mice exposed to 2,000 ppm (26.3 ± 6.7%) or 4,000 ppm (64.8 ± 12.8%), but vacuolated cells were not observed in bronchiolar epithelium of lung sections from control mice or mice exposed to 125, 250, 500, or 1,000 ppm (Foster et al., 1994). Pretreatment with the CYP inhibitor, piperonyl butoxide, counteracted the 2,000 ppm effect (2.4 ± 3.6% vacuolated cells), whereas GSH-depleted mice showed no statistically significant change in percentage of vacuolated cells (32.7 ± 16.9%) compared with the mean percentage in mice exposed to 2,000 ppm without pretreatment. No consistent, statistically significant, exposure-related changes were found in cytosolic GST metabolic activities (with dichloromethane as substrate) or microsomal CYP monooxygenase activities (ethoxycoumarin O-dealkylation), but mean cytosolic levels of nonprotein sulfhydryl compounds were elevated in lungs of mice exposed to 1,000 and 2,000 ppm (134.6 ± 17.1 and 146.4 ± 6.7 nmol/mg protein, respectively) compared with control levels (109.5 ± 7.6 nmol/mg protein). Increased cell proliferation was found in cultured Clara cells isolated from 4,000 ppm mice compared with nonexposed mice; respective values for percentage of cells in S-phase were 18.97 ± 1.18 and 2.02 ± 0.86% (Foster et al., 1994).

Results from the studies by Foster et al. (1994; 1992) indicate that 6-hour exposures of $B6C3F_1$ mice to dichloromethane concentrations ≥2,000 ppm caused transient Clara cell vacuolation in the bronchiolar epithelium, which was not consistently observed following repeated exposures. With repeated exposure to 4,000 ppm, the Clara cell vacuolation did not progress to necrosis, and no hyperplasia of the bronchiolar epithelium was found after up to 13 weeks of exposure. The transient Clara cell vacuolation was decreased by CYP inhibition with piperonyl butoxide and was unaffected by GSH depletion, indicating that a CYP metabolite was involved. Clara cell vacuolation was not found after five consecutive, daily 6-hour exposures to 4,000 ppm but reappeared after 2 days without exposure followed by two additional consecutive, daily exposures (day 9). With repeated exposure, the lesion was detected at a diminished severity on day 44 (but was not found on day 40 or 43) and on day 93 (but was not found on day 89 or 92). The temporal pattern of Clara cell vacuolation with repeated exposure was reflected in the occurrence of transiently decreased CYP metabolic activity after the appearance of vacuolation. Foster et al. (1994; 1992) proposed that the diminishment of severity or the disappearance of the Clara cell vacuolation with repeated exposure was due to the development of a tolerance to dichloromethane, linked with a decrease of CYP metabolism of dichloromethane.

E.7. MECHANISTIC STUDIES OF NEUROLOGICAL EFFECTS

Kanada et al. (1994) examined the effect of dichloromethane on acetylcholine and catecholamines (dopamine, norepinephrine, serotonin) and their metabolites in the midbrain, hypothalamus, hippocampus, and medulla from male Sprague-Dawley rats (4–5/group). The rats

were sacrificed 2 hours after a single gavage dose of 0 or 534 mg/kg of undiluted dichloromethane. Administration of dichloromethane significantly increased the concentration of acetylcholine in the hippocampus and increased dopamine and serotonin levels in the medulla. Dichloromethane decreased norepinephrine levels in the midbrain, and hypothalamus and serotonin levels were decreased in the hypothalamus. There was a trend toward decreased dopamine in the hypothalamus, but the variability between the animals was so high that the effect was not significant. The authors speculated that increased acetylcholine release from dichloromethane administration may be due to decreased acetylcholine release from the nerve terminals. It is unclear as to how these neurochemical changes could be correlated to the neurobehavioral changes observed after dichloromethane exposure.

In a 2-week exposure study, male Wistar rats were exposed to dichloromethane at 500 or 1,000 ppm (6 hours/day, 5 days/week for 1 or 2 weeks) or 1,000 ppm TWA (1 hour at 100 ppm, 1 hour peak at 2,800 ppm, 1 hour at 100 ppm, repeated immediately, 5 days/week for 1 or 2 weeks) (Savolainen et al., 1981). Brains were removed from rats at the end of the study and analyzed. The 1,000 ppm TWA group displayed increases in cerebral RNA. Other changes noted for this group in the cerebrum included significant increases in NADPH-diaphorase and succinate dehydrogenase activity. These changes suggest increased neural activity to possibly offset the overall inhibitory effect of dichloromethane in the CNS. It could also possibly explain why lethargic effects decrease with continued dichloromethane exposure, and this result demonstrates a neuroprotective mechanism resulting from dichloromethane exposure. After a 7-day withdrawal, RNA levels in the cerebrum were significantly lower in the 1,000 ppm group. Succinate dehydrogenase levels remained lowered in the 1,000 ppm TWA group after the 7-day exposure-free period.

Changes in brain catecholamine levels after a subacute exposure to dichloromethane were evaluated using male Sprague-Dawley rats (Fuxe et al., 1984). Rats were exposed to 70, 300, and 1,000 ppm dichloromethane 6 hours/day for 3 consecutive days. At all exposures, there was a significant decrease of catecholamine concentrations in the posterior periventricular region of the hypothalamus. The impact of dichloromethane was also evaluated on the hypothalamic-pituitary gonadal axis. The hypothalamus regulates secretion of reproductive hormones such as follicle-stimulating hormone and luteinizing hormone. The levels of the hormone release were not significantly changed with dichloromethane exposure. In the caudate nucleus, which is involved in memory processes, the catecholamine level initially increased (at 70 ppm) and then was lower (1,000 ppm) in comparison to the control. The study demonstrates significant changes in catecholamine levels in the hypothalamus and caudate nucleus. Catecholamine level changes in the hypothalamus did not have any significant effect on hormonal release, but decreased catecholamine levels in the caudate nucleus at higher exposures may lead to memory and learning impairment.

A series of studies were conducted in male and female Mongolian gerbils exposed continuously to ≥210 ppm dichloromethane for 3 months, followed by a 4-month exposure-free period (Karlsson et al., 1987; Briving et al., 1986; Rosengren et al., 1986). Decreased DNA concentrations were noted in the hippocampus at both the 210 and 350 ppm exposures. At 350 ppm, there was also decreased DNA concentration in the cerebellar hemispheres, indicating a decreased cell density in these regions probably due to cell loss (Rosengren et al., 1986). These findings indicate that the cerebellum, which is the section of the brain that regulates motor control, is a target for dichloromethane. In the same study, increased astroglial proteins were found in the frontal and sensory motor cerebral cortex, which directly correlated to the astrogliosis that was observed in those areas. Up-regulation of these astroglial proteins is a good indicator of neuronal injury (Rosengren et al., 1986).

Karlsson et al. (1987) measured DNA concentrations in different regions of the gerbil brain. The total brain protein concentration per wet weight was not significantly different between dichloromethane-exposed and control animals. However, DNA concentrations per wet weight were significantly decreased in the hippocampus after dichloromethane exposure. No other examined regions demonstrated significant changes in DNA concentrations after dichloromethane exposure. Therefore, this result indicates that the hippocampus, which plays a role in the formation of new memories, is another target for dichloromethane in the CNS. This selective DNA concentration decrease observed in the hippocampus is a sign of neurotoxicity, as noted by the authors, and may possibly explain why some studies have noted memory and learning deficits with dichloromethane exposure.

At a 210 ppm exposure, Briving et al. (1986) observed that dichloromethane decreased glutamate, γ-aminobutyric acid, and phosphoethanolamine levels in the frontal cortex, while glutamate and γ-aminobutyric acid were increased in the posterior cerebellar vermis. Increased levels of glutamate in the posterior cerebellar vermis could reflect an activation of astrocytic glia, since glutamine synthetase is localized exclusively in astrocytes.

APPENDIX F. SUMMARY OF BENCHMARK DOSE (BMD) MODELING OF NONCANCER ENDPOINTS

F.1. ORAL RfD: BMD MODELING OF LIVER LESION INCIDENCE DATA FOR RATS EXPOSED TO DICHLOROMETHANE IN DRINKING WATER FOR 2 YEARS (Serota et al., 1986a)

BMD and BMDL refer to the model-predicted dose (and its lower 95% confidence limit) associated with 10% extra risk for the incidence of liver foci/areas of cellular alteration in male and female F344 rats given dichloromethane in drinking water for 2 years (Serota et al., 1986a) (Table F-1).

Table F-1. Incidence data for liver lesions and internal liver doses based on various metrics in male and female F344 rats exposed to dichloromethane in drinking water for 2 years

Sex	Nominal (actual) daily intake (mg/kg-d)	Rat liver lesion incidence[a]	Rat internal liver dose[b]			
			CYP	GST	GST and CYP	Parent AUC
Male (BW = 380 g)	0 (0)	52/76 (68%)	0	0	0	0
	5 (6)	22/34 (65%)	131.6	2.60	134.2	0.58
	50 (52)	35/38 (92%)[c]	723.9	81.5	805.3	18.1
	125 (125)	34/35 (97%)[c]	1,170.5	276.5	1,446.9	61.3
	250 (235)	40/41 (98%)[c]	1,548.0	612.1	2,160.1	135.7
Female (BW = 229 g)	0 (0)	34/67 (51%)	0	0	0	0
	5 (6)	12/29 (41%)	132.8	2.47	135.3	0.47
	50 (58)	30/41 (73%)[c]	801.4	93.3	894.7	17.8
	125 (136)	34/38 (89%)[c]	1,261.5	307.8	1,569.3	58.6
	250 (263)	31/34 (91%)[c]	1,672.4	705.6	2.378.0	134.4

[a]Liver foci/areas of cellular alteration; number affected divided by total sample size.
[b]Internal doses were estimated using a rat PBPK model from simulations of actual daily doses reported by the study authors. CYP dose is in units of mg dichloromethane metabolized via CYP pathway/L tissue/day; GST dose is in units of mg dichloromethane metabolized via GST pathway/L tissue/day; GST and CYP dose is in units of mg dichloromethane metabolized via CYP and GST pathways/L tissue/day; and Parent AUC dose is in units of mg dichloromethane × hours/L tissue.
[c]Significantly ($p < 0.05$) different from control with Fisher's exact test.

Source: Serota et al. (1986a).

All available dichotomous models in the BMDS (version 2.0) were fit to male and female rat internal tissue doses of dichloromethane metabolized by the CYP pathway and incidences for animals with these liver lesions observed at the time of death (Table F-2). The male rats exhibited a greater sensitivity compared to the female rats (based on lower $BMDL_{10}$ values for all of the models), and thus, the male data are used as the basis for the RfD derivation. The

logistic model was the best fitting model for the male incidence data based on lowest AIC value among models with adequate fit (U.S. EPA, 2000a). (If two or more models share the lowest AIC, $BMDL_{10}$ values from these models may be averaged to obtain a POD. However, this average is no longer a lower confidence bound that provides the stated coverage, and thus should be referred to only as an average of $BMDL_{10}$ values. U.S. EPA does not support averaging BMDLs in situations in which AIC values are similar, but not identical, because the level of stated coverage is lost and no consensus exists regarding a specific cut-off between similar and dissimilar AIC values.) Results for this model are presented below.

Table F-2. BMD modeling results for incidence of liver lesions in male and female F344 rats exposed to dichloromethane in drinking water for 2 years, based on liver-specific CYP metabolism dose metric (mg dichloromethane metabolism via CYP pathway/L liver tissue/day)

Sex and model[a]	BMD_{10}	$BMDL_{10}$	χ^2 goodness of fit *p*-value	AIC
Males				
Gamma[a]	138.124	41.28	0.58	185.46
Logistic[b]	**70.69**	**51.42**	**0.69**	**183.85**
Log-logistic[a]	188.81	38.74	0.80	184.85
Multistage (1)[a]	57.08	39.68	0.64	184.05
Probit	81.83	62.57	0.64	184.01
Log-probit[a]	176.71	66.89	0.77	184.93
Weibull[a]	105.85	40.69	0.52	185.67
Females				
Gamma[a]	287.41	81.95	0.49	233.19
Logistic	137.58	109.45	0.53	231.99
Log-logistic[a]	340.15	95.35	0.57	232.88
Multistage (1)[a]	100.41	74.33	0.40	232.72
Probit	145.33	118.47	0.53	231.97
Log-probit[a]	336.41	143.31	0.57	232.87
Weibull[a]	240.71	80.40	0.43	233.43

[a]These models in U.S. EPA BMDS version 2.0 were fit to the rat dose-response data shown in Table 5-1 by using internal dose metrics calculated with the rat PBPK model. Details of the models are as follows: Gamma and Weibull models restrict power ≥1; log-logistic and log-probit models restrict to slope >1, multistage model restrict betas ≥0; lowest degree polynomial with an adequate fit is reported (degree of polynomial noted in parentheses).
[b]Bolded model is the best-fitting model in the most sensitive sex (males), which is used in the RfD derivation.

Source: Serota et al. (1986a).

Logistic Model, Male Rats (Serota et al., 1986a), CYP Metabolism (Rate of Production) Metric

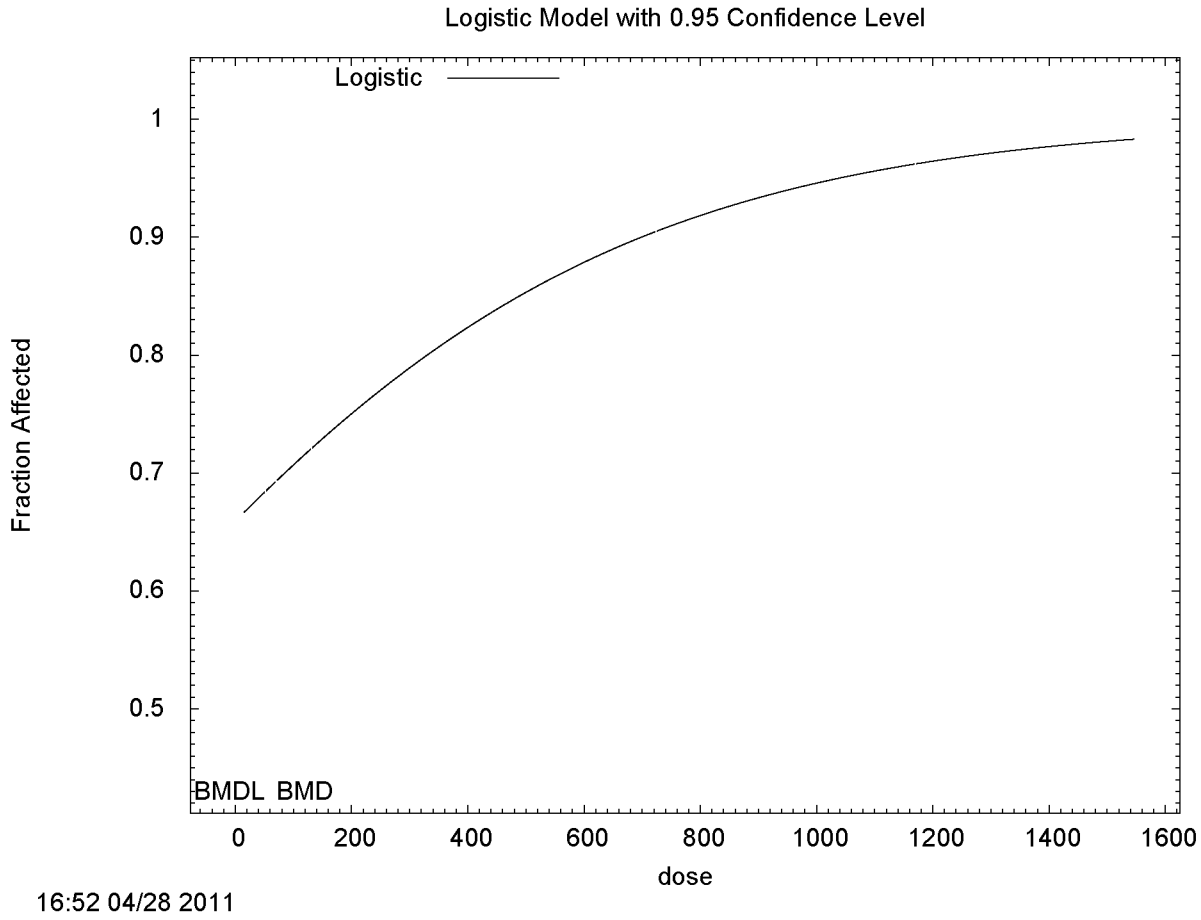


Figure F-1. Predicted (logistic model) and observed incidence of noncancer liver lesions in male F344 rats exposed to dichloromethane in drinking water for 2 years (Serota et al., 1986a).

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 ================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\Usepa\BMDS21\Data\logSerota_new_CYPSetting.(d)
        Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\logSerota_new_CYPSetting.plt
                                              Thu Apr 28 16:52:22 2011
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 BMDS Model Run
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   The form of the probability function is:

   P[response] = 1/[1+EXP(-intercept-slope*dose)]


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
```

Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
background = 0 Specified
intercept = 0.670583
slope = 0.00188727

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.47
slope	-0.47	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	0.659731	0.210128	0.247887	1.07157
slope	0.00220361	0.00049774	0.00122806	0.00317916

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-89.2097	5			
Fitted model	-89.925	2	1.43061	3	0.6984
Reduced model	-106.616	1	34.8133	4	<.0001

AIC: 183.85

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.6592	50.099	52.000	76	0.460
131.6000	0.7211	24.516	22.000	34	-0.962
723.9000	0.9051	34.393	35.000	38	0.336
1170.5000	0.9623	33.680	34.000	35	0.284
1548.0000	0.9832	40.312	40.000	41	-0.380

Chi^2 = 1.48 d.f. = 3 P-value = 0.6880

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 70.6874

BMDL = 51.4235

F.2. INHALATION RfC: BMD MODELING OF LIVER LESION INCIDENCE DATA FOR RATS EXPOSED TO DICHLOROMETHANE VIA INHALATION FOR 2 YEARS (Nitschke et al., 1988a)

BMD and BMDL refer to the model-predicted dose (and its lower 95% confidence limit) associated with 10% extra risk for the incidence of hepatic vacuolation in female F344 rats exposed to dichloromethane via inhalation for 2 years (Nitschke et al., 1988a) (Table F-3).

Table F-3. Incidence data for liver lesions (hepatic vacuolation) and internal liver doses based on various metrics in female Sprague-Dawley rats exposed to dichloromethane via inhalation for 2 years (Nitschke et al., 1988a)

Sex	Exposure (ppm)	Liver lesion incidence[a]	Rat internal liver dose[b]			
			CYP	GST	GST and CYP	Parent AUC
Male	0	22/70 (31)	Not modeled because results from male rats were not provided for the 50 and 200 ppm groups			
	50	Not reported	Not modeled because results for middle two doses were not reported			
	200	Not reported				
	500	28/70 (40)				
Female (BW = 229 g)	0	41/70 (59%)	0	0	0	0
	50	42/70 (60%)	285.3	6.17	291.4	1.18
	200	41/70 (58%)	665.3	93.2	758.5	17.8
	500	53/70 (76%)[c]	782.1	360.0	1,142.1	68.6

[a]Number affected divided by total sample size.
[b]Internal doses were estimated using a rat PBPK model using exposures reported by study authors (50 ppm = 174 mg/m^3, 200 ppm = 695 mg/m^3, and 500 ppm = 1,737 mg/m^3) and are weighted-average daily values for 1 week of exposure at 6 hours/day, 5 days/week. CYP dose is in units of mg dichloromethane metabolized via CYP pathway/L tissue/day; GST dose is in units of mg dichloromethane metabolized via GST pathway/L tissue/day; GST and CYP dose is in units of mg dichloromethane metabolized via CYP and GST pathways/L tissue/day; and Parent AUC dose is in units of mg dichloromethane × hours)/L tissue.
[c]Significantly ($p < 0.05$) different from control with Fisher's exact test.

Source: Nitschke et al. (1988a).

All available dichotomous models in the BMDS (version 2.0) were fit to male and female rat internal tissue doses of dichloromethane metabolized by the CYP pathway and incidences for animals with these liver lesions observed at the time of death (Table F-4). The log-probit model was the best fitting model for the female incidence data based on lowest AIC value among models with adequate fit (U.S. EPA, 2000c). (If two or more models share the lowest AIC, $BMDL_{10}$ values from these models may be averaged to obtain a POD. However, this average is no longer a lower confidence bound that provides the stated coverage, and thus should be referred to only as an average of $BMDL_{10}$ values. U.S. EPA does not support averaging BMDLs in situations in which AIC values are similar, but not identical, because the level of stated

coverage is lost and no consensus exists regarding a specific cut-off between similar and dissimilar AIC values.)

Table F-4. BMD modeling results for incidence of liver lesions in female Sprague-Dawley rats exposed to dichloromethane by inhalation for 2 years, based on liver specific CYP metabolism metric (mg dichloromethane metabolized via CYP pathway/L liver tissue/day)

Model[a]	BMD_{10}	$BMDL_{10}$	χ^2 goodness of fit *p*-value	AIC
Gamma[a]	622.10	227.29	0.48	367.24
Logistic	278.31	152.41	0.14	369.77
Log-logistic[a]	706.50	506.84	0.94	365.90
Multistage (3)[a]	513.50	155.06	0.25	368.54
Probit	279.23	154.52	0.14	369.76
Log-probit[a,b]	**737.93**	**531.82**	**0.98**	**365.82**
Weibull[a]	715.15	494.87	0.95	365.88

[a]These models in U.S. EPA BMDS version 2.0 were fit to the rat dose-response data shown in Table 5-5 by using internal dose metrics calculated with the rat PBPK model. Gamma and Weibull models restrict power ≥1; log-logistic and log-probit models restrict to slope >1, multistage model restrict betas ≥0; lowest degree polynomial with an adequate fit reported (degree of polynomial in parentheses).
[b]Bolded model is the best-fitting model in the most sensitive sex (females), which is used in the RfC derivation.

Source: Nitschke et al. (1988a).

Log Probit Model, Female Rats (Nitschke et al., 1988a), CYP Metabolism (Rate of Production) Metric

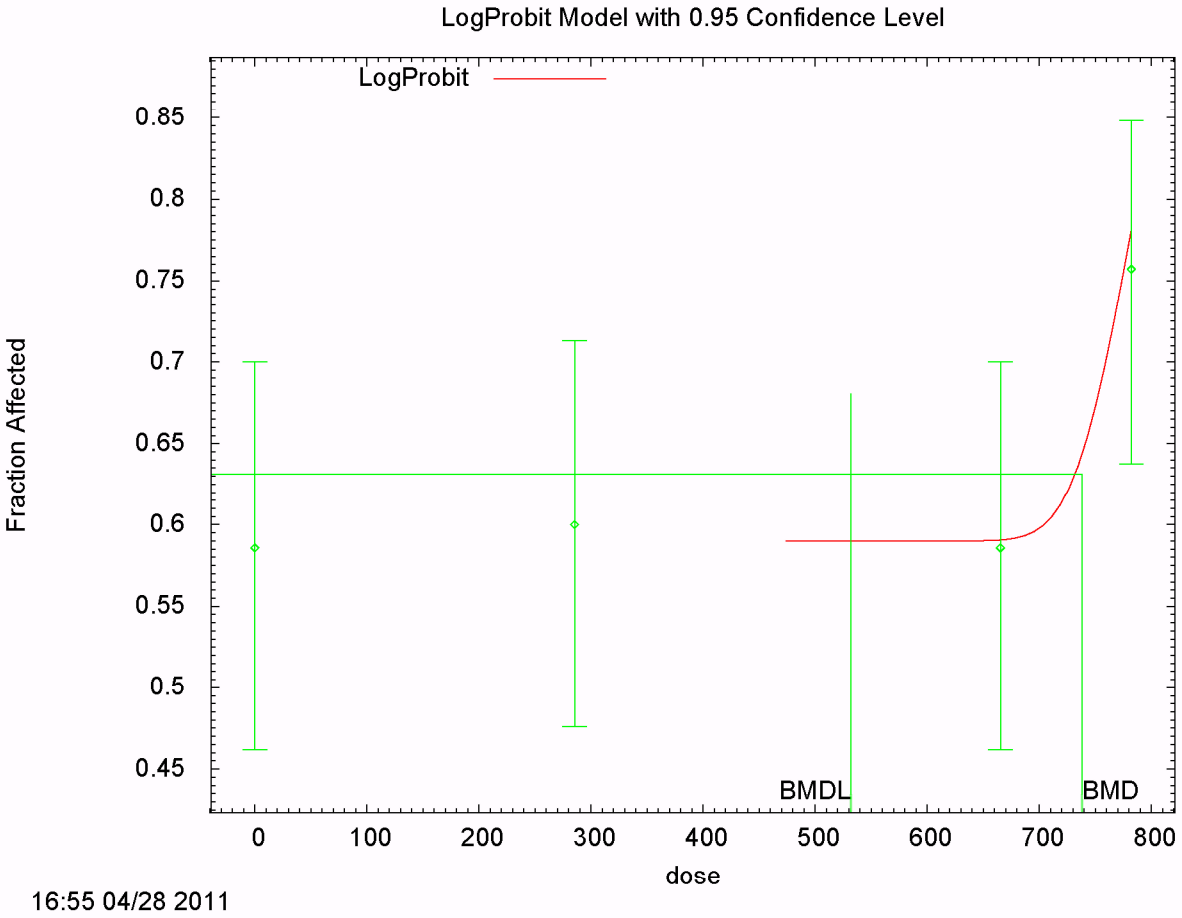

Figure F-2. Predicted (log-probit model) and observed incidence of noncancer liver lesions in female Sprague-Dawley rats inhaling dichloromethane for 2 years (Nitschke et al., 1988a).

```
=================================================================
        Probit Model. (Version: 3.1;  Date: 05/16/2008)
        Input Data File: C:\Usepa\BMDS21\Data\lnpNitschke_new_CYPSetting.(d)
        Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\lnpNitschke_new_CYPSetting.plt
                                          Thu Apr 28 16:55:00 2011
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 BMDS Model Run
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   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1
```

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values
background = 0.585714
intercept = -7.71354
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.37
intercept	-0.37	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.590372	0.0339907	0.523751	0.656992
intercept	-120.151	0.346802	-120.831	-119.471
slope	18	NA		

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-180.889	4			
Fitted model	-180.909	2	0.0403892	2	0.98
Reduced model	-184.186	1	6.5937	3	0.08604

AIC: 365.818

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.5904	41.326	41.000	70	-0.079
285.3000	0.5904	41.326	42.000	70	0.164
665.3000	0.5907	41.350	41.000	70	-0.085
782.1000	0.7571	52.998	53.000	70	0.001

Chi^2 = 0.04 d.f. = 2 P-value = 0.9800

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 737.929

BMDL = 531.817

APPENDIX G. SUMMARY OF BENCHMARK DOSE (BMD) MODELING OF CANCER ENDPOINTS

G.1. ORAL CANCER SLOPE FACTORS: BMD MODELING OF LIVER TUMOR INCIDENCE DATA FOR MICE EXPOSED TO DICHLOROMETHANE IN DRINKING WATER FOR 2 YEARS (Serota et al., 1986b; Hazleton Laboratories, 1983)

BMD_{10} and $BMDL_{10}$ refer to the model-predicted dose (and its lower 95% confidence limit) associated with 10% extra risk for the incidence of hepatocellular adenoma and carcinoma in male mice given dichloromethane in drinking water for 2 years (Serota et al., 1986b; Hazleton Laboratories, 1983) (Table G-1). Multistage models were fit to male mouse internal tissue doses of dichloromethane metabolized by the GST pathway and incidences for animals with liver tumors observed at the time of death. Different polynomial models were compared based on adequacy of model fit as assessed by overall χ^2 goodness of fit (p-value > 0.10) and examination of residuals at the 0 dose exposure (controls) and in the region of the BMR. Due to the lack of a monotonic increase in tumor response at the high dose, the model did not adequately fit the data. In consideration of the region of interest (i.e., low-dose risk estimation), the highest dose group was excluded. The modeling of the remaining four dose groups exhibited an adequate fit to the data. The predicted BMD_{10} and $BMDL_{10}$ for the incidence data are 73.0 and 39.6 mg dichloromethane metabolized via GST pathways/L tissue/day, respectively, for the internal liver metabolism metric, and 3.05 and 1.65 mg dichloromethane metabolized via GST pathway in lung and liver/kg-day, respectively, for the whole-body metabolism metric (Table G-2).

Table G-1. Incidence data for liver tumors and internal liver doses, based on GST metabolism dose metrics, in male B6C3F$_1$ mice exposed to dichloromethane in drinking water for 2 years

Sex	Nominal (actual) daily intake (mg/kg-d)	Mouse liver tumor incidence[a]	Mouse internal liver metabolism dose[b]	Mouse whole body metabolism dose[c]
Male (BW = 37.3 g)	0 (0)	24/125 (19%)	0	0
	60 (61)	51/199 (26%)	17.5	0.73
	125 (124)	30/99 (30%)	63.3	2.65
	185 (177)	31/98 (32%)	112.0	4.68
	250 (234)	35/123 (28%)	169.5	7.1

[a]Hepatocellular carcinoma or adenoma combined. Mice dying prior to 52 weeks were excluded from the denominators. Cochran-Armitage trend p-value = 0.058. p-values for comparisons with the control group were 0.071, 0.023, 0.019, and 0.036 in the 60, 125, 185, and 250 mg/kg-d groups, respectively, based on statistical analyses reported by Hazleton Laboratories (1983).
[b]mg dichloromethane metabolized via GST pathway/L liver/day. Internal doses were estimated from simulations of actual daily doses reported by the study authors.
[c]Based on the sum of dichloromethane metabolized via the GST pathway in the lung plus the liver, normalized to total BW (i.e., [lung GST metabolism (mg/day) + liver GST metabolism (mg/day)]/kg BW). Units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.

Sources: Serota et al. (1986b); Hazleton Laboratories (1983).

Table G-2. BMD modeling results and tumor risk factors for internal dose metric associated with 10% extra risk for liver tumors in male B6C3F$_1$ mice exposed to dichloromethane in drinking water for 2 years, based on liver-specific GST metabolism and whole body GST metabolism dose metrics

Internal dose metric[a]	BMDS model[b]	χ^2 goodness of fit p-value	Mouse BMD_{10}[c]	Mouse $BMDL_{10}$[c]	Allometric-scaled human $BMDL_{10}$[d]	Tumor risk factor[e]	
						Scaling = 1.0	Allometric-scaled
Liver-specific	Mulitstage (1,1)	0.56	73.0	39.6	5.66	2.53×10^{-3}	1.77×10^{-2}
Whole-body	Multistage (1,1)	0.56	3.05	1.65	0.24	–	4.24×10^{-1}

[a]Liver specific dose units = mg dichloromethane metabolized via GST pathway/L tissue/day; Whole-body dose units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day).
[b]The Multistage model in U.S. EPA BMDS version 2.0 was fit to the mouse dose-response data shown in Table 5-11 using internal dose metrics calculated with the mouse PBPK model. Numbers in parentheses indicate: (1) the number of dose groups dropped in order to obtain an adequate fit, and (2) the degree polynomial of the model.
[c]BMD_{10} and $BMDL_{10}$ refer to the BMD-model-predicted mouse internal and its 95% lower confidence limit, associated with a 10% extra risk for the incidence of tumors.
[d]Mouse $BMDL_{10}$ divided by $(BW_{human}/BW_{mouse})^{0.25} = 7$.
[e]Dichloromethane tumor risk factor (extra risk per unit internal dose) derived by dividing the BMR (0.1) by the mouse $BMDL_{10}$ and by the allometric-scaled human $BMDL_{10}$, for the scaling = 1.0 and allometric-scaled risk factors, respectively.

Modeling results are presented in the subsequent sections for the tissue-specific liver-metabolism metric (Section G.1.1) and the whole-body metabolism metric (Section G.1.2).

G.1.1. Modeling Results for the Internal Liver Metabolism Metric

Serota et al. (1986b), Hazleton Laboratories (1983): Internal liver dose-response, highest dose dropped

1-degree polynomial

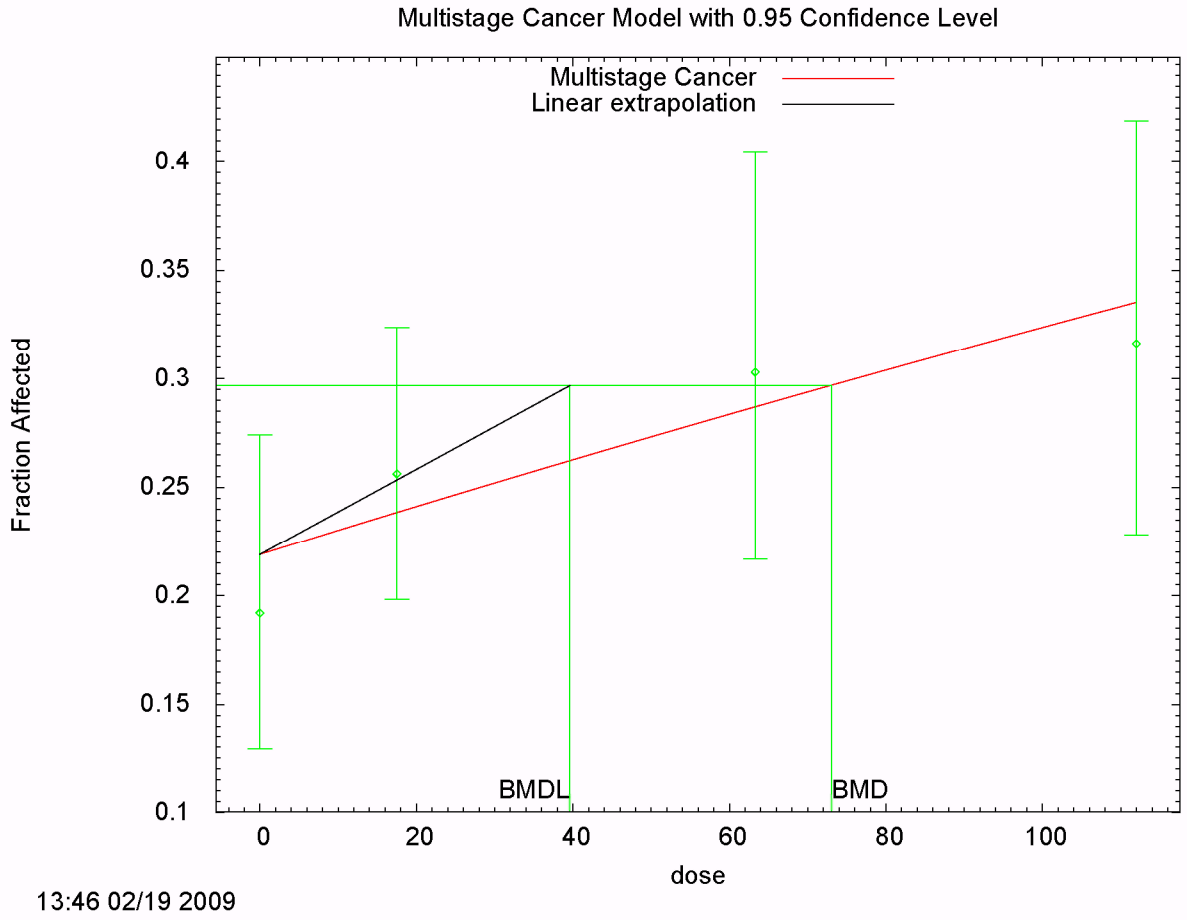

Figure G-1. Predicted and observed incidence of animals with hepatocellular carcinoma or adenoma in male B6C3F$_1$ mice exposed to dichloromethane in drinking water for 2 years, using liver-specific metabolism dose metric (Serota et al., 1986b; Hazleton Laboratories, 1983).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\DCM\Serota\highdosedropped\1MulSerMS_.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\DCM\Serota\highdosedropped\1MulSerMS_.plt
                                        Thu Feb 19 13:46:49 2009
=================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:
   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

Dependent variable = incidence
Independent variable = dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

**** We are sorry but Relative Function and Parameter Convergence ****
**** are currently unavailable in this model. Please keep checking ****
**** the web sight for model updates which will eventually ****
**** incorporate these convergence criterion. Default values used. ****

Default Initial Parameter Values
Background = 0.218634
Beta(1) = 0.00136788

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.7
Beta(1)	-0.7	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.218642	*	*	*
Beta(1)	0.00144288	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-296.282	4			
Fitted model	-296.87	2	1.1754	2	0.5556
Reduced model	-299.126	1	5.68747	3	0.1278

AIC: 597.74

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2186	27.330	24.000	125	-0.721
17.5000	0.2381	47.387	51.000	199	0.601
63.3000	0.2868	28.398	30.000	99	0.356
112.0000	0.3352	32.853	31.000	98	-0.397

Chi^2 = 1.16 d.f. = 2 P-value = 0.5585

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 73.0211

BMDL = 39.6034

BMDU = 335.18

Taken together, (39.6034, 335.18) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00252504

G.1.2. Modeling Results for the Whole-Body Metabolism Metric

Serota et al. (1986b), Hazleton Laboratories (1983): Internal whole-body metabolism dose-response in mice, highest dose dropped

1-degree polynomial

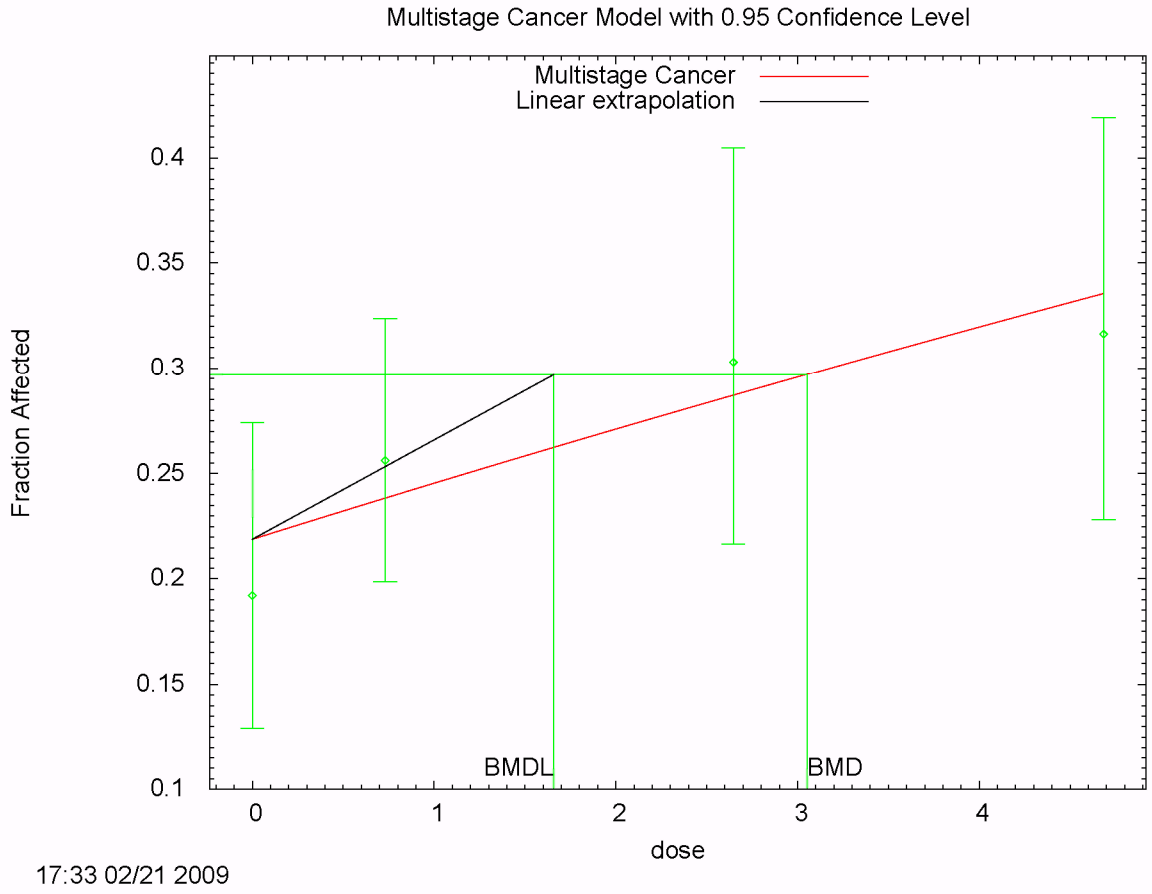

Figure G-2. Predicted and observed incidence of animals with hepatocellular carcinoma or adenoma in male B6C3F$_1$ mice exposed to dichloromethane in drinking water for 2 years, using whole-body metabolism dose metric (Serota et al., 1986b; Hazleton Laboratories, 1983).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\DCM\Serota\highdosedropped\1MulSerMS_.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\DCM\Serota\highdosedropped\1MulSerMS_.plt
                                          Sat Feb 21 17:33:49 2009
=================================================================
 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
   Dependent variable = incidence
   Independent variable = dose
```

```
 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****

                 Default Initial Parameter Values
                    Background =     0.218649
                       Beta(1) =     0.032703

           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1         -0.7

   Beta(1)         -0.7            1
                              Parameter Estimates
                                                    95.0% Wald Confidence Interval
     Variable         Estimate      Std. Err.   Lower Conf. Limit   Upper Conf. Limit
    Background         0.218662         *                *                   *
       Beta(1)        0.0344939         *                *                   *
* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -296.282         4
   Fitted model        -296.871         2       1.17643      2          0.5553
  Reduced model        -299.126         1       5.68747      3          0.1278
           AIC:         597.741
                                  Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.2187        27.333    24.000         125       -0.721
    0.7310     0.2381        47.385    51.000         199        0.602
    2.6470     0.2868        28.397    30.000          99        0.356
    4.6840     0.3352        32.853    31.000          98       -0.396

 Chi^2 = 1.17      d.f. = 2        P-value = 0.5582

   Benchmark Dose Computation
Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95

             BMD =        3.05447

            BMDL =        1.65649

            BMDU =        14.0263

Taken together, (1.65649, 14.0263) is a 90% two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0603686
```

G.2. CANCER INHALATION UNIT RISK: BMD MODELING OF LIVER AND LUNG TUMOR INCIDENCE DATA FOR MALE MICE EXPOSED TO DICHLOROMETHANE VIA INHALATION FOR 2 YEARS (Mennear et al., 1988; NTP, 1986)

BMD_{10} and $BMDL_{10}$ refer to the model-predicted dose (and its lower 95% confidence limit) associated with 10% extra risk for the combined incidence of adenoma and carcinoma of the liver or lung of male $B6C3F_1$ mice inhaling dichloromethane for 2 years (Mennear et al., 1988; NTP, 1986) (Table G-3).

Table G-3. Incidence data for liver and lung tumors and internal doses based on GST metabolism dose metrics in male $B6C3F_1$ mice exposed to dichloromethane via inhalation for 2 years

Sex, tumor type	BW (g)	External dichloromethane concentration (ppm)	Mouse tumor incidence	Mouse internal tissue dose[a]	Mouse whole body metabolism dose[b]
Male, liver[c]	–	0	22/50 (44%)[d]	0	0
	34.0	2,000	24/47 (51%)	2,363.7	100.2
	32.0	4,000	33/47 (70%)	4,972.2	210.7
Male, lung[e]	–	0	5/50 (10%)[d]	0	0
	34.0	2,000	27/47 (55%)	475.0	100.2
	32.0	4,000	40/47 (85%)	992.2	210.7

[a]For liver tumors: mg dichloromethane metabolized via GST pathway/L liver tissue/day from 6 hours/day, 5 days/week exposure; for lung tumors: mg dichloromethane metabolized via GST pathway/L lung tissue/day from 6 hours/day, 5 days/week exposure.
[b]Based on the sum of dichloromethane metabolized via the GST pathway in the lung plus the liver, normalized to total BW (i.e., [lung GST metabolism (mg/day) + liver GST metabolism (mg/d)]/kg BW). Units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.
[c]Hepatocellular carcinoma or adenoma. Mice dying prior to 52 weeks were excluded from the denominators.
[d]Statistically significant increasing trend (by incidental and life-table tests; $p \leq 0.01$).
[e]Bronchoalveolar carcinoma or adenoma. Mice dying prior to 52 weeks were excluded from the denominators.

Sources: Mennear et al. (1988); NTP (1986).

Multistage models were fit to male mouse internal tissue doses of dichloromethane metabolized by the GST pathway and incidences for animals with liver tumors observed at the time of death. The predicted BMD_{10} and $BMDL_{10}$ for the liver tumor incidence data are 913.9 and 544.4 mg dichloromethane metabolized via GST pathways/L liver/day, respectively, for the internal liver metabolism metric, and 38.7 and 23.1 mg dichloromethane metabolized via GST pathway in lung and liver/kg-day, respectively, for the whole-body metabolism metric (Table G-4). For lung tumors, the BMD_{10} and $BMDL_{10}$ are 61.7 and 48.7 mg dichloromethane metabolized via GST pathway/L tissue/day, respectively, for the lung-specific metric, and

13.1 and 10.3 mg dichloromethane metabolized via GST pathway in lung and liver/kg-day, respectively, for the whole-body metabolism metric.

Table G-4. BMD modeling results and tumor risk factors associated with 10% extra risk for liver and lung tumors in male B6C3F$_1$ mice exposed by inhalation to dichloromethane for 2 years, based on liver-specific GST metabolism and whole body GST metabolism dose metrics

Internal dose metric[a]		BMDS model[b]	χ^2 goodness of fit *p*-value	Mouse BMD_{10}[c]	Mouse $BMDL_{10}$[c]	Allometric-scaled human $BMDL_{10}$[d]	Tumor risk factor[e]	
							Scaling = 1.0	Allometric-scaled
Tissue-specific	Male, liver	Multistage (1)	0.40	913.9	544.4	77.8	1.84×10^{-4}	1.29×10^{-3}
	Male, lung	Multistage (1)	0.64	61.7	48.6	7.0	2.06×10^{-3}	1.44×10^{-2}
Whole body	Male, liver	Multistage (1)	0.40	38.7	23.1	3.3	–	3.03×10^{-2}
	Male, lung	Multistage (1)	0.66	13.1	10.3	1.5	–	6.80×10^{-2}

[a]Tissue specific dose units = mg dichloromethane metabolized via GST pathway per L (liver or lung) tissue per day; whole-body dose units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day).
[b]The Multistage model in EPA BMDS version 2.0 was fit to the mouse dose-response data shown in Table 5-17 using internal dose metrics calculated with the mouse PBPK model. Numbers in parentheses indicate: (1) the degree polynomial of the model.
[c]BMD_{10} and $BMDL_{10}$ refer to the BMD-model-predicted mouse internal dose and its 95% lower confidence limit, associated with a 10% extra risk for the incidence of tumors.
[d]Mouse $BMDL_{10}$ divided by $(BW_{human}/BW_{mouse})^{0.25} = 7$.
[e]Dichloromethane tumor risk factor (extra risk per unit internal dose) derived by dividing the BMR (0.1) by the mouse $BMDL_{10}$ and by the allometric-scaled human $BMDL_{10}$, for the scaling = 1.0 and allometric-scaled risk factors, respectively.

Modeling results are presented in the subsequent sections for the tissue-specific liver-metabolism metric for liver tumors (Section G.2.1), tissue-specific lung metabolism metric for lung tumors (Section G.2.2), and the whole-body metabolism metric for liver tumors (Section G.2.3) and lung tumors (Section G.2.4).

G.2.1. Modeling Results for the Internal Liver Metabolism Metric, Liver Tumors. Mennear et al. (1988); NTP (1986): Internal Liver Dose-Response for Liver Tumors in Male Mice

1-degree polynomial

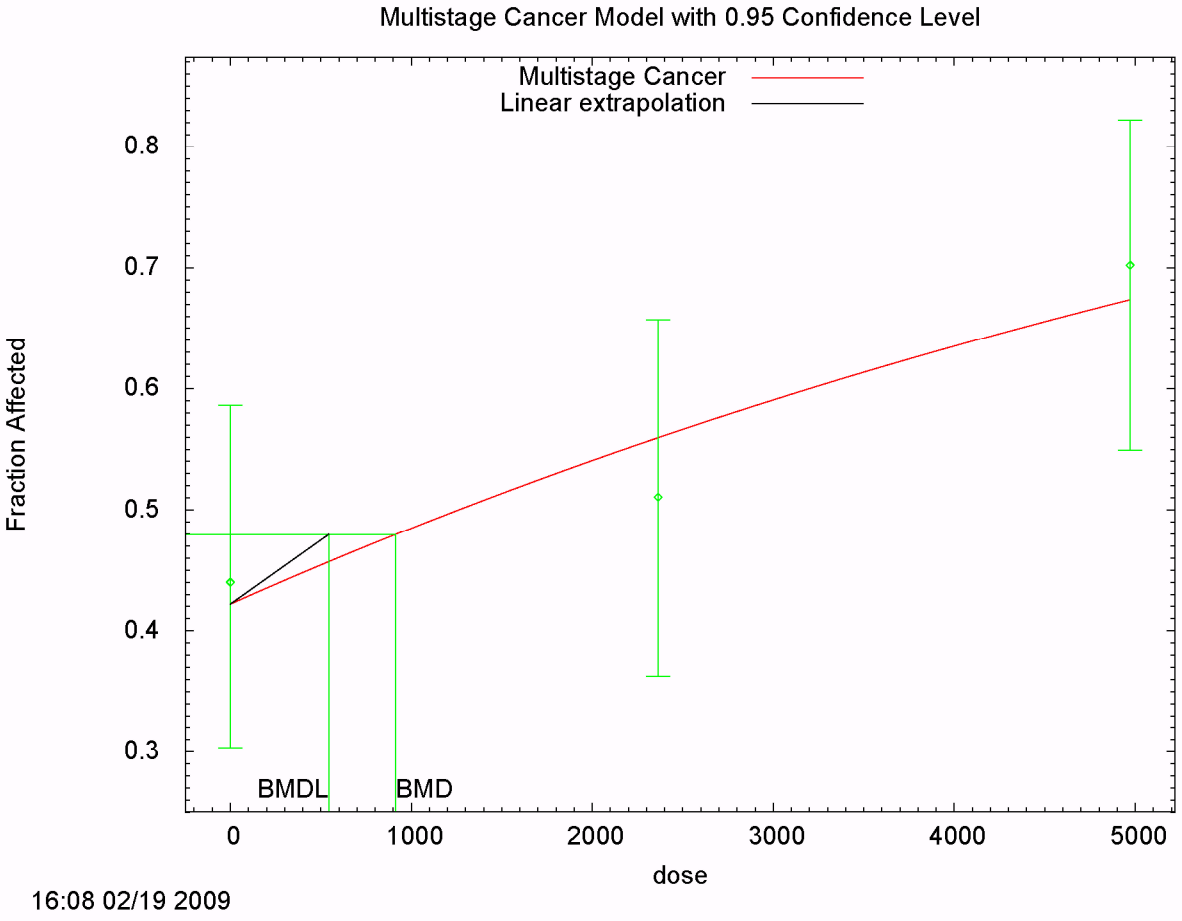

Figure G-3. Predicted and observed incidence of animals with hepatocellular carcinoma or adenoma in male B6C3F$_1$ mice exposed by inhalation to dichloromethane for 2 years, using liver-specific metabolism dose metric (Mennear et al., 1988; NTP, 1986).

```
 ================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\DCM\NTP\lung\male\1MulNTPMS_.(d)
        Gnuplot Plotting File:  C:\USEPA\IRIS\DCM\NTP\lung\male\1MulNTPMS_.plt
                                            Thu Feb 19 16:08:19 2009
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 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive

   Dependent variable = incidence
   Independent variable = dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****

                  Default Initial Parameter Values
                     Background =     0.406706
                        Beta(1) =   0.00012805

           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.69

   Beta(1)        -0.69            1

                                Parameter Estimates

                                                    95.0% Wald Confidence Interval
      Variable         Estimate     Std. Err.   Lower Conf. Limit   Upper Conf. Limit
    Background         0.421771         *              *                   *
       Beta(1)      0.000115283         *              *                   *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -95.4892         3
   Fitted model        -95.8368         2      0.695297      1          0.4044
  Reduced model        -99.1316         1       7.28482      2         0.02619

           AIC:         195.674

                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
```

0.0000	0.4218	21.089	22.000	50	0.261
2363.7000	0.5597	26.305	24.000	47	-0.677
4972.2000	0.6740	31.680	33.000	47	0.411

Chi^2 = 0.70 d.f. = 1 P-value = 0.4042

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 913.932

BMDL = 544.35

BMDU = 2569.01

Taken together, (544.35 , 2569.01) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000183705

G.2.2. Modeling Results for the Internal Lung Metabolism Metric, Lung Tumors. Mennear et al. (1988); NTP (1986): Internal Lung Dose-Response for Lung Tumors in Male Mice

1-degree polynomial

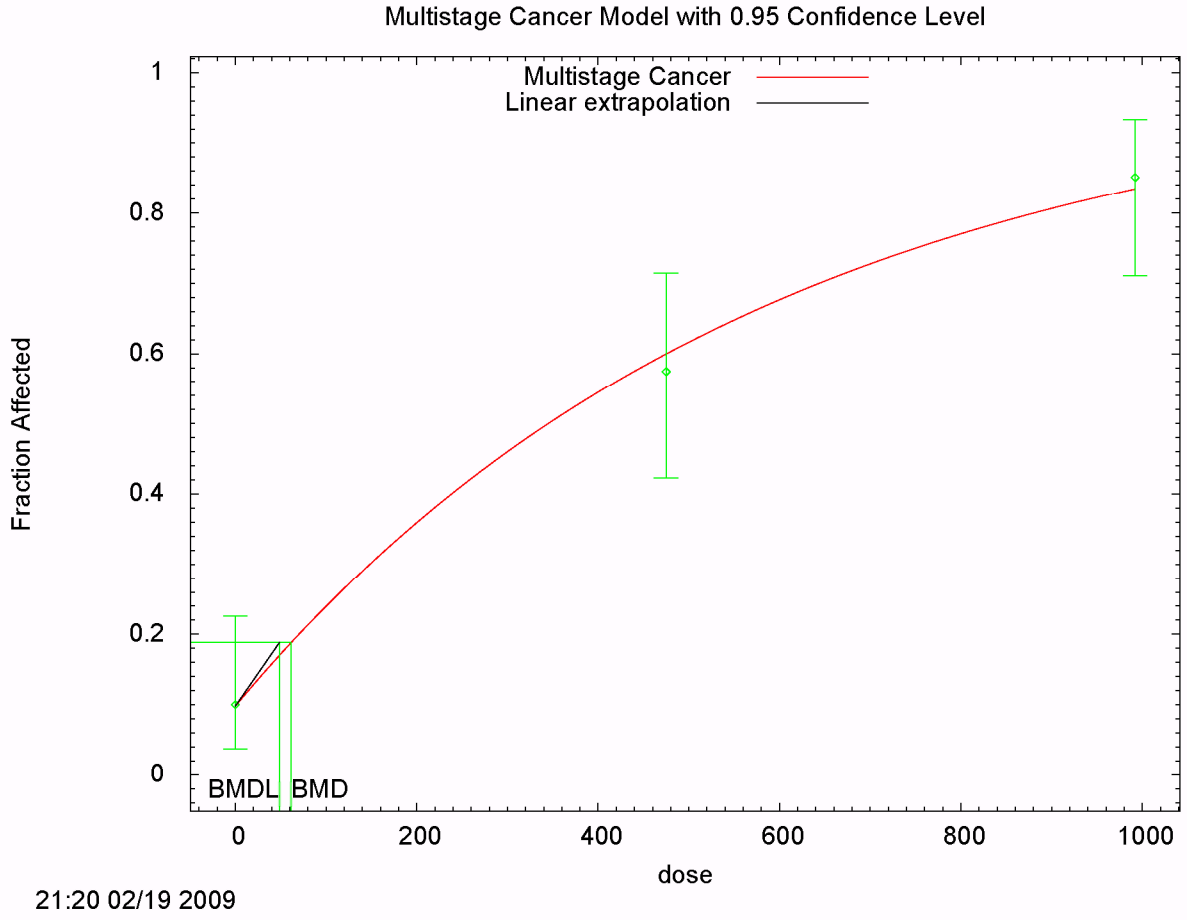


Figure G-4. Predicted and observed incidence of animals with carcinoma or adenoma in the lung of male B6C3F$_1$ mice exposed by inhalation to dichloromethane for 2 years, using liver-specific metabolism dose metric (Mennear et al., 1988; NTP, 1986).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\DCM\NTP\lung\male\1MulNTPMS_.(d)
        Gnuplot Plotting File:  C:\USEPA\IRIS\DCM\NTP\lung\male\1MulNTPMS_.plt
                                          Thu Feb 19 21:20:36 2009
 ================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

Dependent variable = incidence
Independent variable = dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

**** We are sorry but Relative Function and Parameter Convergence ****
**** are currently unavailable in this model. Please keep checking ****
**** the web sight for model updates which will eventually ****
**** incorporate these convergence criterion. Default values used. ****

Default Initial Parameter Values
Background = 0.0642604
Beta(1) = 0.00181622

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.56
Beta(1)	-0.56	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0980033	*	*	*
Beta(1)	0.00170868	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-68.0892	3			
Fitted model	-68.199	2	0.219579	1	0.6394
Reduced model	-99.8132	1	63.4479	2	<.0001

AIC: 140.398

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0980	4.900	5.000	50	0.047
475.0000	0.5994	28.171	27.000	47	-0.349
992.2000	0.8345	39.219	40.000	47	0.306

Chi^2 = 0.22 d.f. = 1 P-value = 0.6408

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk

Confidence level = 0.95

BMD = 61.6618

BMDL = 48.628

```
            BMDU =        80.2137

Taken together, (48.628 , 80.2137) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00205643
```

G.2.3. Modeling Results for the Whole-Body Metabolism Metric, Liver Tumors. Mennear et al. (1988); NTP (1986): Internal Whole-Body Metabolism Dose-Response for Liver Tumors in Male Mice

1-degree polynomial

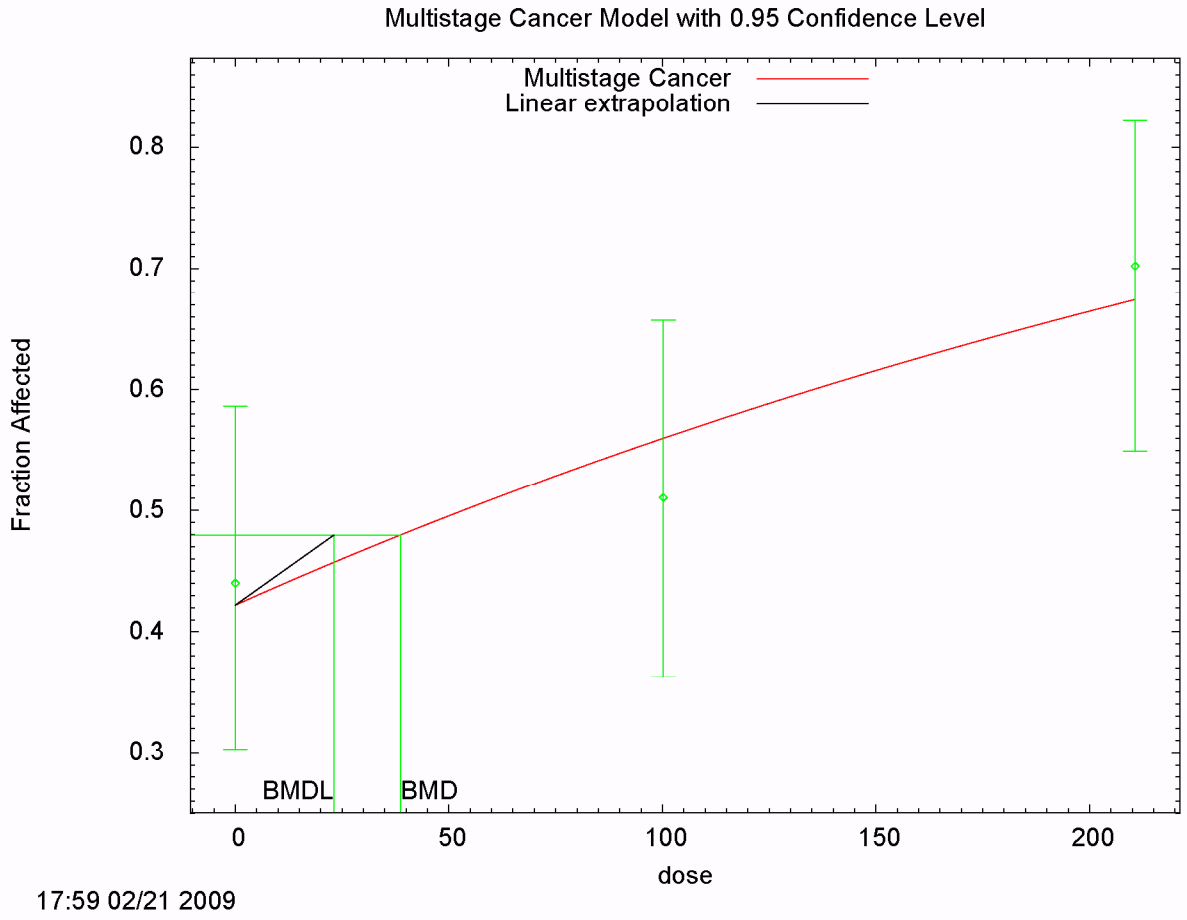


Figure G-5. Predicted and observed incidence of animals with hepatocellular carcinoma or adenoma in male B6C3F$_1$ mice exposed by inhalation to dichloromethane for 2 years, using whole-body metabolism dose metric (Mennear et al., 1988; NTP, 1986).

```
 ======================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\DCM\NTP\liver\male\1MulNTPMS_.(d)
        Gnuplot Plotting File:  C:\USEPA\IRIS\DCM\NTP\liver\male\1MulNTPMS_.plt
                                              Sat Feb 21 17:59:59 2009
 ======================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = incidence
   Independent variable = dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****

                  Default Initial Parameter Values
                     Background =     0.406695
                        Beta(1) =   0.00302163

           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.69

   Beta(1)        -0.69            1

                                Parameter Estimates
                                                    95.0% Wald Confidence Interval
      Variable         Estimate     Std. Err.   Lower Conf. Limit   Upper Conf. Limit
    Background         0.421768         *             *                   *
       Beta(1)       0.00272018         *             *                   *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -95.4892         3
   Fitted model        -95.8372         2      0.696122      1          0.4041
  Reduced model        -99.1316         1       7.28482      2         0.02619

           AIC:         195.674
                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.4218        21.088    22.000          50        0.261
  100.2000     0.5597        26.307    24.000          47       -0.678
```

210.7000	0.6740	31.679	33.000	47	0.411

Chi^2 = 0.70 d.f. = 1 P-value = 0.4039

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 38.733

BMDL = 23.0698

BMDU = 108.885

Taken together, (23.0698, 108.885) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00433467

G.2.4. Modeling Results for the Whole-Body Metabolism Metric, Lung Tumors. Mennear et al. (1988); NTP (1986): Internal Whole-Body Metabolism Dose-Response for Lung Tumors in Male Mice

1-degree polynomial

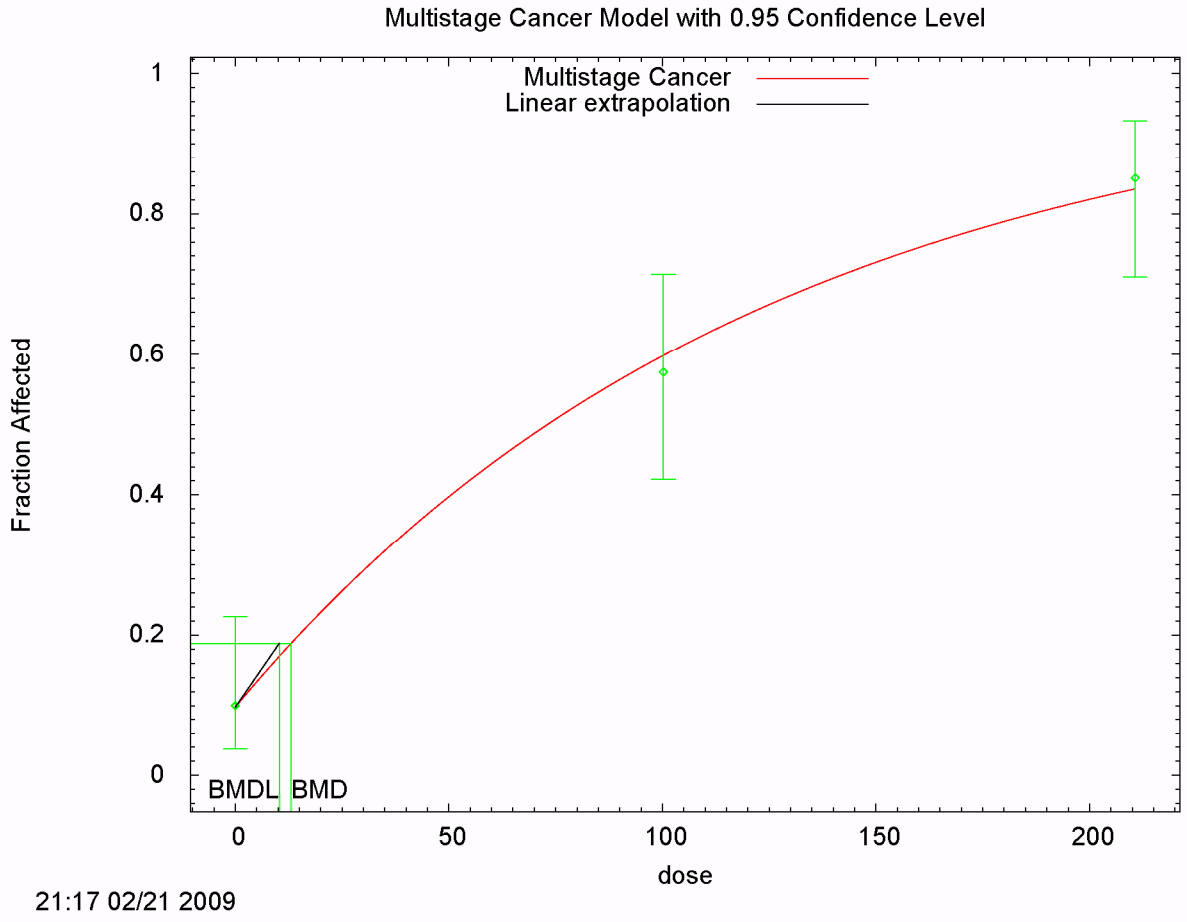


Figure G-6. Predicted and observed incidence of animals with carcinoma or adenoma in the lung of male B6C3F$_1$ mice exposed by inhalation to dichloromethane for 2 years, using whole-body metabolism dose metric (Mennear et al., 1988; NTP, 1986).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\IRIS\DCM\NTP\lung\male\1MulNTPMS_.(d)
        Gnuplot Plotting File:  C:\USEPA\IRIS\DCM\NTP\lung\male\1MulNTPMS_.plt
                                          Sat Feb 21 21:17:36 2009
=================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:
   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

```
   Dependent variable = incidence
   Independent variable = dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****

                 Default Initial Parameter Values
                    Background =    0.0659119
                       Beta(1) =   0.00855407

           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.56

   Beta(1)        -0.56            1

                               Parameter Estimates
                                                    95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0980803            *                *                   *
        Beta(1)       0.00807004            *                *                   *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -68.0892         3
   Fitted model        -68.1887         2      0.198975      1          0.6555
  Reduced model        -99.8132         1       63.4479      2         <.0001

          AIC:         140.377
                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.0981         4.904     5.000          50        0.046
  100.2000     0.5982        28.116    27.000          47       -0.332
  210.7000     0.8353        39.259    40.000          47        0.291

 Chi^2 = 0.20      d.f. = 1        P-value = 0.6569

   Benchmark Dose Computation
Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =        13.0558

            BMDL =        10.2947
```

BMDU = 16.9865

Taken together, (10.2947, 16.9865) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00971371

APPENDIX H. COMPARATIVE CANCER INHALATION UNIT RISK BASED ON FEMALE MICE DATA

Using the male B6C3F$_1$ mouse data from a 2-year inhalation exposure study (Mennear et al., 1988; NTP, 1986), the recommended cancer inhalation unit risks are 7×10^{-9} $(\mu g/m^3)^{-1}$ and 5×10^{-9} $(\mu g/m^3)^{-1}$ for the development of liver and lung cancer, respectively, based on the mean for the GST-T1$^{+/+}$ population. These values were derived using a tissue-specific GST metabolism dose metric with allometric scaling. The combined human equivalent inhalation unit risk values for both tumor types is 1×10^{-8} $(\mu g/m^3)^{-1}$. As described in detail below, the resulting combined human equivalent inhalation unit risk values for both tumors did not differ appreciably by gender.

BMD_{10} and $BMDL_{10}$ refer to the model-predicted dose (and its lower 95% confidence limit) associated with 10% extra risk for the combined incidence of adenoma and carcinoma of the liver or lung of female B6C3F$_1$ mice inhaling dichloromethane for 2 years (Mennear et al., 1988; NTP, 1986) (Table H-1).

Table H-1. Incidence data for liver and lung tumors and internal doses based on GST metabolism dose metrics in female B6C3F$_1$ mice exposed to dichloromethane via inhalation for 2 years

Sex, tumor type	BW (g)	External dichloromethane concentration (ppm)	Mouse tumor incidence	Mouse internal tissue dose[a]	Mouse whole body metabolism dose[b]
Female, liver[c]	–	0	3/47 (6%)[d]	0	0
	30.0	2,000	16/46 (35%)	2,453.2	104.0
	29.0	4,000	40/46 (87%)	5,120.0	217.0
Female, lung[e]	–	0	3/45 (6%)[d]	0	0
	30.0	2,000	30/46 (65%)	493.0	104.0
	29.0	4,000	41/46 (89%)	1,021.8	217.0

[a]For liver tumors: mg dichloromethane metabolized via GST pathway/L liver tissue/day from 6 hours/day, 5 days/week exposure; for lung tumors: mg dichloromethane metabolized via GST pathway/L lung tissue/day from 6 hours/day, 5 days/week exposure.
[b]Based on the sum of dichloromethane metabolized via the GST pathway in the lung plus the liver, normalized to total BW (i.e., [lung GST metabolism (mg/d) + liver GST metabolism (mg/day)]/kg BW). Units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.
[c]Hepatocellular carcinoma or adenoma. Mice dying prior to 52 weeks were excluded from the denominators.
[d]Statistically significant increasing trend (by incidental and life-table tests; $p \leq 0.01$).
[e]Bronchoalveolar carcinoma or adenoma. Mice dying prior to 52 weeks were excluded from the denominators.

Sources: Mennear et al. (1988); NTP (1986).

Multistage models were fit to the female mouse internal tissue doses of dichloromethane metabolized by the GST pathway and incidences for animals with liver tumors observed at the time of death. The predicted BMD_{10} and $BMDL_{10}$ for the liver and lung tumor incidence data are shown in Table H-2.

Table H-2. BMD modeling results and tumor risk factors associated with 10% extra risk for liver and lung tumors in female B6C3F$_1$ mice exposed by inhalation to dichloromethane for 2 years, based on liver-specific GST metabolism and whole-body GST metabolism dose metrics

Internal dose metric[a]		BMDS model[b]	χ^2 goodness of fit *p*-value	Mouse BMD_{10}[c]	Mouse $BMDL_{10}$[c]	Allometric-scaled human $BMDL_{10}$[d]	Tumor risk factor[e]	
							Scaling = 1.0	Allometric-scaled
Liver-specific	Female, liver	Multistage (2)	0.53	1,224.1	659.7	94.2	1.52×10^{-4}	1.06×10^{-3}
	Female, lung	Multistage (1)	0.87	51.2	40.7	5.8	2.46×10^{-3}	1.72×10^{-2}
Whole body	Female, liver	Multistage (2)	0.53	51.9	28.0	4.0	–	2.50×10^{-2}
	Female, lung	Multistage (1)	0.88	10.8	8.6	1.2	–	8.14×10^{-2}

[a]Liver specific dose units = mg dichloromethane metabolized via GST pathway/L tissue per day; whole-body dose units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-d.
[b]The Multistage model in EPA BMDS version 2.0 was fit to the mouse dose-response data shown in Table 5-17 using internal dose metrics calculated with the mouse PBPK model. Numbers in parentheses indicate: (1) the degree polynomial of the model.
[c]BMD_{10} and $BMDL_{10}$ refer to the BMD-model-predicted mouse internal dose and its 95% lower confidence limit, associated with a 10% extra risk for the incidence of tumors.
[d]Mouse $BMDL_{10}$ divided by $(BW_{human}/BW_{mouse})^{0.25} = 7$.
[e]Dichloromethane tumor risk factor (extra risk per unit internal dose) derived by dividing the BMR (0.1) by the mouse $BMDL_{10}$ and by the allometric-scaled human $BMDL_{10}$, for the scaling = 1.0 and allometric-scaled risk factors, respectively.

A probabilistic PBPK model for dichloromethane in humans adapted from David et al. (2006) (see Appendix B) was used with Monte Carlo sampling to calculate distributions of internal lung, liver, or blood doses associated with chronic unit inhalation (1 μg/m^3) exposures. The model was then executed by using the external unit exposure as input, and the resulting human equivalent internal dose was recorded. This process was repeated for 10,000 iterations to generate a distribution of human internal doses. The resulting distribution of inhalation unit risks shown in Table H-3 was derived by multiplying the human internal dose tumor risk factor (in units of reciprocal internal dose) by the respective distributions of human average daily internal dose resulting from a chronic unit inhalation exposure of 1 μg/m^3 dichloromethane. Risk estimates were slightly higher for liver tumors and essentially equivalent for lung tumors in males compared to females.

Table H-3. Inhalation unit risks for dichloromethane based on PBPK model-derived internal liver and lung doses in B6C3F$_1$ female mice exposed via inhalation for 2 years, based on liver-specific GST metabolism and whole-body metabolism dose metrics, by population genotype

Internal dose metric and scaling factor[a]	Population genotype[b]	Tumor type	Human tumor risk factor[c]	Distribution of human internal dichloromethane doses from 1 µg/m^3 exposure[d]			Resulting candidate human inhalation unit risk[e] (µg/m^3)$^{-1}$		
				Mean	95th percentile	99th percentile	Mean	95th percentile	99th percentile
Tissue-specific, allometric-scaled	GST-T1$^{+/+}$	Liver	1.06×10^{-3}	6.61×10^{-6}	2.21×10^{-5}	4.47×10^{-5}	7.0×10^{-9}	2.4×10^{-8}	4.7×10^{-8}
	GST-T1$^{+/+}$	Lung	1.72×10^{-2}	3.89×10^{-7}	1.24×10^{-6}	2.42×10^{-6}	6.7×10^{-9}	2.1×10^{-8}	4.2×10^{-8}
	Mixed	Liver	1.06×10^{-3}	3.71×10^{-6}	1.43×10^{-5}	3.03×10^{-5}	3.9×10^{-9}	1.5×10^{-8}	3.2×10^{-8}
	Mixed	Lung	1.72×10^{-2}	2.20×10^{-7}	8.06×10^{-7}	1.69×10^{-6}	3.8×10^{-9}	1.4×10^{-8}	2.9×10^{-8}
Tissue-specific, scaling = 1.0	GST-T1$^{+/+}$	Liver	1.52×10^{-4}	6.61×10^{-6}	2.21×10^{-5}	4.47×10^{-5}	1.0×10^{-9}	3.4×10^{-9}	6.8×10^{-9}
	GST-T1$^{+/+}$	Lung	2.46×10^{-3}	3.89×10^{-7}	1.24×10^{-6}	2.42×10^{-6}	9.6×10^{-10}	3.1×10^{-9}	6.0×10^{-9}
	Mixed	Liver	1.52×10^{-4}	3.71×10^{-6}	1.43×10^{-5}	3.03×10^{-5}	5.6×10^{-10}	2.2×10^{-9}	4.6×10^{-9}
	Mixed	Lung	2.46×10^{-3}	2.20×10^{-7}	8.06×10^{-7}	1.69×10^{-6}	5.4×10^{-10}	2.0×10^{-9}	4.2×10^{-9}
Whole-body, allometric-scaled	GST-T1$^{+/+}$	Liver	2.50×10^{-2}	1.80×10^{-7}	6.38×10^{-7}	1.41×10^{-6}	4.5×10^{-9}	1.6×10^{-8}	3.5×10^{-8}
	GST-T1$^{+/+}$	Lung	8.14×10^{-2}	1.80×10^{-7}	6.38×10^{-7}	1.41×10^{-6}	1.5×10^{-8}	5.2×10^{-8}	1.2×10^{-7}
	Mixed	Liver	2.50×10^{-2}	1.01×10^{-7}	4.00×10^{-7}	9.43×10^{-7}	2.5×10^{-9}	1.0×10^{-8}	2.4×10^{-8}
	Mixed	Lung	8.14×10^{-2}	1.01×10^{-7}	4.00×10^{-7}	9.43×10^{-7}	8.2×10^{-9}	3.3×10^{-8}	7.7×10^{-8}

[a]Tissue specific dose units = mg dichloromethane metabolized via GST pathway/L tissue (liver or lung, respectively, for liver and lung tumors) per day; whole-body dose units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.
[b]GST-T1$^{+/+}$ = homozygous, full enzyme activity; mixed = population reflecting estimated frequency of genotypes in current U.S. population: 20% GST-T$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$ (Haber et al., 2002).
[c]Dichloromethane tumor risk factor (extra risk per unit internal dose) derived by dividing the BMR (0.1) by the allometric-scaled human $BMDL_{10}$ or by the mouse $BMDL_{10}$ (from Table 5-18) for the allometric-scaled and scaling = 1.0 risk factors, respectively.
[d]Mean, 95th, and 99th percentile of the human PBPK model-derived probability distribution of daily average internal dichloromethane dose resulting from chronic exposure to 1 µg/m^3 (0.00029 ppm).
[e]Derived by multiplying the dichloromethane tumor risk factor by the PBPK model-derived probabilistic internal doses from daily exposure to 1 µg/m^3.

For the female mouse, the combined human equivalent inhalation unit risk values for both tumor types is 1×10^{-8} $(\mu g/m^3)^{-1}$ in the most sensitive (GST-$T1^{+/+}$) population (Table H-4), which is the same value that was obtained using the male mouse data.

Table H-4. Upper bound estimates of combined human inhalation unit risks for liver and lung tumors resulting from lifetime exposure to 1 μg/m^3 dichloromethane based on liver-specific GST metabolism and whole body metabolism dose metrics, by population genotype, using female mouse data for derivation of risk factors

Internal dose metric and scaling factor[a]	Population genotype[b]	Tumor site	Upper bound inhalation unit risk[c]	Central tendency inhalation unit risk[d]	Variance of tissue-specific tumor risk[e]	Combined tumor risk SD[f]	Upper bound on combined tumor risk[g] $(\mu g/m^3)^{-1}$
Tissue-specific, allometric-scaled	GST-T1$^{+/+}$	Liver	7.0×10^{-9}	3.8×10^{-9}	3.87×10^{-18}		
		Lung	6.7×10^{-9}	5.3×10^{-9}	7.12×10^{-19}		
		Liver or lung		9.1×10^{-9}		2.1×10^{-9}	1.3×10^{-8}
	Mixed	Liver	3.9×10^{-9}	2.1×10^{-9}	1.22×10^{-18}		
		Lung	3.8×10^{-9}	3.0×10^{-9}	2.28×10^{-19}		
		Liver or lung		5.2×10^{-9}		1.2×10^{-9}	7.1×10^{-9}
Tissue-specific, scaling = 1.0	GST-T1$^{+/+}$	Liver	1.0×10^{-9}	5.4×10^{-10}	7.89×10^{-20}		
		Lung	9.6×10^{-10}	7.6×10^{-10}	1.42×10^{-21}		
		Liver or lung		1.3×10^{-9}		3.1×10^{-10}	1.8×10^{-9}
	Mixed	Liver	5.6×10^{-10}	3.0×10^{-10}	2.48×10^{-20}		
		Lung	5.4×10^{-10}	4.3×10^{-10}	4.54×10^{-21}		
		Liver or lung		7.3×10^{-10}		1.7×10^{-10}	1.0×10^{-9}
Whole-body, allometric-scaled	GST-T1$^{+/+}$	Liver	4.5×10^{-9}	2.4×10^{-9}	1.59×10^{-18}		
		Lung	1.5×10^{-8}	1.2×10^{-8}	4.11×10^{-18}		
		Liver or lung		1.4×10^{-8}		2.4×10^{-9}	1.8×10^{-8}
	Mixed	Liver	2.5×10^{-9}	1.4×10^{-9}	5.00×10^{-19}		
		Lung	8.2×10^{-9}	6.7×10^{-9}	1.29×10^{-18}		
		Liver or lung		7.9×10^{-9}		1.3×10^{-9}	1.0×10^{-8}

[a]Tissue specific dose units = mg dichloromethane metabolized via GST pathway/L tissue (liver or lung, respectively, for liver and lung tumors) per day; whole-body dose units = mg dichloromethane metabolized via GST pathway in lung and liver/kg-day.
[b]GST-T1$^{+/+}$ = homozygous, full enzyme activity); mixed = population reflecting estimated frequency of genotypes in current U.S. population: 20% GST-T$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$ (Haber et al., 2002).
[c]Estimated at the human equivalent $BMDL_{10}$ (0.1/BMDL10) (see Table H-2).
[d]Estimated at the human equivalent BMD_{10} (0.1/BMD) (see Table H-2).
[e]Calculated as the square of the difference of the upper bound and central tendency inhalation unit risks divided by the *t* statistic, 1.645.
[f]Calculated as the square root of the sum of the variances for liver and lung tumors.
[g]Calculated as the product of the cumulative tumor risk SD and the *t* statistic, 1.645, added to the sum of central tendency inhalation unit risks.

APPENDIX I. COMPARATIVE CANCER INHALATION UNIT RISK BASED ON BENIGN MAMMARY GLAND TUMORS IN RATS

Data for mammary gland tumors in male and female F344 rats following exposure to airborne dichloromethane were used to develop a comparative inhalation unit risk for dichloromethane (Mennear et al., 1988; NTP, 1986) (Table I-1). Significantly increased incidences of mammary gland or subcutaneous tissue adenoma, fibroadenomas, or fibromas were observed in male rats at 4,000 ppm, while mammary gland adenomas or fibroadenomas were increased in female rats exposed 6 hours/day, 5 days/week for 2 years at concentrations ≥1,000 ppm. Significant decreases in survival were observed in the treated groups of both sexes. The at-risk study populations (represented by the denominators in the incidence data) were determined by excluding all animals dying prior to 52 weeks.

Table I-1. Incidence data for mammary gland tumors and internal doses based on different dose metrics in male and female F344 rats exposed to dichloromethane via inhalation for 2 years

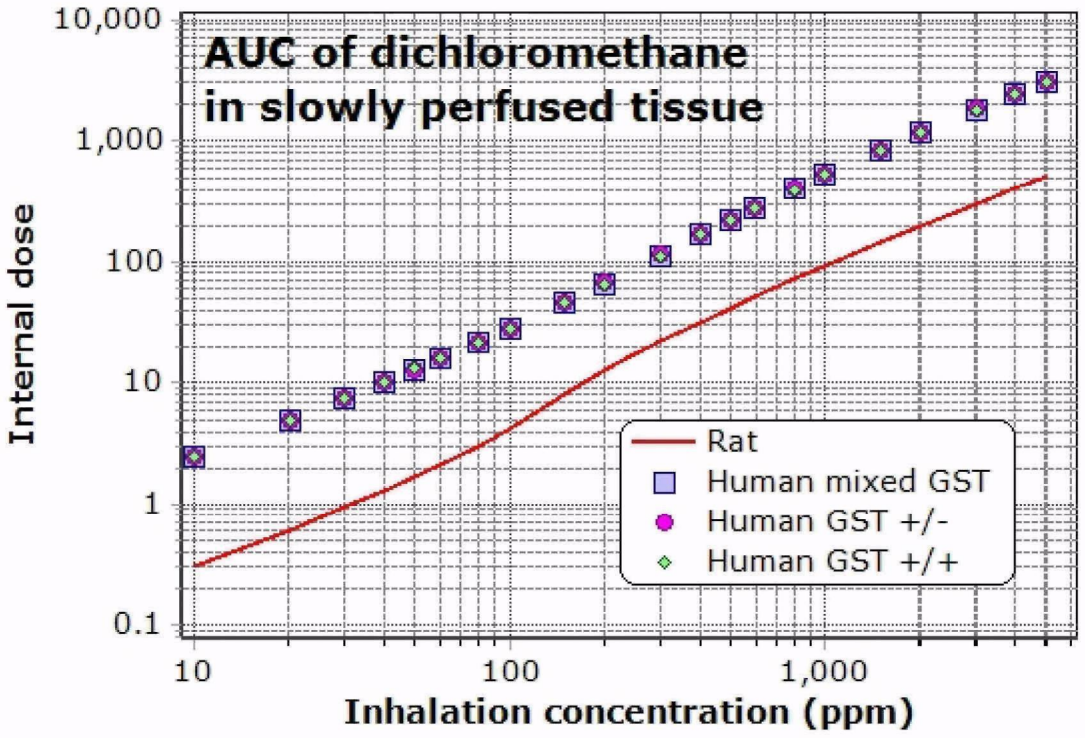
Sex	BW (g)	External dichloromethane concentration (ppm)	Rat tumor incidence[a]	Rat internal dose, AUC in slowly perfused tissue[b]
Male	–	0	1/50 (2%)[c]	0
	390.5	1,000	1/50 (2%)	93.3
	385.2	2,000	4/50 (8%)	196.3
	384.8	4,000	9/50 (18%)	403.2
Female	–	0	6/49 (12%)[c]	0
	245.5	1,000	13/50 (26%)	93.2
	244.3	2,000	14/50 (28%)	196.1
	242.2	4,000	23/50 (46%)	402.7

[a]Male tumors include mammary gland or subcutaneous tissue adenoma, fibroadenomas, or fibroma. Female tumors include mammary gland adenoma or fibroadenomas. Rats dying prior to 52 weeks were excluded from the denominators.
[b]Average daily AUC for dichloromethane in slowly perfused tissue (mg × hour/L) (see text for rationale for using this dose metric).
[c]Statistically significant increasing trend ($p \leq 0.01$).

Sources: Mennear et al. (1988); NTP (1986).

The rat PBPK model of Andersen et al. (1991) (modified as described in Appendix C) was used to simulate inhalation exposures of 6 hours/day, 5 days/week and calculate long-term daily average internal doses for the 2-year bioassays (Mennear et al., 1988; NTP, 1986). Study-, group-, and sex-specific mean BWs for rats were used. The modified PBPK model did not include a compartment for the mammary gland nor did it account for metabolism of dichloromethane occurring in the mammary gland. The selected internal dose metric for

mammary gland tumors was the average daily AUC for dichloromethane in slowly perfused tissue; the mammary gland and the regions around it consist primarily of fatty tissue, which is slowly perfused tissue. The role of CYP- or GST-mediated metabolism in the mammary gland is uncertain. GST-T1 (Lehmann and Wagner, 2008) and CYP2E1 (El-Rayes et al., 2003; Hellmold et al., 1998) expression has been detected in human mammary tissue, and it is also possible that some metabolites enter systemic circulation from the liver and lung where they are formed. Figure I-1 shows the comparison between inhalation external and internal doses in the liver and lung, respectively, using this dose metric for the rat and the human.



Average daily doses were calculated from simulated rat exposures of 6 hours/day, 5 days/week, while simulated human exposures were continuous. The GST metabolism rate in the human population for the mixed GST-T1 group (+/+, +/-, and -/-) in the current U.S. population was estimated as the mean of a simulated sample of 3,000 individuals at each exposure concentration, based on GST-T1 polymorphism data from Haber et al. (2002). The results for the GST-T1 +/- and +/+ subpopulations were then calculated as the means of the subsets of the mixed population sample with the respective genotypes.

Figure I-1. PBPK model-derived internal doses (daily average AUC for dichloromethane in slowly perfused tissue) in rats and humans and their associated external exposures (ppm) used for the derivation of cancer inhalation unit risks based on mammary tumors in rats.

The Multistage model was fit to the rat mammary gland tumor incidence and PBPK model-derived internal dose data to derive rat internal BMD_{10} and $BMDL_{10}$ values associated with 10% extra risk (Table I-2).

Table I-2. BMD modeling results associated with 10% extra risk for mammary gland tumors in F344 rats exposed by inhalation to dichloromethane for 2 years based on AUC for dichloromethane in slowly perfused tissue

Sex	Tumor type	BMDS model[a]	χ^2 goodness of fit *p*-value	Rat BMD_{10}[c]	Rat $BMDL_{10}$[c]	Tumor risk factor[d]
Male	Mammary gland or subcutaneous tissue adenoma, fibroadenoma, or fibroma	Multistage (1)	0.53	275.1	172.2	5.81×10^{-4}
Female	Mammary gland adenoma or fibroadenoma	Multistage (1)	0.72	90.9	61.5	1.63×10^{-3}

[a]The Multistage model in EPA BMDS version 2.0 was fit to each of the two sets of rat dose-response data shown in Table I-1 using internal dose metrics calculated with the rat PBPK model. Numbers in parentheses indicate: (1) the degree polynomial of the model.
[c]BMD_{10} and $BMDL_{10}$ refer to the BMD-model-predicted rat internal dose (average daily AUC for dichloromethane in slowly perfused tissue [mg × hour/L]) and its 95% lower confidence limit associated with a 10% extra risk for the incidence of tumors.
[d]Dichloromethane tumor risk factor (extra risk per average daily AUC for dichloromethane in slowly perfused tissue [mg × hour/L]) was derived by dividing the BMR (0.1) by the rat $BMDL_{10}$. The rat $BMDL_{10}$ is assumed to be equivalent to human $BMDL_{10}$; humans exposed to the same average daily AUC for dichloromethane in slowly perfused tissue as rats will have the same risks for mammary tumors.

Rat mammary gland tumor risk factors (extra risk per unit internal dose) were calculated by dividing 0.1 by the rat internal $BMDL_{10}$. Because this risk factor is based on the internal concentration of dichloromethane rather than a rate of reaction, it is assumed that the human risk factor is equal to that of the rat (i.e., that humans exposed for a 70-year lifetime to the same weekly average AUC of dichloromethane will have the same risk as rats exposed for 2 years). The human PBPK model (David et al., 2006) was used to calculate a distribution of human average daily AUCs for dichloromethane in slowly perfused tissue resulting from chronic inhalation exposure to a unit concentration of 1 μg/m^3 dichloromethane (0.00029 ppm). The distribution of inhalation unit risks for mammary gland tumors was generated by multiplying the human tumor risk factor for each sex by the distribution of internal doses from chronic human exposure to 1 μg/m^3 dichloromethane. Because this analysis is not based on the assumption that either metabolic pathway is or is not influencing the cancer risk, this distribution was derived by using weights reflecting the estimated frequency of GST-T1 genotypes in the current U.S. population (20% GST-T1$^{-/-}$, 48% GST-T1$^{+/-}$, and 32% GST-T1$^{+/+}$). As shown in Table I-3, the mean human inhalation unit risk based on mammary gland tumors in rats is 4×10^{-8} and 1×10^{-7}

$(\mu g/m^3)^{-1}$ based on male and female rat-derived risk factors, respectively. Identical values were obtained using slowly perfused tissue as the internal dose metric.

Table I-3. Inhalation unit risks for dichloromethane based on benign mammary tumors and PBPK model-derived internal doses in F344N rats exposed via inhalation for 2 years based on AUC for dichloromethane in slowly perfused tissue dose metric

Sex, tumor type	Human tumor risk factor[a]	Distribution of human internal dichloromethane doses from 1 µg/m³ exposure[b]			Resulting candidate human inhalation unit risk[c]		
		Mean	95th percentile	99th percentile	Mean	95th percentile	99th percentile
Male	5.81×10^{-4}	6.83×10^{-5}	1.08×10^{-4}	1.33×10^{-4}	3.97×10^{-8}	6.25×10^{-8}	7.76×10^{-8}
Female	1.63×10^{-3}	6.83×10^{-5}	1.08×10^{-4}	1.33×10^{-4}	1.11×10^{-7}	1.75×10^{-7}	2.18×10^{-7}

[a]Dichloromethane tumor risk factor (extra risk per average daily AUC for dichloromethane in slowly perfused tissue [mg × hour/L]) was derived by dividing the BMR (0.1) by the rat $BMDL_{10}$ (from Table I-2). The rat $BMDL_{10}$ is assumed to be equivalent to human $BMDL_{10}$; humans exposed to the same average daily AUC for dichloromethane in slowly perfused tissue as rats will have the same risks for mammary tumors.
[b]Mean, 95th, and 99th percentile of the human PBPK model-derived probability distribution of daily average internal dichloromethane dose (average daily AUC for dichloromethane in slowly perfused tissue [mg × hour/L]) resulting from chronic inhalation exposure to a unit concentration of 1 µg/m³ (0.00029 ppm), based on a distribution of GST-T1 genotypes that reflects the frequency distribution in the current U.S. population (Haber et al., 2002).
[c]Mean, 95th, and 99th percentile of a distribution of human inhalation unit risks (extra risk per µg/m³) derived by multiplying the dichloromethane tumor risk factor by the PBPK model-derived probabilistic distribution of human internal dichloromethane doses from unit dichloromethane inhalation exposure.

APPENDIX J. SOURCE CODE AND COMMAND FILES FOR DICHLOROMETHANE PBPK MODELS

The following is a copy of the primary acslXtreme code (.csl file for implementation under acslXtreme v.3.0.1.6) used for the dichloromethane simulations. Portions of the code that had been commented out (i.e., unused code) were deleted for brevity. Note that most parameters are set in the subsequent script/m-files, used to specify simulations and call the .csl code.

While the code indicates modifications by G, Diamond and M, Lumpkin (current and/or former employees of the EPA contractor, SRC, Inc.), these are changes from the version of the code as received by them to bring it into alignment with the version as described in the publications of Marino et al. (2006) and David et al. (2006) (changes noted by comments). Other changes allow for the calculation of various metrics to improve time-efficiency for computational convergence; for example, calculating an average AUC over only the last week of simulated exposure requires a much shorter overall simulated time for the calculation to give the steady-state or very-long-term average, when the approach to steady-state or repeating periodic solution occurs over the first days or weeks of exposure. Thus, the code exactly replicates the published models when the published parameter values are used.

```
PROGRAM: DCM_2010_EPA.csl
! Code from Reitz et al. 1997, Addressing Data Needs for Methylene Chloride with
        ! Physiologically Based Pharmcokinetic Modeling (Appendix J)
! Translated by GDiamond (05/2004); DCM.CSL.Reitz.
! Revision date 18-Dec-96 by RHR (Peak moved to dynamic)
! Modified by GDiamond (08/2004) based on Andersen et al. 1987 (TAP 87:185-205)
        ! and 1991 (TAP 108:14-27):
        ! Deleted brain compartment
        ! Added lung compartment, with lung metabolism
        ! Adjusted metabolism parameter values to match Andersen et al. 1987
        ! Adjusted physiological parameters to match Andersen et al. 1987
! Modifed by M Lumpkin (11/2005) (MHL) to include extrahepatic MFO metabolism and
        ! CO kinetics in blood
! Additional comments and changes by Paul Schlosser (PS), U.S. EPA (7/2008 - 2/2010)
! Removed unused (legacy) code bits, added comments, PS 9/2009

INITIAL
! Simulation, T=hour
NSTP=1000               ! Initital integration cycle length at CINT/1000
! MERROR ALU=0.00001     ! Error tolerance for Gear
CONSTANT POINTS=96.0    ! Number of points in plot
```

```
CINTERVAL CINT=0.1          ! Communication interval
CONSTANT TEND=25.0          ! Termination time (hr)
TSTOP=TEND
! Initial values for 250 g rat

! Body masses and fractional volumes
CONSTANT BW=0.25            ! Body weight (kg) MHL
CONSTANT VBL2C=0.059        ! Fractional volume of blood MHL
CONSTANT VFC=0.07           ! Fractional volume fat
CONSTANT VLC=0.04           ! Fractional volume of liver
CONSTANT VRC=0.05           ! Fractional volume of rapidly-perfused tissues
CONSTANT VSC=0.75           ! Fractional volume of slowly-perfused tissues **
CONSTANT VLUC=0.0115        ! Allometric scaling factor for lung volume **

! Tissue masses (kg)
VTOT=VFC+VLC+(VLUC/(BW**0.01))+VRC+VSC
        ! Total volume fractions constrained to sum to 0.9215 (7.85% carcass) MHL
VBL2=VBL2C*BW     ! Blood
VF=VFC*BW*0.9215/VTOT   ! Fat
VL=VLC*BW*0.9215/VTOT   ! Liver
VR=VRC*BW*0.9215/VTOT   ! Rapidly-perfused tissue
VS=VSC*BW*0.9215/VTOT   ! Slowly-perfused tissue
VLU=VLUC*(BW**0.99)*0.9215/VTOT         ! Lung

! Flow constants and fractions
CONSTANT VPR=0.42           ! Ventilation/perfusion ratio (for QP calc) MHL
CONSTANT DSPC=0.15          ! Fractional lung dead space MHL
CONSTANT QCC=15.0           ! Allometric constant for cardiac output
CONSTANT QCCR=10.0          ! For 'resting' period PMS 8/4/09
CONSTANT QCSW=0             ! Set to 1 to use QCCR (resting cardiac rate) up to TCHNG
CONSTANT QLC=0.20           ! Fractional flow to liver
CONSTANT QFC=0.09           ! Fractional flow to fat
CONSTANT QSC=0.15           ! Fractional flow to slowly-perfused tissue
CONSTANT QRC=0.56           ! Fractional flow to rapidly-perfused tissue ** MHL

! Flow rates (L/hr)

IF (QCSW) THEN
        QC=QCCR*BW**0.74 ! Cardiac output if resting, PMS 8/4/09
ELSE
        QC=QCC*BW**0.74   ! Cardiac output if not resting, PMS 8/4/09
```

```
ENDIF
QP=QC*VPR                    ! Alveolar ventilation rate MHL
QPDP=QP/DL/PAIR              ! MHL
QTOT=QFC+QLC+QRC+QSC ! MHL
QL=QLC*QC/QTOT               ! Liver MHL
QF=QFC*QC/QTOT               ! Fat MHL
QR=QRC*QC/QTOT               ! Rapidly-perfused tissue MHL
QS=QSC*QC/QTOT               ! Slowly-perfused MHL

! Partition coefficients
CONSTANT PL=0.732            ! Liver:blood
CONSTANT PF=6.19             ! Fat:blood
CONSTANT PS=0.408            ! Muscle:blood
CONSTANT PR=0.732            ! Liver:blood
CONSTANT PLU=0.732           ! Lung:blood **
CONSTANT PB=19.4             ! Blood:air

! Metabolism
CONSTANT VMAXC=4.0           ! Allometric scaling constant for VMAX
CONSTANT KM=0.4              ! Michaelis constant for MFO pathway (mg/L)
CONSTANT FRACR=0.1           ! Oxidative metab in rapidly perfused MHL
CONSTANT KFC=2.56            ! Allometric scaling constant for KF
CONSTANT MOLWT=85.0          ! Molecular weight of DCM
CONSTANT MWCO=28.0           ! Molecular weight of CO MHL
CONSTANT A1=0.416            ! Ratio of specific activities of MFO, lung/liver MHL
CONSTANT A2=0.137            ! Ratio of specific activities of GST, lung/liver MHL

VMAX=VMAXC*BW**0.7           ! Maximum rate of MFO pathway (mg/hr)
VMAXR=VMAX*FRACR             ! MHL
KF=KFC/(BW**0.3)             !First-order rate constant for GSH pathway
CONSTANT AFFG=0.0            ! 1.57e-4          ! Affinity constant (1/Km) for GST pathway
        ! Used to test impact of low affinity / slight saturation PMS 12/09
! CO Submodel params MHL
CONSTANT SOLCO=0.03                ! mg/L/mmHG, MHL
CONSTANT DLC=0.060           ! MHL
CONSTANT RENCOC=0.035  ! Rate of endogenous CO production MHL
CONSTANT ABCOC=0.117     ! Conc of background CO (mg/kg) MHL
CONSTANT HBTOT=10.0          ! Conc of hemoglobin (mmoles/L) MHL
CONSTANT P1=0.80             ! CO yield factor MHL
L12=P1*MWCO/MOLWT                  ! PMS
CONSTANT F1=1.21             ! CO elimination factor MHL
```

```
CONSTANT MMM=197                ! Haldane coefficient, PMS 1/11
CONSTANT COINH=0.0      ! Controlled CO inhalation concentration (ppm)  MHL
PICO=COINH/1402.5  ! Controlled CO concentration in inhaled air (mg/L) MHL
CONSTANT COBGD=2.2      ! Background CO conc. in air (ppm), PMS 1/11
CONSTANT O2=0.13        !4/16/32 mmoles/L MHL
BO2=O2/MMM              ! MHL
CONSTANT PAIR=713.0     ! Pressure of air (mm Hg) MHL
CONSTANT RHO=1102.0     ! Density of CO (mg/L) MHL
CONSTANT SOL=0.03       ! mg/L/mmHG, MHL
DL=DLC*(BW**0.92)       ! MHL

CONSTANT PCTHBCO0=1.0  ! Endogenous/background HBCO, PMS 2/10
CONSTANT RENCOS=1.0     ! Switch to selectively turn off bgd CO, PMS 2/10

if (RENCOC .EQ. 0.0) THEN
! Calculations of initial amounts of CACO/RENCO, PMS 2/10
        PCOBG=COBGD*RENCOS/1402.5     ! Background CO conc. in air (mg/L), PMS 1/11
        F0 = RENCOS*PCTHBCO0/100.0
        HBCO0=F0*HBTOT    ! PMS, 2/10
        CACO0 = HBCO0 + BO2*F0/(1.0-F0)
        ABL20=CACO0*VBL2C*BW*MWCO ! PMS, 2/10
        COFREE0=(O2/MMM)*F0/(1.0-F0)     ! Conc of free CO in blood (mmol/L) MHL
        PCCO0=COFREE0*MWCO/SOLCO
        RENCO=DL*RHO*F1*QPDP*(PCCO0 - PCOBG)/(1.0+QPDP)
ELSE
        PCOBG=COBGD*RENCOS/1402.5     ! Background CO conc. in air (mg/L), PMS 1/11
        RENCO=RENCOC*(BW**0.7)! MHL
        ABL20=ABCOC*BW
        ADTCO0=0.0
END IF

        PEAKPCTHBCO = RENCOS*PCTHBCO0 !Capture peak CO concentration

! Exposure schedule
CONSTANT CONC=0.0       ! Inhaled concentration (ppm)
CONSTANT TCHNG=6.0      ! Exposure pulse 1 width (hr)
CONSTANT TDUR=24.0      ! Exposure duration (hr)
CONSTANT TCHNG2=120.0  ! Exposure pulse 2 width (hr)
CONSTANT TDUR2=168.0    ! Exposure duration 2 (hr)
CIZONE = 1.0            ! Start with inhalation on
PEAK = 0.0              ! Zero peak concentration in brain
```

```
CONSTANT IVDOSE=0.0       ! IV infusion (mg/kg) MHL
CONSTANT TIV=0.0028       ! IV dosing time (hrs, or 10 seconds) MHL
CONSTANT VCHC=10.0        ! Uncorrected rat chamber volume (L) MHL
CONSTANT CC=0             ! Flag for closed chamber MHL
CONSTANT NCH=5            ! Number of animals in chamber MHL
CONSTANT KL=0.0           ! MHL
VCH=VCHC-(NCH*BW)

! Oral Gavage Dosing added by MHL
CONSTANT BOLUS=0.0
TOTALBOLUS=BOLUS*BW
TOTALDOSE=(IVDOSE+BOLUS)*BW
IVORBOL=(TOTALDOSE.GT.0.0)
CONSTANT DRCONC=0.0     ! User-specified concentration in drinking water (mg/L)
CONSTANT FIXDRDOSE=0.0! Set to constant DW dose in mg/kg-day
! Assume 70 kg human drinks 2 L/day, calculate rodent allometrically
constant RSTDY=0.0    ! Same except for zero-order infusion
ZLSTDY=RSTDY*BW/24.0
DRVOL=0.102*BW**0.7       ! Daily water intake, based on body weight
DRDOSE=DRVOL*DRCONC + FIXDRDOSE*BW     ! Total dose from water (mg (in a day))
DDOSE=DRDOSE/BW           ! Daily dose (mg/kg/day)
NEWDAY=0.0 ! To reset area-under-curve values each 24 hours
PEAKPCTHBCO=0.0
CONSTANT KA=5.0           ! Rate constant for absorption
CONSTANT KA2=1.67
CONSTANT K12=35
! Periodic drinking water intake schedule
! Assume T=0 is 7 AM (T+4=11 AM)
INTEGER I
I=2
DIMENSION DRTIME(32)              ! Store drinking times in array
DIMENSION DRPCT(32)               ! Store drinking percentages array
CONSTANT DRTIME=32*0.0, DRPCT=32*0.0
        GASD=MOLWT/24450.0

IF (DRDOSE.GT.0.0) SCHEDULE drink.AT.DRTIME(2)        ! Assume DRTIME(1)=0 ???
        ! and initial drink applied here.
CUMORALDOSE=DRPCT(1)*DRDOSE       !To calculate input to GI (mg)

! Switching 'lastday' and 'lastwk' to be defined by discrete blocks, below, PS 3/2009
lastday=0.0
```

```
IF (TSTOP.GT.24) SCHEDULE lday .AT. TSTOP-24
lastwk=0.0
if (TSTOP.GT.168) SCHEDULE lwk .AT. TSTOP-168

CIDAY=1.0
CIWK=1.0
SCHEDULE idayoff .AT.TCHNG
SCHEDULE idayon .AT.TDUR
SCHEDULE iwkoff.AT.TCHNG2
SCHEDULE iwkon .AT.TDUR2
!zzstopflag = .FALSE.
END    ! End of INITIAL section of program

DYNAMIC
        DISCRETE idayoff
                CIDAY=0.0
                QC=QCC*BW**0.74
                SCHEDULE idayoff .AT. (T+TDUR)
        END
        DISCRETE idayon
                CIDAY=1.0
                IF (QCSW) THEN
                        QC=QCCR*BW**0.74
                ENDIF
                SCHEDULE idayon .AT. (T+TDUR)
        END
        DISCRETE iwkoff
                CIWK=0.0
                SCHEDULE iwkoff .AT. (T+TDUR2)
        END
        DISCRETE iwkon
                CIWK=1.0
                SCHEDULE iwkon .AT. (T+TDUR2)
        END
        CIZONE = CIDAY*CIWK

        DISCRETE lday          ! Value -> 1 for last 24 h, PS 3/2009
                lastday=1.0
        end    ! of lday
        DISCRETE lwk           ! Value -> 1 for last 168 h, PS 3/2009
                lastwk=1.0
```

```
    end     ! of lwk

    DISCRETE drink      ! Loop for drinking water intake schedule
        CUMORALDOSE = CUMORALDOSE + DRPCT(I)*DRDOSE
        I=I+1
        IF (I .EQ. 32) THEN
            I=1
            NEWDAY=NEWDAY + 24.0
        ENDIF
        SCHEDULE drink.AT.(NEWDAY+DRTIME(I))
    End     ! Drink

DERIVATIVE
    ALGORITHM IALG=2 ! Gear for stiff systems

    ! Following are daily averages over final week of simulations, PS 8/2008
    WAVGLIVCYPDOSE=integ(lastwk*RAM1L,0.0)/(7.0*VL)
    WAVGLIVGSTDOSE=integ(lastwk*RAM2L,0.0)/(7.0*VL)
    WAVGLUNGGSTDOSE=integ(lastwk*RAM2LU,0.0)/(7.0*VLU)
    WAVGLUNGCYPDOSE=integ(lastwk*RAM1LU,0.0)/(7.0*VLU)
    WAVGWBDYGSTDOSE=integ(lastwk*(RAM2L+RAM2LU),0.0)/(7.0*BW)
    WAVGWBDYCYPDOSE=integ(lastwk*(RAM1L+RAM1LU),0.0)/(7.0*BW)
    WAVGAUCV=integ(lastwk*CV,0.0)/7.0
    WAVGAUCS=integ(lastwk*CS,0.0)/7.0
    WAVGAUCL=integ(lastwk*CL,0.0)/7.0

    ! Following are daily averages calculated from final day's dose-rate, PS 8/2008
    LDAYLIVCYPDOSE=integ(lastday*RAM1L,0.0)/VL
    LDAYLIVGSTDOSE=integ(lastday*RAM2L,0.0)/VL
    LDAYLUNGGSTDOSE=integ(lastday*RAM2LU,0.0)/VLU
    LDAYWBDYGSTDOSE=integ(lastday*(RAM2L+RAM2LU),0.0)/BW
    LDAYWBDYCYPDOSE=integ(lastday*(RAM1L+RAM1LU),0.0)/BW
    LDAYLIVAUC=integ(lastday*CL,0.0)
    LDAYAUCV=integ(lastday*CV,0.0)
    LDAYAUCS=integ(lastday*CS,0.0)
    LDAYPCTHB=integ(lastday*PCTHBCO,0.0)/24.0
    AVRFROMDCM=1000.0*integ(lastday*RFROMDCM,0.0)/(BW*24.0)

    ! GI compartment for drinking water inputs
    RSTOM=-(KA+K12)*STOM
    R12=K12*STOM
```

```
        RLGY=KA2*LGY
        STOM=INTEG(RSTOM,TOTALBOLUS)+CUMORALDOSE
        LGY=INTEG(R12-RLGY,0.0)
R= 0.21*pulse(0.0,1.0,0.015)*pulse(0.0,24.0,12.0)/(12*0.015) + &
        0.79*pulse(0.0,0.6,0.015)*pulse(12.0,24.0,12.0)/(20*0.015)

        RIV=IVDOSE*BW/TIV          ! IV dose rate (mg/hr) MHL
        blip=STEP(tiv)
        IV=RIV*(1.0-blip)

! Chamber calcs MHL
        RACH=CC*NCH*QP*((CA1/PB)-CCH)-(KL*ACH)    ! MHL
        ACH=INTEG(RACH,CONC*GASD*VCH)     ! MHL added initial condition
        CCH=CC*ACH/VCH   ! MHL
        CCHPPM=CCH/GASD ! MHL original

! If/else statements removed by use of multipliers; PMS 7-23-08
        CI=(1.0-CC)*CONC*CIZONE*GASD  ! Convert to mg/L
        DIDOSE=24.0*QP*INTEG(CI,0.0)/(BW*(T+1.0E-8))   ! Daily inhaled dose (mg/kg-d)

! CA1=Arterial blood concentration from gas exchange region
! to lung tissue compartment (mg DCM/L)
CA1=(QP*CI+QP*CCH+QC*CV)/((QP/PB)+QC)        !**

! AX=Amount eliminated by exhalation (mg DCM)
CX=CA1/PB    ! Concentration in air leaving gas exchange region
RAX=QP*CX
AX=INTEG(RAX, 0.0)

! Concentration DCM in exhaled air
CEX1=0.7*CX + 0.3*CI
        ! Assumes mixing with 30% of inhaled that only goes to deadspace
RAEX1=CEX1*QP      ! MHL
AEX1=INTEG(RAEX1,0.0)     ! MHL

PCTIVEXH=100.0*IVORBOL*AX/(TOTALDOSE + 1.0E-8)
        ! % IV/BOLUS dose exhaled as DCM, MHL

! Amount in lung tissue (mg DCM)
RAM1LU=A1*VMAX*CA*(VLU/VL)/(KM+CA)
AM1LU=INTEG(RAM1LU, 0.0)
```

```
RAM2LU=A2*KF*CA*VLU/(1.0+AFFG*CA)
RALU=QC*(CA1-CA)-RAM1LU-RAM2LU
ALU=INTEG(RALU, 0.0)
CLU=ALU/VLU
AUCLU=INTEG(CLU,0.0)
CA=CLU/PLU  ! Amount in arterial blood to body

! AF=Amount in fat (mg DCM)
RAF=QF*(CA-CVF)
AF=INTEG(RAF, 0.0)
CF=AF/VF
CVF=CF/PF

!AL=Amount in liver (mg DCM)
RAL=QL*(CA-CVL)-RAM1L-RAM2L+KA*STOM+KA2*LGY+ZLSTDY
RAM1L=VMAX*CVL/(KM+CVL)      !**
RAM2L=KF*CVL*VL/(1.0+AFFG*CVL)       !**
AL=INTEG(RAL, 0.0)
CL=AL/VL
CVL=CL/PL
AUCL=INTEG(CL, 0.0)          !**

!AS= Amount in slowly-perfused tissues
RAS=QS*(CA-CVS)    !-RAMS          ! VMAXS=0, so not needed, PS 7/2008
AS=INTEG(RAS, 0.0)
CS=AS/VS
CVS=CS/PS

!AR=Amount in rapidly-perfused tissues
RAMR=VMAXR*CVR/(KM+CVR)  ! MHL
AMR=INTEG(RAMR,0.0)       ! Total metabolized (MFO) in RP, MHL
RAR=QR*(CA-CVR)-RAMR
AR=INTEG(RAR, 0.0)
CR=AR/VR
CVR=CR/PR

! AM1=Amount metabolized in MFO pathway (mg DCM)
RAM1=RAM1LU+RAM1L+Ramr       !+ams   ! AMS = 0, term not needed, PS 7/2008
TAM1=INTEG(RAM1,0.0)
! DDAM2=Amount metabolized in GSH pathway (mg DCM)
RAM2=RAM2LU+RAM2L
```

```
TAM2=INTEG(RAM2,0.0)
! Total rate/amount metabolized (MFO plus GSH pathways)
RAMTOT=RAM1+RAM2        !**
AMTOT=INTEG(RAMTOT, 0.0)

! CV=Average venous blood concentration (mg DCM/L)
CV=(QF*CVF+QL*CVL+QS*CVS+QR*CVR+IV)/QC !MHL added RIV
CVP=0.23*CV ! Venous plasma conc
ABL=(CA+CV)*VBL2
AUCBL=INTEG(ABL, 0.0)
AUCV=INTEG(CV, 0.0)  ! GLD

! CO appearence in and elimination from blood, MHL
ACO=ABL2/(VBL2*MWCO) !MHL
BO2=O2/Mmm          ! MHL
CHBT=HBTOT          ! MHL
HBCO=((ACO+BO2+CHBT)-SQRT((ACO+BO2+CHBT)*(ACO+BO2+CHBT)-4.0*ACO*CHBT))/2.0
        ! Conc of HB-bound CO (mmol/L) MHL
COFREE=ACO-HBCO ! Conc of free CO in blood (mmol/L) MHL
PICO=COINH/1402.5   ! CO concentration in inhaled air (mg/L) MHL

PCTHBCO=100.0*HBCO/HBTOT        ! Percent carboxyhemoglobin in blood
PEAKPCTHBCO = MAX(LDAYPCTHB,PCTHBCO)   ! Capture peak CO concentration
WAVGHBCO=integ(lastwk*PCTHBCO,0.0)/168.0
! COE = Amount of expired CO, MHL
PCCO=COFREE*MWCO/SOL
PACO=(PCCO+(CIZONE*PICO+PCOBG)*QPDP)/(1.0+QPDP)
! Background term, PCOBG, added to above, PMS 1/11
RCOE=DL*RHO*(PCCO-PACO)*F1
COE=INTEG(RCOE,0.0)         ! Expired amount of CO (mg) MHL
COEC=0.7*COE/QP             ! Conc expired CO (mg/L)
        ! Multiplier changed from 2/3 to 0.7 in above to be consistent with...
        ! other expired concentration calculations, PS 7/2008.
COECPPM=COEC*24450.0/MWCO
PCTIVCOEXH=100.0*IVORBOL*COE/(TOTALDOSE*MWCO/MOLWT + 1.0E-8) ! % dose exhaled
as CO MHL

! ABL2 = Amount of CO in blood (mg) MHL
RFROMDCM=(RAM1L+RAM1LU+RAMR)*L12 ! RAMS left out since not used, =0
! Inputs calculated for mass balance
AENCO = INTEG(RENCO, 0.0)                   ! Amount produced endogenously (mg)
```

```
AFROMDCM = INTEG(RFROMDCM, 0.0)      ! Amount produced from metabolism of DCM
RABL2=RFROMDCM+RENCO-RCOE
ABL2=INTEG(RABL2,ABL20)          ! MHL
CABL2=ABL2/VBL2              ! MHL

! AI=Total mass input (mg DCM)
RAI=(QP*(CI+CCH))+RIV !MHL
AI=INTEG(RAI, 0.0)

! TMASS=Mass balance (mg DCM)
MASSBAL=MASSIN-STOM-AF-AL-ALU-AS-AR-ABL-MASSOUT
MASSIN=AI+CUMORALDOSE + TOTALBOLUS
MASSOUT=AX+AMTOT

! Mass Balance for CO
MASSBALCO = 100*(-ABL2+AENCO-COE+AFROMDCM)/(AENCO+AFROMDCM+1e-10)

! IF (T .GE. TSTOP) zzstopflag = .TRUE.          ! Termination condition for model run
END    ! End of DERIVATIVE section of program
TERMT(T.GE.TEND)
END    ! End of Dynamic section of program

TERMINAL
        AVGT= 24.0/(T+1.0e-8)
  METDOS = AVGT*RAMTOT/VL
        LDAYREFDOSE = LDAYLIVCYPDOSE + LDAYLIVGSTDOSE        ! MHL
        WAVGREFDOSE = WAVGLIVCYPDOSE + WAVGLIVGSTDOSE    ! PS, 8/2008
        LDAYWBDYCYPGSTDOSE = LDAYWBDYCYPDOSE + LDAYWBDYGSTDOSE
        METABRATIO = RAM1/(RAM2+1.0E-12)
        DDOSECHECK = CUMORALDOSE*avgt/BW ! Should equal DDOSE
END    ! End of TERMINAL section of program
END    ! End of PROGRAM
```

Computational Files (.m files)

The following are files (functions) created as .m files to support the Monte Carlo statistical sampling and subsequent calculations for the DCM analysis.

Utility files used for all human simulations

```
function v=normbnd(mu, sigma, lo, up)
% ------------------------------------------------------------
```

```
% FILE: normbnd.m
%
% NORMBND - Returns a random sample from a truncated normal distribution with
%         mean = mu, standard deviation = sigma, lower bound = lo, & upper bound = up.
% Modified by Paul Schlosser, U.S. EPA, 7/2008
% ---------------------------------------------------------------
        if (lo >= up)
                v = NaN; disp('Lower bound must be less than upper bound.')
                return;
        end
        FL = normcdf(lo, mu, sigma);    % normal probability of lower bound
        FU = normcdf(up, mu, sigma);    % normal probability of upper bound
        p = rand*(FU - FL) + FL;        % rand = uniform random(0,1)
        v = norminv(p, mu, sigma);
end
```

```
function v=lnormbnd(mu, sigma, lo, up)
% ---------------------------------------------------------------
% FILE: lnormbnd.m
%
% LNORMBND - Returns a truncated LOGnormal distribution with distribution with
%         mean = mu, standard deviation = sigma, lower bound = lo, & upper bound = up.
% Modified by Paul Schlosser, U.S. EPA, 7/2008
% ---------------------------------------------------------------
v = exp(normbnd(log(mu), log(sigma), log(lo), log(up)));
end
```

```
function v=prctile(y,x)
% ---------------------------------------------------------------
% FILE: prctile.m
% Computes percentiles of vector y at percentile values x
% If x = [50 90 95], that's 50th, 90th, 95th
% Created by Paul Schlosser, U.S. EPA, July, 2008
% ---------------------------------------------------------------
if any((x<0) | (x>100))
        disp('Error!  All percentiles must be between 0 and 100!');
        return
end
dy = sort(y);
ps = 100*((1:length(dy))-0.5)/length(dy);
v = x;
for id=1:length(x)
        if x(id)<ps(1)
                v(id)=dy(1);
        elseif x(id)>max(ps)
                v(id)=max(dy);
        else
                v(id)=interp1(ps,dy,x(id));
        end
end
```

```
% ---------------------------------------------------------------
% File clearT.m
```

```
% Creates list of variable names for tracking, rnames, and
% performs other initializations for human Monte Carlo chains.
% Created by Paul Schlosser, U.S. EPA, 7/2008
% ----------------------------------------------------------------
rnames=["age";"BW";"DRVOL";"DRCONC";"DDOSE";"CONC";"VMAXC";"KM";"FRACR";"KFC";
"A1";"A2";"BW";"VLC";"VFC";"VRC";"VLUC";"VSC";"QAlvC";"QCC";"QLC";"QFC";"QSC";
"QRC";"PL";"PLU";"PF";"PS";"PR";"PB";"GSTGT";"CV";"LDAYLIVGSTDOSE";
"LDAYLIVCYPDOSE";"LDAYLUNGGSTDOSE";"LDAYWBDYGSTDOSE";"LDAYREFDOSE";"L
DAYAUCV";
"LDAYAUCS";"PEAKPCTHBCO";"LDAYPCTHB";"AVRFROMDCM";"WAVGAUCV";"WAVGAU
CS"];
sr='['; rns=length(rnames);
for ir=1:(rns-1)
        sr=[sr,ctot(rnames(ir)),', '];
end
sr=[sr,ctot(rnames(rns)),'];'];
% save current model values before perturbations
save @file='dcm_human.sav'
runo=[];
% prepare time history values.
prepare @clear T CV
WESITG=0; WEDITG=0; CONC=0.0; IVDOSE=0.0; DRCONC=0.0; FIXDRDOSE=0.0;
IVORBOL=0.0;
COINH=0; RENCOS=1;
DRPCT = [0.25, 0.1, 0.25, 0.1, 0.25, 0.05,zeros(1,26)];
DRTIME= [0.0, 3.0, 5.0, 8.0, 11.0, 15.0, 16+[1:26]/10];
TEND=600; TCHNG=2000; TCHNG2=2000; TDUR=2000; TDUR2=2000;
start @NoCallback
exist popn;       % Test to see if 'popn' defined.
if ~ans  % If not...
        popn="mix"; % Mix of GST types.
end
exist agem;       % Check if agem defined ...
if ~ans  % If not...
        agem=0;
end
exist gendm;     % Check if gendm defined ...
if ~ans  % If not...
        gendm="both"; % males and females
end
```

```
function nn=findnames(nm,rnames)
% ----------------------------------------------------------------
% File findnames.m
%
% Function to find indices of specific names in list nm
% within the larger list (of all variables), rnames.
% Created by Paul Schlosser, U.S. EPA, 7/2008
% ----------------------------------------------------------------
        nn=[];
        for n=1:length(nm)
                n1=find(rnames==nm(n));
                if isempty(n1)
```

```
                    disp(['Error, ',ctot(nm(n)),' not in saved variable list.']); return
                end
                nn=[nn,n1];
        end
end

% File human_par1.m
%
% Code for selecting human model parameters from MC distribution for
% dichlormethane PBPK model from probability density functions as
% described by David et al (2006), with *most of* the revisions as
% described in the U.S. EPA IRIS Toxicological Review for Dichloromethane,
% Appendix B.
% ** This code uses "one-dimensional" sampling distributions for CYP
% (VMAXC) and GSTT-1 (KFC).  The sampling of KFC is as described by David
% et al (2006).  The sampling for VMAXC uses the mean value of David et
% al (2006) but substitutes a larger geometric standard deviation and
% uses log-normal distribution without bounds.  Further details below.
% Genotypic distribution of GSTT-1 activity taken from Tables 8 and 9
% of Environ report for Eastman Kodak.
% Arithmetic means and SDs for lognormal distributions converted
% by ML to geometric mean/SD to match acsl m-file lnormbnd.m values.
%
% Programmed by Michael Lumpkin (ML)
% Syracuse Research Corporation, 11/2005
% Modified by Paul Schlosser (PS), U.S. EPA 7/2008 and 1/2010
%
% Gender parameter 'gendm' can be "both" (default), "male", or "female".
% Age parameter 'agem' can be 0 (default) to simulate the full range
% from 0.5-80 years, or any value in that range for a specific age.
% Specific parameters, as noted below, set for those life-stages.
% ------------------------------------------------------------------

% choose uniform discrete distribution for GSTT-1 genotypes.
if popn=="++"
        GSTGT = 0; % for +/+ only
elseif popn=="+-"
        GSTGT = 1; % for +/- only
elseif popn=="--"
        GSTGT = 2; % for -/- only
else
        r=rand(1)*gsmult; GSTGT=(r>0.32)+(r>0.8); %For general/mixed population
        popn = "mix of +/+, +/-, and -/-";
end

% selection of GSTT1 activity distribution based on ethnic distribution
% upper bounds changed from mean + 3*SD to mean + 5*SD, P.S., 2/2009
if (GSTGT == 0)
        KFC = (5.87/0.852)*normbnd(1.31, 0.167, 0.0, 2.145);          %
elseif (GSTGT == 1)
        KFC = (5.87/0.852)*normbnd(0.676, 0.123, 0.0, 1.291);          %
else
        KFC = 0.0;
end
```

```
AFFG=0.0; % 1.57e-4   % Affinity constant (1/Km) for GSTT-T1 pathway
        % Allows for some saturation of the GSTT-1 pathway if set > 0, P.S., 1/2010

KA=5.0; %From Reitz et al (1997); this parameter has no variability data and is
        % described as point estimate
VMAXC=lognorm(9.34, 1.73); %lnormbnd(9.34, 1.73, 3.8, 23.0);
        %geometric mean and GSD, converted from arith mean/SD of 9.42 and 1.23
        % VMAXC switched to unbounded distribution, PS, 2/2009
KM=lnormbnd(0.410, 1.39, 0.154, 1.10);
        %geometric mean and SD, converted from arith mean/SD of 0.433 and 1.46
FRACR=lnormbnd(0.0152, 2.0, 0.0019, 0.122);
        %geometric mean and SD, converted from arith mean/SD of 0.0193 and 0.0152
A1=lnormbnd(0.00092, 1.47, 0.000291, 0.00292);
        %geometric mean & SD, converted from arith mean/SD of 0.000993 & 0.000396
A2=lnormbnd(0.0083, 1.92, 0.00116, 0.0580);
        %geometric mean and SD, converted from arith mean/SD of 0.0102 and 0.00739

age=agem;
if age==0       % agem=0 => age = random from population, otherwise leave as set
        p=rand;
        age = 165.86*p^4 - 253.19*p^3 + 113.27*p^2 + 53.356*p + 0.5;
end
VMP = 0.7 + 0.18*(age<18);
        % power for scaling Vmax is 0.88 if age < 18, otherwise 0.7
        % Change in VMP introduced 2/2009, PS

gend=gendm;
if ~((gendm=="male")|(gendm=="female"))       % if not defined as one
        gend="female";
        if rand()<(0.513*((125.3 - age)^4)/(33.7^4 + (125.36 - age)^4));
                gend="male";
        end
end

if mod(RUNN,50)==0
        disp(['GSTGT group: ',ctot(popn),'.  GSTGT = ',num2str(GSTGT),'.']);
        disp(['Simulation for ',num2str(age),' year old ',ctot(gend),'; gender mix: ',ctot(gendm)]);
        disp(' ');
end

% Human physiologic parameters

if gend=="female"
        aged=(16-age)/10;
        bwmean = 4.146 - 0.147*aged - 1.36*aged^2 + 0.44*aged^3;
        if aged<0
                bwmean = 4.146 - 0.147*aged - 0.0278*aged^2 - 0.00095*aged^3;
        end
        aged=(13-age)/10;
        bwSD = 2.574 - 0.358*aged - 2.55*aged^2 + 1.16*aged^3;
        if aged<0
                bwSD = 2.574 - 0.358*aged - 0.0861*aged^2 - 0.00469*aged^3;
        end
else % males
```

```
        aged=(21-age)/10;
        bwmean = 4.406 - 0.0285*aged - 0.729*aged^2 + 0.115*aged^3;
        if aged<0
                bwmean = 4.406 - 0.0285*aged + 0.0048*aged^2 + 0.0018*aged^3;
        end
        aged=(16-age)/10;
        bwSD = 2.87 + 0.06*aged - 2.56*aged^2 + 0.96*aged^3;
        if aged<0
                bwSD = 2.87 + 0.06*aged + 0.0448*aged^2 + 0.0067*aged^3;
        end
end
BW=norminv((0.01+0.98*rand),exp(bwmean),exp(bwSD));

QAlvmean = 13.6 + 13.3*exp(-0.05*age);
if gend=="female"
        QAlvmean = 10.7 + 22.1*exp(-0.08*age);
end
Qgsd = -0.1948*(age/10)^3 + 0.6095*(age/10)^2 - 0.3978*(age/10) + 1.4261;
if age>16.81
        Qgsd=1.554;
end
QAlvC = QAlvmean*exp(norminv((0.05+0.9*rand),0,log(Qgsd)));

QCCmean = 56.906*(1.0 - exp(-0.681*exp(0.0454*QAlvC))) - 29.747;
QCC = QCCmean/lnormbnd(1.0, 0.203, 0.69, 1.42);
VPR = QAlvC/QCC;

vlm=-0.0036*(age/10)^2 - 0.0051*(age/10) + 0.0395;
if age>17
        vlm=-0.0004*(age/10)^2 + 0.0034*(age/10) + 0.0169;
end
VLC=vlm*normbnd(1.0, 0.05, 0.85, 1.15);

vfm=0.1612*(age/10)^3 + 0.0846*(age/10)^2 - 0.3083*(age/10) + 0.2709;
if ((age>7)&(age<=20))
        vfm=-0.0458*(age/10)^2 + 0.2082*(age/10) + 0.0274;
        if gend=="male"
                vfm= -0.0057*(age/10)^2 + 0.0293*(age/10) + 0.1303;
        end
elseif age>20
        vfm=-0.0024*(age/10)^3 + 0.0355*(age/10)^2 - 0.115*(age/10) + 0.3678;
        if gend=="male"
                vfm= -0.0015*(age/10)^2 + 0.0384*(age/10) + 0.0908;
        end
end
VFC=vfm*normbnd(1.0, 0.3, 0.1, 1.9);

VRC=normbnd(0.064, 0.0064, 0.0448, 0.0832);
VLUC=normbnd(0.0115, 0.00161, 0.00667, 0.0163);
VSC=normbnd(0.63, 0.189, 0.431, 0.829);
vtr=0.9215/(VFC+VLC+VLUC+VRC+VSC);
VFC = VFC*vtr; VLC=VLC*vtr; VLUC=VLUC*vtr; VRC=VRC*vtr; VSC=VSC*vtr;
VBL2C=0.059;
```

```
DSPC=0.15;
QLC=normbnd(0.26, 0.0910, 0.010, 0.533);
QFC=normbnd(0.05, 0.0150, 0.0050, 0.0950);
QSC=normbnd(0.19, 0.0285, 0.105 ,0.276);
QRC=normbnd(0.50 ,0.10, 0.20, 0.80);

%Human partition coefficients for DCM
PL=lnormbnd(1.43, 1.22, 0.790, 2.59);
        %geometric mean and SD, converted from arith mean/SD of 1.46 and 0.292
PLU=lnormbnd(1.43, 1.22, 0.790, 2.59);
        %geometric mean and SD, converted from arith mean/SD of 1.46 and 0.292
PF=lnormbnd(11.9, 1.34, 4.92, 28.7);
        %geometric mean and SD, converted from arith mean/SD of 12.4 and 3.72
PS=lnormbnd(0.80, 1.22, 0.444, 1.46);
        %geometric mean and SD, converted from arith mean/SD of 0.82 and 1.64
PR=lnormbnd(1.43, 1.22, 0.790, 2.59);
        %geometric mean and SD, converted from arith mean/SD of 1.46 and 0.292
PB=lnormbnd(9.7, 1.10, 7.16, 13.0);
        %geometric mean and SD, converted from arith mean/SD of 9.7 and 0.97
```

```
% File human_par2.m
%
% Code for selecting human model parameters from MC distribution for
% dichlormethane PBPK model from probability density functions as
% described by David et al (2006), with revisions as described in the
% U.S. EPA IRIS Toxicological Review for Dichloromethane, Appendix B.
%
% ** This code uses "two-dimensional" sampling distributions for CYP
% (VMAXC) and GSTT-1 (KFC), as described in the current assessment.
% Also see comments further details below.
%
% Genotypic distribution of GSTT-1 activity taken from Tables 8 and 9
% of Environ report for Eastman Kodak.
% Arithmetic means and SDs for lognormal distributions converted
% by ML to geometric mean/SD to match acsl m-file lnormbnd.m values.
%
% Programmed by Michael Lumpkin (ML)
% Syracuse Research Corporation, 11/2005
% Modified by Paul Schlosser (PS), U.S. EPA 7/2008 and 1/2010
%
% Gender parameter 'gendm' can be "both" (default), "male", or "female".
% Age parameter 'agem' can be 0 (default) to simulate the full range
% from 0.5-80 years, or any value in that range for a specific age.
% Specific parameters, as noted below, set for those life-stages.
% ----------------------------------------------------------------
%'par2'
% choose uniform discrete distribution for GSTT-1 genotypes.
if popn=="++"
        GSTGT = 0; % for +/+ only
elseif popn=="+-"
        GSTGT = 1; % for +/- only
elseif popn=="--"
        GSTGT = 2; % for -/- only
```

```
else
        r=rand(1)*gsmult; GSTGT=(r>0.32)+(r>0.8); %For general/mixed population
        popn = "mix of +/+, +/-, and -/-";
end
% selection of GSTT1 activity distribution based on ethnic distribution
% upper bounds changed from mean + 3*SD to mean + 5*SD, P.S., 2/2009
% From Table 4 of David et al., for kfc, mu = 0.852, CV = 0.711
% Lognorm tranform: m = mu/sqrt(CV^2 + 1) = 0.6944
        % and s = exp(sqrt(ln(CV^2 + 1))) = 1.896
        % kfcm=lnormbnd(m,s,m/(s^2),m*(s^2))/((0.48/2)+0.32);
        % The last term (divisor) accounts for relative weighting and activity
        % of the three genotypes.
        kfcm=lnormbnd(0.6944,1.896,0.1932,2.496);
if (GSTGT == 0)
        KFC = kfcm*normbnd(1.786, 0.2276, 0.0, 2.924); % Relative activity in +/+ pop'n
                %=kfcm*normbnd(1, 0.167/1.31, 0.0, 1.0+5*0.167/1.31)/0.56;
                % 0.167 and 1.31 are s.d and mean, respectively, for +/+ from Table 2 of David et al.
elseif (GSTGT == 1)
        KFC = kfcm*normbnd(0.8929, 0.1622, 0.0, 1.704); % Relative activity in +/- pop'n
                %=kfcm*normbnd(1, 0.123/0.676, 0.0, 1.0+5*0.123/0.676);
                % 0.123 and 0.676 are s.d and mean, respectively, for +/- from Table 2 of David et al.
else
        KFC = 0.0;
end
AFFG=0.0; % 1.57e-4   % Affinity constant (1/Km) for GSTT-T1 pathway
        % Allows for some saturation of the GSTT-1 pathway if set > 0, P.S., 1/2010
KA=4.31; 5.0; %from Reitz et al (1997); this parameter has no variability data and is described as point
estimate
K12=0; KA2=0;
vmaxcm=lnormbnd(9.34,1.14,7.20,12.11);
        %geometric mean and GSD, converted from arith mean/SD of 9.42 and 1.23
        % lower/upper bounds = GM/(GSD^2) and GM*(GSD^2); i.e., +/- 2 SD in log-space
        % bound calculations done with GM and GSD *not rounded* to 2 decimal places
VMAXC=lognorm(vmaxcm, 1.73); %lnormbnd(9.34, 1.73, 3.8, 23.0);
        % VMAXC switched to unbounded distribution, PS, 2/2009
KM=lnormbnd(0.410, 1.39, 0.154, 1.10);          %geometric mean and SD, converted from arith
mean/SD of 0.433 and 1.46
FRACR=lnormbnd(0.0152, 2.0, 0.0019, 0.122);     %geometric mean and SD, converted from arith
mean/SD of 0.0193 and 0.0152
A1=lnormbnd(0.00092, 1.47, 0.000291, 0.00292);          %geometric mean & SD, converted
from arith mean/SD of 0.000993 & 0.000396
A2=lnormbnd(0.0083, 1.92, 0.00116, 0.0580);     %geometric mean and SD, converted from arith
mean/SD of 0.0102 and 0.00739
%If un-commented, the block rescales the parameters to the means obtaine
% for the DiVincenzo and Kaplan (1981) data set; PS 4/2011
%       VMAXC = VMAXC*10.2/9.42;                %
%       KM = KM*2.06/0.433;                             %
%       KFC = KFC*5.87/0.852;                           %
%       A1 = A1*0.00111/0.000993;               %
%       A2 = A2*0.0177/0.0102;                          %
%       FRACR = FRACR*0.0379/0.0193;    %
% ----- END OF RESCALING BLOCK ----
age=agem;
if age==0       % agem=0 => age = random from population, otherwise leave
```

```
        p=rand;
        age = 165.86*p^4 - 253.19*p^3 + 113.27*p^2 + 53.356*p + 0.5;
end
VMP = 0.7 + 0.18*(age<18);     % power for scaling Vmax is 0.88 if age < 18, otherwise 0.7
% Change in VMP introduced 2/2009, PS
gend=gendm;
if ~((gendm=="male")|(gendm=="female"))        % if not defined as one
        gend="female";
        if rand()<(0.513*((125.3 - age)^4)/(33.7^4 + (125.36 - age)^4));
                gend="male";
        end
end
if mod(RUNN,50)==0
        disp(['GSTGT group: ',ctot(popn),'.  GSTGT = ',num2str(GSTGT),'.']);
        disp(['Simulation for ',num2str(age),' year old ',ctot(gend),'; gender mix: ',ctot(gendm)]);
        disp(' ');
end
        % Human physiologic parameters
if gend=="female"
        aged=(16-age)/10;
        bwmean = 4.146 - 0.147*aged - 1.36*aged^2 + 0.44*aged^3;
        if aged<0
                bwmean = 4.146 - 0.147*aged - 0.0278*aged^2 - 0.00095*aged^3;
        end
        aged=(13-age)/10;
        bwSD = 2.574 - 0.358*aged - 2.55*aged^2 + 1.16*aged^3;
        if aged<0
                bwSD = 2.574 - 0.358*aged - 0.0861*aged^2 - 0.00469*aged^3;
        end
else % males
        aged=(21-age)/10;
        bwmean = 4.406 - 0.0285*aged - 0.729*aged^2 + 0.115*aged^3;
        if aged<0
                bwmean = 4.406 - 0.0285*aged + 0.0048*aged^2 + 0.0018*aged^3;
        end
        aged=(16-age)/10;
        bwSD = 2.87 + 0.06*aged - 2.56*aged^2 + 0.96*aged^3;
        if aged<0
                bwSD = 2.87 + 0.06*aged + 0.0448*aged^2 + 0.0067*aged^3;
        end
end
BW=norminv((0.01+0.98*rand),exp(bwmean),exp(bwSD));
QAlvmean = 13.6 + 13.3*exp(-0.05*age);
if gend=="female"
        QAlvmean = 10.7 + 22.1*exp(-0.08*age);
end
Qgsd = -0.1948*(age/10)^3 + 0.6095*(age/10)^2 - 0.3978*(age/10) + 1.4261;
if age>16.81
        Qgsd=1.554;
end
QAlvC = QAlvmean*exp(norminv((0.05+0.9*rand),0,log(Qgsd)));
QCCmean = 56.906*(1.0 - exp(-0.681*exp(0.0454*QAlvC))) - 29.747;
QCC = QCCmean/lnormbnd(1.0, 0.203, 0.69, 1.42);
VPR = QAlvC/QCC;
```

```
vlm=-0.0036*(age/10)^2 - 0.0051*(age/10) + 0.0395;
if age>17
        vlm=-0.0004*(age/10)^2 + 0.0034*(age/10) + 0.0169;
end
VLC=vlm*normbnd(1.0, 0.05, 0.85, 1.15);
vfm=0.1612*(age/10)^3 + 0.0846*(age/10)^2 - 0.3083*(age/10) + 0.2709;
if ((age>7)&(age<=20))
        vfm=-0.0458*(age/10)^2 + 0.2082*(age/10) + 0.0274;
        if gend=="male"
                vfm= -0.0057*(age/10)^2 + 0.0293*(age/10) + 0.1303;
        end
elseif age>20
        vfm=-0.0024*(age/10)^3 + 0.0355*(age/10)^2 - 0.115*(age/10) + 0.3678;
        if gend=="male"
                vfm= -0.0015*(age/10)^2 + 0.0384*(age/10) + 0.0908;
        end
end
VFC=vfm*normbnd(1.0, 0.3, 0.1, 1.9);
VRC=normbnd(0.064, 0.0064, 0.0448, 0.0832);
VLUC=normbnd(0.0115, 0.00161, 0.00667, 0.0163);
VSC=normbnd(0.63, 0.189, 0.431, 0.829);
vtr=0.9215/(VFC+VLC+VLUC+VRC+VSC);
VFC = VFC*vtr; VLC=VLC*vtr; VLUC=VLUC*vtr; VRC=VRC*vtr; VSC=VSC*vtr;
DSPC=0.15;
QLC=normbnd(0.26, 0.0910, 0.010, 0.533);
QFC=normbnd(0.05, 0.0150, 0.0050, 0.0950);
QSC=normbnd(0.19, 0.0285, 0.105 ,0.276);
QRC=normbnd(0.50 ,0.10, 0.20, 0.80);

%Human partition coefficients for DCM
PL=lnormbnd(1.43, 1.22, 0.790, 2.59);   %geometric mean and SD, converted from arith mean/SD of
1.46 and 0.292
PLU=lnormbnd(1.43, 1.22, 0.790, 2.59);%geometric mean and SD, converted from arith mean/SD of
1.46 and 0.292
PF=lnormbnd(11.9, 1.34, 4.92, 28.7);    %geometric mean and SD, converted from arith mean/SD of
12.4 and 3.72
PS=lnormbnd(0.80, 1.22, 0.444, 1.46);   %geometric mean and SD, converted from arith mean/SD of
0.82 and 1.64
PR=lnormbnd(1.43, 1.22, 0.790, 2.59);   %geometric mean and SD, converted from arith mean/SD of
1.46 and 0.292
PB=lnormbnd(9.7, 1.10, 7.16, 13.0);     %geometric mean and SD, converted from arith mean/SD of 9.7
and 0.97
% CO sub-model
   RENCOS=0;
   VBL2C=normbnd(0.059, 0.0177, 0.0148, 0.103);
   DLC=normbnd(0.058, 0.02262, 0.01, 0.1);
   ABCOC=0; %normbnd(0.1, 0.75, 0.01, 1);
   RENCOC=0; %normbnd(0.05, 0.75, 0.01, 1);
   HBTOT=normbnd(10, 0.5, 9, 11);
   MMM=normbnd(178.3, 14.264, 152, 210);
   P1=normbnd(0.71, 0.1278, 0.5, 1);
   F1=normbnd(0.85, 0.1275, 0.5, 1);
   COBGD=0; %normbnd(2.2, 2.2, 0, 4.4);
```

```
% ------------------------------------------------------------------
% File: finish.m
% Programmed by Paul Schlosser, U.S. EPA, 8/2008, rev. 1/2010
% Performs final analysis on saved results in runo from simulations.
% ------------------------------------------------------------------
disp([num2str(size(runo,1)),' simulations completed.']);
contsim=0; p=ctot(popn); p=p(1:min([3,length(p)]));
eval(['save runo @file=',astp,num2str(agem),p,'_',ctot(gendm), ...
        '_',model(7:10),'.csv @format=ascii @separator=comma']);
eval(['save @file=',astp,num2str(agem),p,'_',ctot(gendm),'_',model(7:10),'.mat']);
disp(['GSTT-1 group: ',ctot(popn)]);
aget=['a ',num2str(agem),' year-old; '];
if agem==0
        aget='0.5-80 years of age; ';
end
gendt=[ctot(gendm),' population'];
if gendm=="both"
        gendt='males and females';
end
disp(['Simulation for ',aget,gendt,'.']);
disp(['Metric =',dtxt,'.']);
res=[];
pcs=num2str(percs(1));
for n=2:length(percs)
        pcs=[pcs,', ',num2str(percs(n))];
end
for n=1:length(nm)
        disp([ctot(nm(n)),' mean, median, percentiles = ',pcs]);
        r=[mean(runo(:,nn(n))),median(runo(:,nn(n))),prctile(runo(:,nn(n)),percs/gsmult)]*mult;
        disp(r); disp(' '); res=[res;r];
end
```

Files used specifically for cancer analysis

```
% ------------------------------------------------------------------
% File: straightsims.m
% Created by Paul Schlosser, U.S. EPA, 8/2008
% Runs Monte Carlo (MC) simulations withOUT search (for human equivalent exposures)
% Requires set exposure, NRUNS and rnames (list of variables to save)
% PBPK parameters set using MC selection by file human_pars.m.
% ------------------------------------------------------------------
exist contsim;    % Test to see if contsim defined.
if ~ans  % If not...
        contsim=0; % Not a continuation.
end
if contsim==0    % When starting a new set of simulation;
        % set contsim=1 if continuing an interupted chain.
        runo=[]; rns=length(rnames); ns=1;
else
        ns=RUNN;
end
for RUNN = ns:NRUNS
```

```
        if mod(RUNN,50)==0
                disp(['Remaining runs = ',num2str(NRUNS-RUNN)])
        end
        human_pars; start @NoCallback
        runo=[runo;eval(sr)];
end
```

```
% ------------------------------------------------------------------
% File: Human drinking water MCA OSF.m
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 10/2007
% Modified by Paul Schlosser, U.S. EPA 8/2008, rev. 1/2010
%
% This script file sets up the control parameters to simulate human unit
% drinking water exposure (1 mg/kg/day) and calls straightsim which
% generates a Monte-Carlo chain for the internal doses (identified
% in text array nn) to be used in calculating oral slope factors (OSFs).
% ------------------------------------------------------------------
% Test to see if contsim defined.
exist contsim
if ~ans  % If not...
        contsim=0; % Not a continuation.
end
if contsim==0   % When starting a new set of simulation;
        % set contsim=1 if continuing an interrupted chain.
        clearT
        nm=["LDAYPCTHB";"PEAKPCTHBCO";"AVRFROMDCM"]; FIXDRDOSE=0.006; dtxt='
avg % HbCO; Peak %'; mult=1;
                % Calculate distrib of PCTHBCO given RfD (0.006 mg/kg-day), PMS 2-14-11
        nm=["LDAYLIVGSTDOSE";"LDAYWBDYGSTDOSE"]; dtxt='n/a';
                FIXDRDOSE=1.0; mult=1.0/FIXDRDOSE;       % Drinking fixed mg/kg-day
        nn=findnames(nm,rnames);
        if (length(nn) ~= length(nm))
                disp("Not all names in list nm.")
                return
        end
        TEND=95.0; NRUNS=10000; CINT=0.1; % Total iterations for Monte Carlo analysis
        gendm="both";  % Gender mix; choose "male, "female", or "both"
        agem=0;                 % Age "mix"; if 0 draws from distribution (0.5-80 years)
                        % otherwise agem value is used exclusively
        popn="++"; % GSTGT "++", "+-", or "mix" of +/+, +/-, and -/-
        model='human_par2'; % Choose model between 'human_par1' (original) and 'human_par2' (+
uncertainty)
        astp='OSF_age'; percs=[95 99]; gsmult=1.0;
end
straightsims;
finish
```

```
% ------------------------------------------------------------------
% File: Human inhalation MCA IUR.m
```

```
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 11/2007
% Modified by Paul Schlosser, 7/2008, rev. 1/2010
%
% This script file sets up the control parameters to simulate human unit
% inhalation exposures (1 mcg/m3) and calls straightsim which generates
% a Monte-Carlo chain for the internal doses (identified in text array
% nn) to be used in calculating inhalation unit risks (IURs).
% ------------------------------------------------------------------------
exist contsim;    % Test to see if contsim defined.
if ~ans  % If not...
        contsim=0; % Not a continuation.
end
if contsim==0    % When starting a new set of simulation;
        % set contsim=1 if continuing an interupted chain.
        clearT; CINT=1.0;
        TEND=24*15; CONC=1.0e-6/GASD; CC=0;      % Daily dose-rates now calculated from
                % final day of simulations, & only need to go to 60 hr to reach SS
                % CONC in ppm; 0.00029 ppm = 1 ug/m3 DCM = 1e-3 ug/L = 1e-6 mg/L
        nm=["LDAYLIVGSTDOSE";"LDAYLUNGGSTDOSE";"LDAYWBDYGSTDOSE";"CV";"LD
AYAUCS"];
        astp='IUR_age'; mult= 1.0e-6/(CONC*GASD); percs=[95 99]; dtxt=' n/a';
        %CONC=2/(1000*GASD); nm=["LDAYPCTHB";"PEAKPCTHBCO";"AVRFROMDCM"];
dtxt=' avg % HbCO; Peak %';    mult=1;
                % Calculate distrib of PCTHBCO given RfC (0.6 mg/mm^3), PMS 2-14-11
        nn=findnames(nm,rnames);
        if (length(nn) ~= length(nm))
                disp("Not all names in list nm.")
                return
        end
        NRUNS=10000;  %Total iterations for Monte Carlo analysis
        gendm="both";  % Gender mix; choose "male, "female", or "both"
        agem=0;                 % Age "mix"; if 0 draws from distribution (0.5-80 years)
                        % otherwise agem value is used exclusively
        popn="++"; % GSTGT "++", "+-", or "mix" of +/+, +/-, and -/-
        gsmult = 1.0; % Multiplies draw for GST individual selection, divides the percentiles at end.
        model='human_par2'; % Choose model between 'human_par1' (original) and 'human_par2' (+
uncertainty)
end
straightsims; finish
```

Files used specifically for non-cancer analysis

```
% ------------------------------------------------------------------
% File: searchsim_refdose.m
% Created by Paul Schlosser, U.S. EPA, 8/2008
% Runs Monte Carlo (MC) simulations WITH search for human equivalent
% exposures, using variable LDAYREFDOSE ('reference dose based on
% last day of simulated exposure -- presumed "periodicity").
% Requires exposure variable named = expnm = "DRCONC" or "CONC",
% target value = heqt, relative tolerance = hetol, initial set
% exposure (CONC or DRCONC value >0) , NRUNS, and rnames (list of
```

```
% variables to save)
% PBPK parameters set using MC selection by file human_pars.m.
% ----------------------------------------------------------------
% set contsim=1 if continuing an interupted chain.
if contsim==0   % When starting a new set of simulations
        if ~(expnm=="CONC" | expnm=="DRCONC")
                disp('Variable expnm must be "CONC" or "DRCONC", in double quotes.');
                return
        end
        if expnm=="CONC"      % atxt used as variable command below
                DRCONC=0.0; atxt='CONC = c1;'; c2=CONC;
        else
                CONC=0.0; atxt='DRCONC = c1;'; c2=DRCONC;
        end
        c1=c2; runo=[]; rns=length(rnames); ns=1; c1s=['c1 = c2*heqt/',dtxt,';'];
        v1s=['v1 = ',dtxt,';']; v2s=['v2 = ',dtxt,';'];
else    % Continuing a set of simulations
        ns=RUNN;
end
for RUNN = ns:NRUNS
        disp(['Remaining runs = ',num2str(NRUNS-RUNN)])
        eval(model);
        % Following block calculates daily dose resulting in specified internal dose
        start @NoCallback
        nstep=1; eval(v2s); eval(c1s); eval(atxt);
        start @NoCallback
        eval(v1s); het1=hetol;
        while (abs((v1/heqt)-1)>hetol);  % Specify corresponding dose metric
                cn = abs((heqt-v2)*(c1-c2)/(v1-v2) + c2);          % Linear interpolation to heqt
                c2=c1; v2=v1; c1=cn; eval(atxt);           % Assign values
                start @NoCallback
                eval(v1s);
                nstep=nstep+1;
                if nstep==20
                        hetol=hetol*10; nstep=1;
                        disp('WARNING! hetol increased to allow convergence!')
                end
        end
        hetol=het1; runo=[runo;eval(sr)];
end
```

```
% ----------------------------------------------------------------
% File: human drinking water MCA RfD.m
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 11/2005
% Modified by Paul Schlosser, U.S. EPA 8/2008 and 1/2010
%
% This script file sets up the control parameters to simulate human
% drinking water exposure for a given internal dose (heqt) and calls
% searchsim_refdose which generates a Monte-Carlo chain for the human
% equivalent administered daily dose (HEAD; mg/kg/d).
% ----------------------------------------------------------------
```

```
exist contsim;
if ~ans
        contsim=0;
end
if contsim==0   % When starting a new set of simulation;
        % set contsim=1 if continuing an interupted chain.
        clearT
        nm=["DDOSE"]; nn=findnames(nm,rnames);
        if (length(nn) ~= length(nm))
                disp("Not all names in list nm.")
                return
        end
        hetol=1.0e-4; %relative tolerance
        expnm="DRCONC"; DRCONC=9.0; % 35 mg/L is 1 mg/kg/day for 70 kg human drinking 2L
        TEND=95.0; NRUNS=10000;  %Total iterations for Monte Carlo analysis
        gendm="both";  % Gender mix; choose "male, "female", or "both"
        agem=0;                 % Age "mix"; if 0 draws from distribution (0.5-80 years)
                                % otherwise agem value is used exclusively
        popn="mix"; % GSTGT "++", "+-", "--", or "mix" of +/+, +/-, and -/-
        % dtxt = dose metric to use
        % heqt = target for HEQ search; set value for specific dose metric
        dtxt='LDAYREFDOSE'; dt1='W';
        dtxt='LDAYLIVGSTDOSE'; dt1='G';
        dtxt='LDAYLIVAUC'; dt1='A'; heqt=11.1;
        dtxt='LDAYLIVCYPDOSE'; dt1='Y'; heqt=13.31;
        model='human_par2'; % Choose model between 'human_par1' (original) and 'human_par2' (+
uncertainty)
        astp=['RfD_',dt1,'_',num2str(heqt),'_age']; percs=[5 1]; mult=1; gsmult=1;
end
searchsim_refdose; finish
```

```
% ----------------------------------------------------------------
% File: Human inhalation MCA RfC.m
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 11/2005
% Modified by Paul Schlosser, U.S. EPA, 7/2008 and 1/2010
%
% This script file sets up the control parameters to simulate human
% inhalation exposure for a given internal dose (heqt) and calls
% searchsim_refdose which generates a Monte-Carlo chain for the human
% equivalent cocnetration (HEC; mg/m^3).
% ----------------------------------------------------------------
exist contsim;
if ~ans
        contsim=0;
end
if contsim==0   % When starting a new set of simulation;
        % set contsim=1 if continuing an interupted chain.
        use clearT
        nm=["CONC"]; nn=findnames(nm,rnames);
        if (length(nn) ~= length(nm))
                disp("Not all names in list nm.")
                return
        end
        TEND=96; NRUNS=10000;  %Total iterations for Monte Carlo analysis
        expnm="CONC"; CONC=67; % CONC in ppm; 0.29 ppm = 1 mg/m3 DCM
        hetol=1.0e-5; %relative tolerance
        gendm="both";  % Gender mix; choose "male, "female", or "both"
        agem=0;                 % Age "mix"; if 0 draws from distribution (0.5-80 years)
                                % otherwise agem value is used exclusively
        popn="mix"; % GSTGT "++", "+-", "--", or "mix" of +/+, +/-, and -/-
        gsmult = 1.0; % Multiplies draw for GST individual selection, divides the percentiles at end.
        % dtxt = dose metric to use
        % heqt = target for HEQ search; set value for specific dose metric
        % The last statement below is the metric and target value used.
        % Move desired one to end and save this file before running.
        dtxt='LDAYLIVAUC'; dt1='A'; heqt=0.0562;
        dtxt='LDAYLIVGSTDOSE'; dt1='G'; heqt=10.97;
        dtxt='LDAYREFDOSE'; dt1='W'; heqt=76.71;
        dtxt='LDAYLIVCYPDOSE'; dt1='Y'; heqt=130.03;
        model='human_par2'; % Choose model between 'human_par1' (original) and 'human_par2' (+
uncertainty)
        astp=['RfC_',num2str(heqt),'_age']; mult=GASD*1000; percs=[5 1];
end
searchsim_refdose; finish
```

```
% ----------------------------------------------------------------
% File: mouse_set.m
% Programmed by Paul Schlosser, U.S. EPA, 7/2008
% Clears previous variables and sets parameters for mouse simulations.
% ----------------------------------------------------------------
% prepare time history values.
prepare @clear @all
CINT=0.1; CONC=0.0; IVDOSE=0.0; BOLUS=0.0; DRCONC=0.0; FIXDRDOSE=0.0;
WESITG=0; WEDITG=0; CC=0;
% from Reitz et al. 1997, Table 1
DRPCT = [0.233, 0.1, 0.1, 0.1, 0.233, 0.234];
DRTIME= [0.0, 4.0, 8.0, 12.0, 16.0, 20.0];
TEND=336;      % 2 weeks
TCHNG=6;       % daily exposure duration = 6 hours/day
TCHNG2=120; % weekly dose width = 5 days/week = 120 hours
TDUR=24;       % daily dose period = 24 hours
TDUR2=168;    % weekly exposure period = 7 days = 168 hours

% U.S. EPA (1988) reference value for B6C3F1 mice: males=0.0373, females=0.0353
BW=0.0373;
% Mouse uptake & metabolism parameters (defined by MCMC calibration)
KA=5.0; VMAXC=9.27; KM=0.574; FRACR=0.0; KFC=1.41; AFFG=0.0; A1=0.207; A2=0.196;
% Mouse physiologic parameters (from prior distributions or MCMC
% calibration)
VLC=0.04; VFC=0.04; VRC=0.05; VLUC=0.0115; VBL2C=0.059; VSC=0.78;
QCC=24.2; VPR=1.45; DSPC=0.15; QLC=0.24; QFC=0.05; QSC=0.19; QRC=0.52;
%Mouse partition coefficients for DCM
PL=1.6; PLU=0.46; PF=5.1; PS=0.44; PR=0.52; PB=23.0;
start @NoCallBack
```

```
% ----------------------------------------------------------------
% File: Mouse drinking water National Coffee 1983.m
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 01/2007
% Modified by Paul Schlosser, U.S. EPA, 8/2008
%
% This run time file sets species-specific constants and exposure
% values for National Coffee Association study of DCM in drinking water
        % in the mouse; parameterized to run with DCM.07.rev3.csl.
% ----------------------------------------------------------------
use mouse_set
BW=0.0373; TEND=4*7*24; runo=[];

% Mouse exposure parameters: DDOSE males: 61, 124, 177, or 244 mg/kg-day
for FIXDRDOSE=[61, 124, 177, 244]
        start @NoCallback;
        runo=[runo;[BW,DDOSE,WAVGLIVGSTDOSE,WAVGLUNGGSTDOSE,
WAVGWBDYGSTDOSE]];
end
runo
```

```
save runo @file='mouseOSFdrink.csv' @format=ascii @separator=comma
```

```
% ----------------------------------------------------------------
% File: Mouse Inhalation NTP 1986.m
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 11/2007
% Modified by Paul Schlosser, U.S. EPA, 7/2008
%
%This run time file sets species-specific constants and exposure
%values for NTP (1986) inhalation exposures of DCM in the mouse.
% ----------------------------------------------------------------
use mouse_set
CINT=0.01;
% Kodak value; study average male: 2k=0.034, 4k=0.032; female 2k=0.030, 4k=0.029
TEND=5*7*24; Bws=[0.034, 0.032; 0.030,0.029]; runo=[]; Cs=[2000, 4000];
for s=[1 2]
        for c=[1,2];
                BW=Bws(s,c); CONC=Cs(c);
                start @NoCallback
runo=[runo;[BW,CONC,WAVGLIVGSTDOSE,WAVGLUNGGSTDOSE,WAVGWBDYGSTDOSE]];
        end
end
runo
%%% Saving last simulation results to file
run=[_t _cv _cvl _cvf _cvs _cvr _cabl2 _wavglivgstdose _wavglunggstdose _ddose];
save run @file='RUNOUT_NTP_inhal.csv' @format=ascii @separator=comma
```

Files used for rat dose analyses

```
% ----------------------------------------------------------------
% File: rat_set_D.m
% Programmed by Paul Schlosser, U.S. EPA, 9/2009
% Clears previous variables and sets parameters for rat simulations.
% Rat model "D" is a baseline for other models.
% ----------------------------------------------------------------
% prepare time history values.
prepare @clear @all
WESITG=0; WEDITG=0;
% Rat exposure controls
CINT=0.01; CONC=0; IVDOSE=0; DRCONC=0; FIXDRDOSE=0; BOLUS=0; CC=0; RSTDY=0;
%DRTIME=[0:12, 12.6:0.6:23.4];
%DRPCT=[(0.21/12)*ones(12,1);(0.79/20)*ones(20,1)];
DRPCT = [0.233, 0.1, 0.1, 0.1, 0.233, 0.234, zeros(1,26)];
DRTIME = [0.0, 4.0, 8.0, 12.0, 16.0, 20.0, 21.1:0.1:23.6];
TEND=25;        % hours
TCHNG=6;    % daily exposure = 6 hours
TCHNG2=120;  % weekly dose width = 5 days/week = 120 hours
TDUR=24;     % daily dose period = 24 hours
TDUR2=168;   % weekly exposure period = 7 days = 168 hours
% Rat UPTAKE & metabolism parameters
FRACR=0; AFFG=0; KFEC=0; K12=0; PSCO=0;
```

```
VMAXC=3.992; KM=0.4; A1=0.002; A2=0.149; KFC=1.917; KZERC=0;
% Rat physiologic parameters (from Andersen et al 1991)
BW=0.220; % males:0.380 kg, females:0.229 kg, from EPA (1988)
VLC=0.04; VFC=0.07; VRC=0.05; VLUC=0.0115; VBL2C=0.059; VSC=0.75;
QCC=15.9; VPR=0.94; DSPC=0.15; QLC=0.20; QFC=0.09; QSC=0.15; QRC=0.56;
%Rat partition coefficients for DCM (Andersen et al 1991)
PL=0.732; PLU=0.46; PF=6.19; PS=0.408; PR=0.732; PB=19.4;
%CO submodel parameters
DLC=0.060; HBTOT=10; P1=0.80; F1=1.21; MMM=197.0; COINH=0;
COBGD=2.2; O2=0.13; PAIR=713; RHO=1102; SOL=0.03;
ABCOC=0; RENCOC=0; RENCOS=1; PCTHBCO0=0.7;  % Bgd total blood CO ...
% ... and production to be calculated in csl to match PCTHBCO0
start @NoCallBack
VMAXC = 3.97; KM = 0.51; KFC = 2.47; P1 = 0.68;
KA = 4.31; K12 = 35.0; KA2 = 1.67;
```

```
% ------------------------------------------------------------------
% File: rat_set_A.m
% Programmed by Paul Schlosser, U.S. EPA, 2/2011
% Clears previous variables and sets parameters for rat simulations.
% Rat model "A".
% ------------------------------------------------------------------
use rat_set_D
VMAXC=3.992; KM=0.4; A1=0.002;
KFC=1.917; A2=0.149; KA=5.0; K12=0;
P1=0.8; ABCOC=0.117; RENCOS=1;
```

```
RENCOC=0.035; COBGD=2.2;% File: rat_set_B.m
% Programmed by Paul Schlosser, U.S. EPA, 2/2011
% Clears previous variables and sets parameters for rat simulations.
% Rat model "B".
% ------------------------------------------------------------------
use rat_set_D
VMAXC = 3.97; KM = 0.509; KFC = 2.46; P1 = 0.68;
%use inhivfit_rat-params % Un-comment this to use acslX-saved params
```

```
% ------------------------------------------------------------------
% File: rat_set_C.m
% Programmed by Paul Schlosser, U.S. EPA, 2/2011
% Clears previous variables and sets parameters for rat simulations.
% Rat model "C".
% ------------------------------------------------------------------
use rat_set_D
VMAXC = 3.97; KM = 0.51; KFC = 2.47; P1 = 0.68;
KA = 4.31; K12 = 35.0; KA2 = 1.67;
K12=0; KA2=0;  % For 2nd GI compartment [not used]
```

```
% ------------------------------------------------------------------
% File: Rat inhalation Nitschke 1988a.M
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 10/2007
% Modified by Paul Schlosser, U.S. EPA, 7/2008
%
% This run time file sets species-specific constants values for modified
```

```
% Andersen et al. (1987; 1991) simulating Nitschke et al. (1988) exposures
% of DCM in the male and female rats.
% --------------------------------------------------------------------
use rat_set_C
TEND=5*7*24; runo=[]; BW=0.229; TCHNG=6; TCHNG2=120;
% males:0.380 kg, females:0.229 kg, from U.S. EPA (1988)
for CONC=[50, 200, 500]
        start @NoCallback
        runo=[runo;[BW,CONC,DIDOSE,WAVGREFDOSE,WAVGLIVGSTDOSE,
                WAVGLIVCYPDOSE,WAVGDAILYAUCL]];
end
runo
save runo @file='Rat_inhal_Nitschke.csv' @format=ascii @separator=comma
```

```
% --------------------------------------------------------------------
% File: Rat inhalation Burek et al. (1984).m
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 10/2007
% Modified by Paul Schlosser, U.S. EPA, 7/2008
%
% This run time file sets species-specific constants values for modified Andersen et al
% (1987, 1991) simulating Burek et al. (1984) exposures of DCM in male & female rats
% --------------------------------------------------------------------
use rat_set_C
TEND=5*7*24; TCHNG=6; TCHNG2=120; runo=[];
for BW=[0.523, 0.338]
        for CONC=[500, 1500, 3500]
                start @NoCallback
                runo=[runo;[BW,DIDOSE,CONC,WAVGREFDOSE,WAVGLIVGSTDOSE,
                        WAVGLIVCYPDOSE,WAVGDAILYAUCL]];
        end
end
runo
save runo @file='Rat_inhal_Burek.csv' @format=ascii @separator=comma
```

```
% --------------------------------------------------------------------
% File: Rat drinking water Serota 1986a.m
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 1/2007
% Modified by Paul Schlosser, U.S. EPA, 7/2008
%
% This run time file sets species-specific constants values for modified Andersen et al
% (1987, 1991) simulating Serota et al. (1986) exposures of DCM to male & female rats
% --------------------------------------------------------------------
use rat_set_C
%DRCONC values from Serota et al. (1986)
% males:0, 44.00, 381.36, 916.74, 1723.47
% females: 0, 37.80, 365.41, 856.82, 1656.94
TEND=7*24; runo=[]; BW=0.380; % Males
for FIXDRDOSE=[6, 52, 125, 235]
        start @NoCallBack
```

```
        runo=[runo;[BW,DDOSE,LDAYREFDOSE,LDAYLIVGSTDOSE,LDAYLIVCYPDOSE,
                LDAYLIVAUC]];
end
BW=0.229; % Females
for FIXDRDOSE=[6, 58, 136, 263]
        start @NoCallBack
        runo=[runo;[BW,DDOSE,LDAYREFDOSE,LDAYLIVGSTDOSE,LDAYLIVCYPDOSE,
                LDAYLIVAUC]];
end
runo
save runo @File="Serota_rat_DW.csv" @Format=ascii @Separator=comma
```

```
% ----------------------------------------------------------------
% File: Rat inhalation NTP 1986.M
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 11/2007
% Modified by Paul Schlosser, U.S. EPA, 7/2008
%
% This run time file sets species-specific constants values for modified Andersen et al
% (1987, 1991) simulating NTP (1986) exposures of DCM to male & female rats
% ----------------------------------------------------------------
use rat_set_A
use rat_set_C
CINT=0.1;
bws=[0.3905, 0.3852, 0.3848, 0.2455, 0.2443, 0.2422];
TEND=6*24*7; concs=[1 2 4 1 2 4]*1000; runo=[];
for n=1:length(bws)
        BW=bws(n); CONC=concs(n); start @NoCallback
        runo=[runo;[BW,CONC, WAVGLIVCYPDOSE,WAVGLIVGSTDOSE, WAVGREFDOSE,
                (WAVGLIVCYPDOSE/WAVGLIVGSTDOSE),WAVGAUCV,WAVGAUCS]];
end
runo
save runo @File="Rat_inhal_NTP.csv" @Format=ascii @Separator=comma
```

```
% ----------------------------------------------------------------
% File: Figure 5_3.m
% Programmed by Paul Schlosser, U.S. EPA, 9/2009
% Creates dichloromethane exposure-dose relationship for humans
% versus the rat shown in Figure 5-3 of the IRIS assessment
% ----------------------------------------------------------------
use rat_set_C
ods=[10:10:100,120,140,170,200,220,250];
TEND=7*24; ratr=[]; BW=0.380; % Male rats
for FIXDRDOSE=ods
        start @NoCallBack
        ratr=[ratr;[LDAYLIVGSTDOSE,LDAYLIVCYPDOSE,LDAYREFDOSE]];
        disp(['Rat, oral dose = ',num2str(FIXDRDOSE),', livGSTdose =
',num2str(LDAYLIVGSTDOSE),'.']);
end

clearT; TEND=95.0; humr=[]; contsim=0;
```

```
NRUNS=1000;  % NRUNS = Total iterations for Monte Carlo analysis
gendm="both";          % Gender mix; choose "male, "female", or "both"
agem=0;                % Age "mix"; if 0 draws from distribution (0.5-80 years)
                       % otherwise agem value is used exclusively
popn="mix"; gsmult=1; % GSTGT "++", "+-", or "mix" of +/+, +/-, and -/-
model='human_par2'; % Choose between 'human_par1' (original) and 'human_par2' (+
uncertainty)
nm=["LDAYLIVGSTDOSE";"LDAYLIVCYPDOSE";"LDAYREFDOSE"];
nn=findnames(nm,rnames);
        if (length(nn) ~= length(nm))
                disp("Not all names in list nm.")
                return
        end
for FIXDRDOSE=ods
        rres=[FIXDRDOSE]; straightsims;
        for n=1:length(nm)
                rres=[rres,[mean(runo(:,nn(n))),prctile(runo(:,nn(n)),[5 95])]];
        end
        disp(['Human, dose = ',num2str(FIXDRDOSE),', internal doses = ...']); rres
        humr=[humr;rres];
end
save @File="Fig5_3.mat"
plot(ods,ratr(:,1),ods,humr(:,2),ods,humr(:,4),'Fig5_3a.aps');
plot(ods,ratr(:,2),ods,humr(:,5),ods,humr(:,6),ods,humr(:,7),'Fig5_3b.aps');
plot(ods,ratr(:,3),ods,humr(:,8),ods,humr(:,9),ods,humr(:,10),'Fig5_3c.aps');
```

```
% ---------------------------------------------------------------------
% File: Figure 5_7.m
% Programmed by Paul Schlosser, U.S. EPA, 9/2009
% Creates dichloromethane exposure-dose relationship for humans
% versus the rat shown in Figure 5-7 of the IRIS assessment
% ---------------------------------------------------------------------
use rat_set_C
ccs=[10:10:60,80,100,150,200:100:600,800,1000]; %,1500,2000:1000:5000];
TEND=2*7*24; ratr=[]; BW=0.229; TCHNG=6; TCHNG2=120;
for CONC=ccs
        start @NoCallBack
        ratr=[ratr;[CONC,WAVGLIVGSTDOSE,WAVGLIVCYPDOSE,WAVGREFDOSE]];
disp(['Rat, conc = ',num2str(CONC),', livGSTdose = ',num2str(WAVGLIVGSTDOSE),'.']);
end
save ratr @file='Fig5_7-rat.csv' @format=ascii @separator=comma
clearT; TEND=95.0; hum1=[]; contsim=0;
NRUNS=1000;          % NRUNS = Total iterations for Monte Carlo analysis
gendm="both";        % Gender mix; choose "male, "female", or "both"
agem=0;              % Age "mix"; if 0 draws from distribution (0.5-80 years)
                     % otherwise agem value is used exclusively
popn="mix"; gsmult=1; % GSTGT "++", "+-", or "mix" of +/+, +/-, and -/-
model='human_par2';
        % Choose between 'human_par1' (original) and 'human_par2' (+ uncertainty)
```

```
nm=["LDAYLIVGSTDOSE";"LDAYLIVCYPDOSE";"LDAYREFDOSE";
        "LDAYLUNGGSTDOSE"];
nn=findnames(nm,rnames);
        if (length(nn) ~= length(nm))
                disp("Not all names in list nm.")
                return
        end
for CONC=ccs
        rres=[CONC]; straightsims;
        for n=1:length(nm)
                rres=[rres,[mean(runo(:,nn(n))),prctile(runo(:,nn(n)),[5 95])]];
        end
        disp(['Human, conc = ',num2str(CONC),', internal doses = ...']); rres
        hum1=[hum1; rres];
end
save hum1 @file='Fig5_7-human.csv' @format=ascii @separator=comma
save @File="Fig5_7.mat"
plot(ccs,ratr(:,2),ccs,hum1(:,2),ccs,hum1(:,4),'Fig5_7a.aps')
plot(ccs,ratr(:,3),ccs,hum1(:,5),ccs,hum1(:,6),ccs,hum1(:,7),'Fig5_7b.aps')
plot(ccs,ratr(:,4),ccs,hum1(:,8),ccs,hum1(:,9),ccs,hum1(:,10),'Fig5_7c.aps')
```

```
% ------------------------------------------------------------------
% File: Figure 5_9n10_rat_sense.m
% Programmed by Paul Schlosser, U.S. EPA, 9/2009
% Performs sensitivity analysis for rat oral (Figure 5-9) and inhalation
% (Figure 5-10) dichloromethane exposures, and writes results to file
% 'ratsense_Figs59n10.csv' for plotting in Excel
% ------------------------------------------------------------------
use rat_set_C

FIXDRDOSE=10; TEND=7*24; ratr=[]; BW=0.380; % Male rats
start @nocallback
r0=[LDAYLIVGSTDOSE,LDAYLIVCYPDOSE,LDAYREFDOSE];
pars=["QCC","VPR","VLC","VSC","PB","VMAXC","KA","A2","KFC"];
for pt=pars
        p0=eval(pt); setbase(pt,1.01*p0); start @nocallback
        r=[LDAYLIVGSTDOSE,LDAYLIVCYPDOSE,LDAYREFDOSE];
        setbase(pt,0.99*p0); start @nocallback
        r= 50*(r-[LDAYLIVGSTDOSE,LDAYLIVCYPDOSE,LDAYREFDOSE])./r0;
        setbase(pt,p0);
        ratr=[ratr;r];
end

FIXDRDOSE=0; CONC=500; TEND=5*7*24; BW=0.229; TCHMG=6; TCHNG2=120;
start @nocallback
r0=[WAVGLIVGSTDOSE,WAVGLIVCYPDOSE,WAVGREFDOSE];
for pt=pars
        p0=eval(pt); setbase(pt,1.01*p0);  start @nocallback
        r=[WAVGLIVGSTDOSE,WAVGLIVCYPDOSE,WAVGREFDOSE];
```

```
        setbase(pt,0.99*p0); start @nocallback
        r= 50*(r-[WAVGLIVGSTDOSE,WAVGLIVCYPDOSE,WAVGREFDOSE])./r0;
        setbase(pt,p0);
        ratr=[ratr;r];
end

ratr
save ratr @file="ratsense_Figs59n10.csv" @format=ascii @separator=comma
```

```
% ------------------------------------------------------------------
% File: Figure 5_14.m
% Programmed by Paul Schlosser, U.S. EPA, 9/2009
% Creates dichloromethane exposure-dose relationship for humans versus the mouse
% shown in Figure 5-14 of the IRIS assessment; results are written to file
% "Fig5-14_human.csv" for plotting in Excel
% ------------------------------------------------------------------
use mouse_set
ods=[10:10:100,120,140,170,200,220,250];
TEND=7*24; mour=[]; BW=0.033; TCHNG=2000; TCHNG2=2000;
model='human_par2';
for FIXDRDOSE=ods
        start @NoCallBack
        mour=[mour;WAVGLIVGSTDOSE];
        disp(['Mouse, dose = ',num2str(FIXDRDOSE),',
                livGSTdose = ',num2str(WAVGLIVGSTDOSE),'.']);
end
save mour @file="Fig5-14_mouse.csv" @format=ascii @separator=comma
clearT; TEND=95.0; hum2=[]; contsim=0;
NRUNS=1000;  % NRUNS = Total iterations for Monte Carlo analysis
gendm="both";          % Gender mix; choose "male, "female", or "both"
agem=0;                % Age "mix"; if 0 draws from distribution (0.5-80 years)
                       % otherwise agem value is used exclusively
gsmult=1; nm=["LDAYLIVGSTDOSE"]; nn=findnames(nm,rnames);
        if isempty(nn)
                disp("Variable name not in list nm.")
                return
        end
for FIXDRDOSE=ods
        rres=[FIXDRDOSE]
        for popn=["mix","+-","++"] % GSTGT "++", "+-", or "mix" of +/+, +/-, and -/-
                straightsims;
                for n=1:length(nm)
                        rres=[rres,[mean(runo(:,nn(n))),prctile(runo(:,nn(n)),[5 95])]]
                end
        disp(['Human',ctot(popn),', dose = ',num2str(FIXDRDOSE),', internal doses = ...']); rres
        end
        hum2=[hum2; rres];
end
save hum2 @file="Fig5-14_human.csv" @format=ascii @separator=comma
```

```
save @file="Fig5_14.mat"
plot(ods,mour,ods,hum2(:,2),ods,hum2(:,4), ods,hum2(:,5),ods,hum2(:,6),ods,hum2(:,7), ...
        ods,hum2(:,8),ods,hum2(:,9),ods,hum2(:,10),'Fig5_14.aps')
```

```
% ----------------------------------------------------------------
% File: Figure 5_15.m
% Programmed by Paul Schlosser, U.S. EPA, 9/2009
% Creates dichrlomethane exposure-dose relationship for humans versus the
% mouse shown in Figure 5-15 (panels A and B) of the IRIS assessment;
% results are written to file "Fig5-15_human.csv" for plotting in Excel
% ----------------------------------------------------------------
use mouse_set
ccs=[10:10:100,120,150,200,230,300,330,400,430,500:100:1000,1300,2000:1000:5000];
TEND=5*7*24; mour=[]; BW=0.031; TCHNG=6; TCHNG2=120;
for CONC=ccs
        start @NoCallBack
        mour=[mour;[CONC,WAVGLIVGSTDOSE,WAVGLUNGGSTDOSE]];
disp(['Mouse, conc = ',num2str(CONC),', livGSTdose = ',num2str(WAVGLIVGSTDOSE),'.']);
end

clearT; TEND=95.0; hum3=[]; contsim=0;
NRUNS=1000;         % NRUNS = Total iterations for Monte Carlo analysis
gendm="both";       % Gender mix; choose "male, "female", or "both"
agem=0;             % Age "mix": draws from distribution (0.5-80 years)
model='human_par2'; gsmult=1;
nm=["LDAYLIVGSTDOSE";"LDAYLUNGGSTDOSE"]; nn=findnames(nm,rnames);
        if (length(nn) ~= length(nm))
                disp("Not all names in list nm.")
                return
        end
for CONC=ccs
        rres=[CONC];
        for popn=["+-","++"] % GSTGT "++", "+-", or "mix" of +/+, +/-, and -/-
                straightsims;
                for n=nn
                        rres=[rres,[mean(runo(:,n)),prctile(runo(:,n),[5 95])]];
                end
                if popn=="mix"
                        for ij=[0,1]
                                runo2=runo(runo(:,31)==ij,:);
                                for n=1:length(nm)
                                rres=[rres,[mean(runo2(:,nn(n))),prctile(runo2(:,nn(n)),[5 95])]];
                                end
                        end
                end
                disp(['Human, conc = ',num2str(CONC),', internal doses = ...']); rres
        end
        hum3=[hum3;rres];
end
```

```
save hum3 @file="Fig5-15_human.csv" @format=ascii @separator=comma
save @File="Fig5_15.mat"
% Liver dose plot...
%plot(ccs,mour(:,2),hum1(:,1),hum1(:,2),hum1(:,1),hum1(:,4), ...
%       ccs,hum3(:,2),ccs,hum3(:,3),ccs,hum3(:,4), ...
%       ccs,hum3(:,8),ccs,hum3(:,9),ccs,hum3(:,10),'Fig4_3.aps')
plot(ccs,mour(:,2),hum1(:,1),hum1(:,2),ccs,hum3(:,2),ccs,hum3(:,8),'Fig5_15a.aps')
% Lung dose plot...
%plot(ccs,mour(:,1),hum1(:,1),hum1(:,11),hum1(:,1),hum1(:,13), ...
%       ccs,hum3(:,5),ccs,hum3(:,6),ccs,hum3(:,7), ...
%       ccs,hum3(:,11),ccs,hum3(:,12),ccs,hum3(:,13),'Fig4_4.aps')
plot(ccs,mour(:,3),hum1(:,1),hum1(:,11),ccs,hum3(:,5),ccs,hum3(:,11),'Fig5_15b.aps')
```

```
% ------------------------------------------------------------------
% File: Fig5_17to19_mouse_sense.m
% Programmed by Paul Schlosser, U.S. EPA, 9/2009
% Runs sensitivity analyses for dichloromethane exposures in mice,
% producing results plotted in Figures 5-17 to 5-19 of the IRIS assessment.
% Values saved to file "mousesense_Figs5_17to19.csv" for plotting in Excel.
% ------------------------------------------------------------------
use mouse_set
CINT=0.001; TEND=(3*7*24)-CINT;
CONC=2000; TCHNG=6; TCHNG2=120; mour=[]; BW=0.031; % average mouse
start @nocallback
r0=WAVGLIVGSTDOSE;
pars=["QCC","VPR","VLC","VSC","PB","VMAXC","KA","A2","KFC"];
for pt=pars
        p0=eval(pt);
        setbase(pt,1.01*p0); start @nocallback
        r=WAVGLIVGSTDOSE;
        setbase(pt,0.99*p0); start @nocallback
        r= 50*(r-WAVGLIVGSTDOSE)/r0;
        setbase(pt,p0);
        mour=[mour,r];
end

DRCONC=500; CONC=0; start @nocallback
r0=LDAYLIVGSTDOSE; m5=[];
for pt=pars
        p0=eval(pt); setbase(pt,1.01*p0); start @nocallback
        r=LDAYLIVGSTDOSE;
        setbase(pt,0.99*p0); start @nocallback
        r= 50*(r-LDAYLIVGSTDOSE)/r0;
        setbase(pt,p0);
        m5=[m5,r];
end
mour=[mour;m5];

CONC=500; DRCONC=0; start @nocallback
```

```
r0=WAVGLUNGGSTDOSE; m5=[];
for pt=pars
        p0=eval(pt); setbase(pt,1.01*p0); start @nocallback
        r=WAVGLUNGGSTDOSE;
        setbase(pt,0.99*p0); start @nocallback
        r= 50*(r-WAVGLUNGGSTDOSE)/r0;
        setbase(pt,p0);
        m5=[m5,r];
end
mour=[mour;m5]
save mour @file="mousesense_Figs5_16to18.csv" @format=ascii @separator=comma
```

```
% ----------------------------------------------------------------------
% File: FigC3 rat inhal Gargas 86.m
% Figure C-3 (creates 2 sub-plots)
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 10/2007
% Modified by Paul Schlosser, U.S. EPA, 9/2009
%
% This run time file sets species-specific constants values for modified
% Andersen et al (1987, 1991) simulating Burek et al. (1984) exposures
% of dichloromethane in the male and female rats
% ----------------------------------------------------------------------
dch1=[0.103, 93.73; 0.2178, 81.12; 0.3707, 66.26; 0.5543, 52.58; 0.7227, 41.73;
                0.8834, 34.085; 1.044, 27.84; 1.22, 22.095; 1.3807, 18.312; 1.55, 14.532;
                1.717, 12.58; 1.893, 10.28; 2.061, 8.3948];

dch5=[0.1015, 448.9; 0.216, 400.0; 0.3843, 326.7; 0.5522, 282.85; 0.7128, 234.4;
                0.8806, 208.9; 1.056, 183.5; 1.231, 163.5; 1.384, 145.7; 1.5514, 133.7;
                1.719, 119.14; 1.887, 106.2; 2.0395, 95.99; 2.192, 85.5385; 2.360, 75.13;
                2.5276, 65.99; 2.688, 57.96; 2.8484, 49.45; 3.024, 40.99; 3.1996, 36.00;
                3.360, 31.165; 3.5356, 25.46; 3.719, 21.10; 3.864, 18.00; 4.025, 15.585;
                4.193, 13.297; 4.353, 11.51];

dch10=[0.0973, 927.1; 0.2195, 826.0; 0.3957, 627.6; 0.5556, 575.7; 0.7235, 513.1;
                0.8836, 457.2; 1.059, 419.5; 1.226, 401.9; 1.3936, 379.6; 1.561, 348.2;
                1.721, 333.66; 1.873, 329.1; 2.0556, 301.9; 2.200, 285.1; 2.3754, 265.4;
                2.5426, 258.0; 2.695, 236.7; 2.87, 226.8; 3.030, 211.1; 3.1975, 193.66;
                3.365, 182.9; 3.532, 175.24; 3.7076, 156.175; 3.875, 145.4; 4.035, 135.3;
                4.195, 124.1; 4.340, 112.2; 4.515, 101.5; 4.683, 89.12];

dch30=[0.1058, 2958.6; 0.2288, 2280; 0.3819, 1862.5; 0.5419, 1684; 0.7095, 1545;
                0.8848, 1397; 1.067, 1338.6; 1.227, 1264; 1.387, 1229; 1.5615, 1195;
                1.7136, 1178.5; 1.881, 1129; 2.056, 1051; 2.208, 1052; 2.383, 1008;
                2.5424, 994.2; 2.6945, 966.46; 2.869, 939.6; 3.0366, 913.45; 3.196, 914.2;
                3.356, 863.3; 3.531, 851.6; 3.713, 816.1; 3.880, 805.0; 4.032, 794.0;
                4.192, 760.75; 4.352, 750.4; 4.5114, 708.6; 4.686, 709.2; 4.853, 699.6;
                5.0055, 670.3; 5.173, 642.26; 5.34, 633.53];
```

```
for model=['A' 'C']
        eval(['use rat_set_',model]);
        BW=0.225; TEND=6; TCHNG=7; CC=1; VCHC=9; NCH=3; CINT=0.05;
        prepare @clear T CCHPPM
        res=[];
        for CONC=[107, 498, 1028, 3206]
                start @nocallback
                res=[res,_t,_cchppm];
        end
 eval(['save res @file=gargas-inh-sim-',model,'.csv @format=ascii @separator=comma'])
        plot(dch1(:,1),dch1(:,2),dch5(:,1),dch5(:,2),dch10(:,1),dch10(:,2), ...
                dch30(:,1),dch30(:,2),res(:,1),res(:,2),res(:,3),res(:,4), ...
                res(:,5),res(:,6),res(:,7),res(:,8),['gargas86',model,'.aps'])
end

dch1=[dch1;dch5;dch10;dch30];
save dch1 @file='gargas-inh-dat.csv' @format=ascii @separator=comma
```

```
% ------------------------------------------------------------------
% File: FigC4n5 Angelo_IV_comp.m
% Figures C-4 and C-5 (creates up to 4 sub-plots for each)
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 10/2007
% Modified by Paul Schlosser, U.S. EPA, 9/2009
%
% This run time file sets species-specific constants values for the
% modified Andersen et al (1987, 1991) PBPK model for dichloromethane,
% simulating Angelo et al. (1986b) IV exposures of DCM in rats
% ------------------------------------------------------------------
d10bl=[2, 17.624; 5, 8.6635; 10, 3.533; 15, 2.858; 20, 1.7125;
        30, 0.826; 40, 0.6192];
d50bl=[2, 62.686; 5, 34.105; 10, 16.56; 15, 12.33; 20, 12.80;
        30, 10.25; 40, 5.561];
d10ex=[0.3333, 32.245; 0.6667, 38.56; 1, 41.396; 4, 44.42];
d50ex=[0.3333, 35.38; 0.6667, 45.95; 1, 51.38; 4, 57.59];

for model=['A' 'C'] %['A' 'B' 'C' 'D'] % Use set of letters for models to test
        eval(['use rat_set_',model]);
        prepare @clear T CV PCTIVEXH
        TEND=10; CINT=0.01;
        IVDOSE=10
 start @nocallback
        res=[_t,_cv,_pctivexh]; r2=[_t*60,_cv,_t,_pctivexh];
        IVDOSE=5; start @nocallback
        res=[res,_t,_cv,_pctivexh]; r2=[r2,_t*60,_cv,_t,_pctivexh];
        IVDOSE=25; start @nocallback
        res=[res,_t,_cv,_pctivexh]; r2=[r2,_t*60,_cv,_t,_pctivexh];
```

```
        IVDOSE=50; start @nocallback
        res=[res,_t,_cv,_pctivexh];r2=[r2,_t*60,_cv,_t,_pctivexh];
        plot(d10bl(:,1),d10bl(:,2),d50bl(:,1),d50bl(:,2), ...
                res(:,1)*60,res(:,2),_t*60,_cv,res(:,4)*60,res(:,5), ...
                res(:,7)*60,res(:,8),['angivbl',model,'.aps'])
        plot(d10ex(:,1),d10ex(:,2),d50ex(:,1),d50ex(:,2), ...
                res(:,1),res(:,3),_t,_pctivexh,res(:,4),res(:,6), ...
                res(:,7),res(:,9),['angivex',model,'.aps'])
eval(['save r2 @file=Angelo_IV_sims_',model,'.csv @format=ascii @separator=comma'])
end
```

```
% ------------------------------------------------------------------
% File: FigC6 rat_inhal_Andersen.m
% Figures C-6 (creates multiple sub-plots)
%
% Programmed by Michael Lumpkin
% Syracuse Research Corporation, 10/2007
% Modified by Paul Schlosser, U.S. EPA, 9/2009
%
% This run time file sets species-specific constants values for the
% modified Andersen et al (1987, 1991) PBPK model for dichloromethane,
% simulating Andersen et al. (1987) inhalation exposures of DCM in rats
% ------------------------------------------------------------------
dan2=[1, 2.6634; 2, 4.290; 3, 4.9915; 4, 4.464; 4.25, 1.3334; 4.5, 0.5513;
        4.75, 0.4234; 5, 0.2618; 5.25, 0.1206; 5.5, 0.1049];
dan10=[1, 29.34; 2, 49.50; 3, 47.10; 4, 50.72; 4.25, 14.92; 4.5, 9.225;
        4.75, 6.260; 5, 3.811; 5.25, 1.3085; 5.5, 0.6515; 5.75, 0.6025];
can2=[0.9942, 3.967; 1.027, 4.121; 1.039, 3.798; 2.045, 7.151;
        2.0565, 6.598; 2.057, 5.7836; 3.053, 8.4295; 3.076, 7.493;
        3.086, 8.7215; 4.073, 9.094; 4.074, 8.003; 4.084, 8.940; 4.5545, 5.302;
        4.597, 6.853; 4.608, 7.1145; 5.078, 3.5525; 5.079, 2.723; 5.0795, 2.124;
        5.302, 1.464; 5.313, 1.971; 5.325, 1.142];
can10=[1.006, 4.004; 1.018, 4.305; 1.021, 5.114; 2.0494, 9.257;
        2.061, 9.488; 2.068, 7.9395; 3.047, 10.42; 3.0535, 8.777; 3.055, 9.170;
        4.030, 6.449; 4.030, 10.31; 4.036, 12.11; 5.0275, 7.448; 5.061, 7.656;
        5.067, 9.598; 5.5285, 4.978; 5.540, 5.001; 5.552, 9.115; 6.028, 1.999;
        6.030, 6.460; 6.049, 1.653; 6.510, 0.4305; 6.525, 2.072; 6.539, 2.973];
Tab3=[];
for model=['A' 'C']; %['A' 'B' 'C' 'D']
        eval(['use rat_set_',model]);
        prepare @clear T CV PCTHBCO
        start @nocallback
        QCSW=0; % No work/rest change
        TEND=7; CONC=200; CINT=0.05; TCHNG=4; BW=0.237; start @nocallback
        start @nocallback
        res=[_t,_cv,_pcthbco];
        TEND=4*24; start @nocallback
Tab3=[Tab3;[CONC,LDAYLIVCYPDOSE, LDAYLIVGSTDOSE,(LDAYLIVCYPDOSE + ...
        LDAYLIVGSTDOSE),(LDAYLIVCYPDOSE/LDAYLIVGSTDOSE)]];
```

```
        if model=='C'
                CONC=950; TEND=7; start @nocallback
                res=[res,_cv];
        end

        %CONC=750; start @nocallback
CONC=1000; TEND=7; start @nocallback
        res2=[_t,_cv,_pcthbco]; res=[res,res2];
                plot(dan2(:,1),dan2(:,2),dan10(:,1),dan10(:,2), ...
                res(:,1),res(:,2),res2(:,1),res2(:,2),res(:,1),res(:,4),['andinhbl',model,'.aps'])
        plot(can2(:,1),can2(:,2),can10(:,1),can10(:,2), ...
                res(:,1),res(:,3),_t,_pcthbco,['andinhhb',model,'.aps'])
        eval(['save res @file=Andersen87_inh_sims_',model,'00.csv @format=ascii …
                @separator=comma'])
TEND=4*24; start @nocallback
Tab3=[Tab3;[CONC,LDAYLIVCYPDOSE, LDAYLIVGSTDOSE,(LDAYLIVCYPDOSE + …
        LDAYLIVGSTDOSE),(LDAYLIVCYPDOSE/LDAYLIVGSTDOSE)]];
end
Tab3=Tab3([1,3,2,4],:)
save can2 @file=andersen_200ppm_co_data.csv @Format=Ascii @Separator=comma
```

```
% ----------------------------------------------------------------------
% File: FigC7_Andersen_91_Fig4.m
% Programmed by Paul Schlosser, U.S. EPA, 1/2011
% Creates plot shown in Figure C-7 of the dichloromethane IRIS
% assessment.  Figure C-7 shows model A and C simulations versus
% % HbCO data of Andersen et al (1991) for a 30-min inhalation
% exposure to 5159 ppm DCM.  The model A simulations use the
% parameters as listed in Table 1 of Andersen et al. (1991) rather
% than those listed in the caption of Figure 4, for a fair "apples
% to apples" comparison to model C; i.e., in both cases this is a
% validation of the parameters/model fit to other data, rather than
% an alternate model w/ the same struchture.
% ----------------------------------------------------------------------
prepare @clear T PCTHBCO
% Anderse et al. (1991) rat timecourse data for inhalation of
% 5159 ppm DCM for 30 min.
% time (hr), % HbCO
load @file=Andersen_91_Fig4.csv @Format=Ascii; dat=Andersen_91_Fig4;

use rat_set_A
TEND=7; BW=0.24; CINT=0.1; CONC=5159; TCHNG=0.5; start @nocallback
start @nocallback
t1=_t; p1=_pcthbco;
use rat_set_C
TEND=7; BW=0.24; CINT=0.1; CONC=5159; TCHNG=0.5; start @nocallback
start @nocallback
plot(dat(:,1),dat(:,2),t1,p1,_t,_pcthbco,[0,0.5],[0.3,0.3],'and91fig4.aps')
```

```
% ----------------------------------------------------------------
% File: FigC8 rat inhal exp_dose.m
% Programmed by Paul Schlosser, U.S. EPA, 1/2011
% Creates exposure-dose relationship for rat inhalation exposures to
% dichloromethane, for models A and C, as shown in Figure C-8 of the
% IRIS assessment
% ----------------------------------------------------------------
use rat_set_C
prepare @clear T CV PCTHBCO

bwm=mean([0.3905, 0.3852, 0.3848, 0.2455, 0.2443, 0.2422]); res=[];
for model=['A' 'C'];
        eval(['use rat_set_',model]);
        prepare @clear T CV PCTHBCO
        TEND=6*24*7; CINT=0.1; TCHNG=6.0; BW=bwm; r=[0,0,0]; start @nocallback
        for CONC=[200,1000,2000,4000] %[10:10:100, 100:20:300, 400:100:1000, …
                1200:200:2000]
                start @nocallback
                r=[r;[CONC,WAVGLIVGSTDOSE,WAVGLIVCYPDOSE]];
        end
        res=[res,r]
end
plot(res(:,1),res(:,2),res(:,1),res(:,3), ...
        res(:,4),res(:,5),res(:,4),res(:,6),'ratlinear.aps')
```

```
% ----------------------------------------------------------------
% File: FigC9n10 rat_oral_fits.m
% Programmed by Paul Schlosser, U.S. EPA, 2/2011
% Creates plots shown in Figure C-9 (4 panels) and Figure C-10 of the
% dichloromethane IRIS assessment.  Figure C-9 shows model C fits to
% data of Angelo et al. (1986b) for 50 and 200 mg/kg bolus oral
% exposures.  Figure C-10 is fit of model D with KA = 4.31 or = 0.62.
% Results now written to an Excel file where plots were created.
% ----------------------------------------------------------------
load @file=angelo_86_50mg.csv @Format=Ascii; da50=angelo_86_50mg; % 50 mg/kg
load @file=angelo_86_200mg.csv @Format=Ascii; da200=angelo_86_200mg; % 200 mg/kg
% Angelo et al. (1986b) rat timecourse data from
% oral dosing of 50 or 200 mg/kg
% time (hr), blood DCM, liver DCM, % exhaled DCM, % exh CO
fit=0 % Set to 0 to not use all_fit-params
use rat_set_C
if fit
        use allfit_rat-params
end

prepare @clear T CV CL PCTIVEXH PCTIVCOEXH PCTHBCO HBCO
RENCOC=0; RENCOS=0; TEND=25; BW=0.259; BOLUS=50; CINT=0.05; start @nocallback
start @nocallback
ra50=[_t, _cv, _cl, _pctivexh, _pctivcoexh];
```

```
pth="\\AA.AD.EPA.GOV\ORD\RTP\USERS\K-Q\pschloss\Net MyDocuments\DCM\rat
PBPBK DCM paper figures\Rat DCM PBPK Figure 8.xls"
xlsWrite(pth,"gargas-oral-dat","G1:K501",ra50)
use KAfit-params
start @nocallback
ra50=[ra50, _cv, _cl, _pctivexh, _pctivcoexh];
xlsWrite(pth,"gargas-oral-dat","R1:U501",ra50(:,6:9))
use rat_set_D
if fit
        use allfit_rat-params
end
RENCOC=0; RENCOS=0; TEND=25; BW=0.259; BOLUS=200; CINT=0.05; start
@nocallback
ra200=[_t, _cv, _cl, _pctivexh, _pctivcoexh];
xlsWrite(pth,"gargas-oral-dat","M1:P501",ra200(:,2:5))
use KAfit-params
start @nocallback
ra200=[ra200, _cv, _cl, _pctivexh, _pctivcoexh];
xlsWrite(pth,"gargas-oral-dat","W1:Z501",ra200(:,6:9))
plot(da50(:,1),da50(:,2),ra50(:,1),ra50(:,2),da200(:,1),da200(:,2), ...
        ra200(:,1),ra200(:,2),ra50(:,1),ra50(:,6),ra200(:,1),ra200(:,6),'angbl.aps')
plot(da50(:,1),da50(:,3),ra50(:,1),ra50(:,3),da200(:,1),da200(:,3), ...
        ra200(:,1),ra200(:,3),ra50(:,1),ra50(:,7),ra200(:,1),ra200(:,7),'angli.aps')
plot(da50(:,1),da50(:,4),ra50(:,1),ra50(:,4),da200(:,1),da200(:,4), ...
        ra200(:,1),ra200(:,4),ra50(:,1),ra50(:,8),ra200(:,1),ra200(:,8),'angexd.aps')
plot(da50(:,1),da50(:,5),ra50(:,1),ra50(:,5),da200(:,1),da200(:,5), ...
        ra200(:,1),ra200(:,5),ra50(:,1),ra50(:,9),ra200(:,1),ra200(:,9),'angexc.aps')

% Pankow et al. '91; COHb following a single gavage dose
% of 6.2 mmol/kg DCM (526 mg/kg) in 259 gram male Wistar rats
load @file=Pankow_91_526mgHb.csv @Format=Ascii; dpank=Pankow_91_526mgHb;
use rat_set_C
if fit
        use allfit_rat-params
end
RENCOS=1; TEND=12.5; BOLUS=526; BW=0.259; PCTHBCO0=1.0; CINT=0.05;
start @nocallback
ps=[_t,_pcthbco];
%use KAfit-params
KA=0.62;
start @nocallback
% See comment on line 15 above re. KA
plot(dpank(:,1),dpank(:,2),ps(:,1),ps(:,2),_t,_pcthbco,'pankhb.aps')
ra50=[ra50,ra200];
save ra50 @file=Angelo-oral-sim.csv @format=ascii @separator=comma
```

```
% ---------------------------------------------------------------
% File: FigC11_McKennay_oral_rat.m
% Programmed by Paul Schlosser, U.S. EPA, 1/2011
```

```
% Creates plots shown in Figure C-11 of the dichloromethane IRIS
% assessment.  Figure C-11 shows model C fits to data of McKenna
% and Zempel (1981) for disposition of 14C-DCM for 1 and 50 mg/kg
% bolus oral exposures.
% ---------------------------------------------------------------
use rat_set_C
prepare @clear T PCTIVEXH
TEND=4.7; BW=0.25; CINT=0.1;
RENCOS=0.0; COINH=0.0; PSCO=0.0;  PCTHBCO0=0.0; RENCOC=0.0;
% McKenna and Zempel (1981) rat timecourse data from
% oral dosing of 1 or 50 mg/kg
% time (hr), % exhaled DCM in 30-min interval, ...
%                 cumulative % exhaled DCM
load @file=McKenna81_1mg_exhDCM.csv @Format=Ascii; da1=McKenna81_1mg_exhDCM;
        % 1 mg/kg data
load @file=McKenna81_50mg_exhDCM.csv @Format=Ascii;
da50=McKenna81_50mg_exhDCM; % 50 mg/kg data
% Also showing Angelo et al. (1986b) data, also 50 mg/kg, for comparison
load @file=angelo_86_50mg.csv @Format=Ascii; d50=angelo_86_50mg; % 50 mg/kg

BOLUS=1; start @nocallback
t=_t, cex= _pctivexh;
TEND=48; start @nocallback
p1=PCTIVCOEXH;
BOLUS=50; start @nocallback
p1=[1,50;p1,PCTIVCOEXH]
TEND=6.2; start @nocallback
plot(da1(:,1),da1(:,3),t,cex,da50(:,1),da50(:,3),_t,_pctivexh, d50(:,1), d50(:,4),'mckenna.aps')
```

```
% ---------------------------------------------------------------
% File: FigureI-1_rat-human-inahl-AUC-dosim.m
% Programmed by Paul Schlosser, U.S. EPA, 4/2011
% Creates exposure-dose relationship for humans versus the rat
% shown in Figure I-1 of the IRIS assessment.
% ---------------------------------------------------------------
ccs=[10:10:60,80,100,150,200:100:600,800,1000,1500,2000:1000:5000];
clearT; TEND=95.0; hum1=[]; contsim=0;
NRUNS=3000;  % NRUNS = Total iterations for Monte Carlo analysis
gendm="both";          % Gender mix; choose "male, "female", or "both"
agem=0;                % Age "mix"; if 0 draws from distribution (0.5-80 years)
                       % otherwise agem value is used exclusively
popn=["mix"]; % "mix" of +/+, +/-, and -/-
        % +/+ and +/- subsets are sampled in for-CONC loop below;
model='human_par2'; % Choose between 'human_par1' (original) and 'human_par2' (+
uncertainty)
nm=["LDAYAUCS";"GSTGT"]; nn=findnames(nm,rnames); gsmult=1;
        if (length(nn) ~= length(nm))
                disp("Not all names in list nm.")
                return
```

```
        end
for CONC=ccs
        straightsims; res=mean(runo(:,nn(1))); rres=[CONC,res]; % Results for full population
        disp(['Human [',ctot(popn),'], conc = ',num2str(CONC),', AUCSlowly = ',num2str(res)]);
        res=mean(runo(runo(:,nn(2))==1,nn(1))); rres=[rres,res]; % Results for full GST +/-
        % nn(2) is the column index for the GST type identifier (GSTGT), which has values:
                % 0 for +/+, 1 for +/-, and 2 for -/-.  runo(:,nn(2))==x then returns a column of
                % 1's and 0's that selects the rows when the index is x.
        disp(['Human [+/-], conc = ',num2str(CONC),', AUCSlowly = ',num2str(res)]);
        res=mean(runo(runo(:,nn(2))==0,nn(1))); rres=[rres,res]; % Results for full GST +/+
        disp(['Human [+/+], conc = ',num2str(CONC),', AUCSlowly = ',num2str(res)]);
        hum1=[hum1; rres];
end
save hum1 @file='FigG-1_human.csv' @format=ascii @separator=comma
save @File="FigG-1.mat"

% Then rat simulations
use rat_set_C
TEND=2*7*24; ratr=[]; BW=0.229; TCHNG=6; TCHNG2=120;
for CONC=ccs
        start @NoCallBack
        ratr=[ratr;[CONC,WAVGAUCS]];
        disp(['Rat, conc = ',num2str(CONC),', AUCSlowly = ',num2str(WAVGAUCS),'.']);
end
save ratr @file='FigG-1_rat.csv' @format=ascii @separator=comma

plot(ccs,ratr(:,2),ccs,hum1(:,2),ccs,hum1(:,3),ccs,hum1(:,4),'FigG_1.aps')
```